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$^{15}N/^{14}N$ ratio and quality control of Iberian pig carcasses

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SUMMARY – The aim of this study is to evaluate the possible use of the $^{15}N/^{14}N$ index as a means of determining the feeding regime of Iberian pigs. Two batches of Iberian pigs (B1 and B2) were fed on fallen acorns and fodder (*montanera*) during their final fattening stage and two batches (C1 and C2) ate only commercial feed. The feed consumed by batch C2 was a special feed, designed to achieve a fatty acid profile similar to that produced when the animals eat acorns (*bellota*). The $^{15}N/^{14}N$ isotope ratio was determined in each animal, in loin samples in addition to the fatty acid content in samples of subcutaneous fat taken from the animals' hindquarters. According to the Official Sales Contract of Iberian pigs (OSC), the batches were classified as "Recebo" (B1 and C2), "Bellota" (B2) and "Cebo" (C1). The δ^{15} N isotope values enables the groups of animals to be classified according to their feeding regime, including the two batches fed on commercial feed.

Keywords: Iberian pig, isotope d15, loin, fatty acids.

RESUME – *"Le rapport $^{15}N/^{14}N$ et le contrôle de qualité de carcasses de porcs Ibériques". Le but de ce travail est d'évaluer la possibilité de différencier le régime d'alimentation de porcs Ibériques au moyen de l'indice $^{15}N/^{14}N$. On a utilisé deux lots de porcs Ibériques finis en montanera (B1 et B2) et deux lots qui ont consommé seulement un aliment composé (C1 et C2); l'aliment consommé par le lot C2 était fabriqué spécialement pour obtenir un profil d'acides gras semblable à celui que produit le gland chez les animaux. Pour chaque animal, on a déterminé la relation isotopique $^{15}N/^{14}N$ sur des échantillons de longe, et la composition en acides gras d'échantillons de graisse sous-cutanée pris vers la croupe. Selon le Contrat Officiel d'Achat de porcs Ibériques, les lots B1 et C2 ont été classés comme "Recebo", le B2 comme "Bellota" et le C1 comme "Cebo". Les valeurs de l'isotope δ^{15} N ont clairement permis de différencier les groupes d'animaux selon l'alimentation reçue, même les deux lots qui ont consommé l'aliment composé.*

Mots-clés : *Porc Ibérique, isotope d15 N, longe, acides gras.*

Introduction

Pigs used for the production Iberian pork have traditionally eaten the natural resources that abound in the Spanish countryside: fodder, grains and mainly acorns, which have fallen from the *Quercus* trees (oak, cork oak, gall-oak, etc). This system of production means that animals fattened in this way produce high quality meat and therefore meat products. The limitation of acorn production on the one hand and the high product price on the other, have led to a search for alternative products to acorns, attempting recreate the characteristics of the animals fattened on acorns and fodder, although the quality of the meat is not as good as that of animals fattened on *montanera*.

In order to distinguish which carcasses belong to animals that have not been fattened on *montanera*, several analytical techniques have been developed to determine certain characteristic parameters for each feeding regime. Some of these parameters are very easy to determine, but have a high error rate, such as tactile sensation, slip temperature and iodine levels. Others, such as the determination of fatty acids, triglycerides or phospholipids, have led to a more accurate identification of the animals' feeding regime (De Pedro, 2001). However, the development of special commercial feeds that create lipids profiles in the animals similar to those provided by acorns, have cast doubt on the reliability of these techniques.

Confronted with this situation, recently other techniques have been proposed such as the determination of isotopes (Gonzalez-Martín *et al*., 1999; Gonzalez-Martín *et al*., 2001) in order to identify the animals' feeding regime.

Isotope composition is a natural characteristic that can only be changed by processes of tissue formation, and therefore, could be used as a natural "fingerprint". Living organisms more or less reflect the isotope composition of the substances they ingest and which are present in their surroundings. This technique has been applied to animal-derived produce, such as dairy (Manca *et al*., 2001; Rossmann *et al*., 2000; Camin *et al*., 2001; Rossmann *et al*., 1998) and meat products (Delgado y Garcia, 2001. Renou *et al*., 2003, Piasentier *et al*., 2003), in addition to other products such as fruit juices (Bricout y Koziet, 1985; Kornexl *et al*., 1995; Rossmann *et al*., 1994), honey (Giraudon, *et al*., 2000; Reniero, *et al*., 1997; White *et al*., 1998), wine (Martin *et al*., 1988; Versini *et al*, 1997) and oil (Kelly, 2003; Krueger, 1998; Rossmann, 2001).

Hence the aim of this study is to assess how the feeding regime given to Iberian pigs affects their isotope ratio $^{15}N/^{14}N$ and hence the possibility of using this parameter to identify the animals' feeding regime, and therefore, the quality of their carcasses and their products.

Material and methods

For this study, four batches of Iberian pigs were used. Two batches were fattened at the Dehesón del Encinar Research Centre, which belongs to the Department of Agriculture of Castilla La Mancha; one of them (B1; n=43 pigs) was fed exclusively on pastureland and acorns (the production of acorns was somewhat scarce), whereas the other batch (C1; n=15 pigs) was fed on commercial feed; in both cases, the fattening period was 115 days. The other two batches of animals were part of a genetic study carried out by AECERIBER in two areas of pastureland in Badajoz. One of them (B2; n=9 pigs) was fattened exclusively on *montanera* for 104 days, in which the production of acorns was abundant; the other batch (C2; n=10 pigs) was only fed commercial feed. This feed was special since one of the raw materials used was high-oleic sunflower flour, in order to product high levels of this fatty acid in the animals' subcutaneous fat.

Once the animals were slaughtered, a sample of subcutaneous fat was taken from the animals' hindquarters. The sample contained skin, fat between the skin and the lean meat and a little lean meat. Subsequently, the loins were separated from the carcass and a sample (50 g) was taken from the anterior end of one of them, so that only the *Longissimus dorsi* muscle was included, without any external or intermuscular fat.

In the subcutaneous fat sample, the fat was extracted using a microwave oven following the methodology explained by De Pedro (1997), and the fatty acids composition was determined using gas chromatography according to the official methods for oil and fat analysis (BOE, 1979).

The loin sample was ground to homogenise it, and a sample of 0.05 mg was taken to determine its isotopes. This was carried out using a Carlo Erba Elemental Analyser coupled with an isotope ratio mass spectrometer (IRMS).

The isotope composition of $\delta^{15}N$ refers to the stable isotope composition of nitrogen in the air. The value of $d^{15}N$ is calculated using the following equation,

$$\delta^{15} N = (Ra/Rsa-1) * 1000 \ (^{0}/_{00})$$

where Ra and Rsa is the proportion $\delta^{15} N / \delta^{14} N$ of the air and the sample.

The batch classification criteria used were the field information and the Official Sales Contract (OSC) of Iberian pigs in force at the time of the trials (BOE, 2000).

Results

Table 1 shows the results obtained from the determination of the subcutaneous fat composition in the experimental batches. The table only shows the main fatty acids, which are those included in the OSC (BOE, 2000) for the determination of animal feeding regime certificates.

In accordance with the OSC criteria, based on their average fatty acids composition, batches B1 and C1 would be classified as "Cebo" quality. In the case of batch C1, this classification would agree with the field information. However, for the batch B1, the high value of linoleic acid means that it would not even be classified in the "Recebo" category.

In the case of batch B2, the classification based on its fatty acid composition agrees with the field information (Bellota). However, Batch C2 would have been overvalued using this method, since having consumed commercial feed, it should be classified as "Recebo".

We see therefore that classification based on fatty acids composition can undervalue or overvalue certain batches of meat, either to the detriment of the producer (for batch B1) or the manufacturer who buys the animals or the consumer who acquires the end product (for batch C2).

Table 1. Mean composition and standard deviation of subcutaneous fat of the experimental batches and their classification according to the OSC criteria

Batch	Palmitic acid			Stearic acid			Oleic acid			Linoleic acid		
	Mean	Min.	Max.	Mean	Min.	Max.	Mean	Min.	Max.	Mean	Min.	Max.
B1	19.5	18.2	21.0	9.4	7.8	11.0	53.4	51.9	55.0	11.2	9.9	12.0
C1	20.8	20.0	22.5	10.9	9.9	11.7	50.7	50.5	51.6	11.2	10.0	12.0
B2	19.0	17.9	20.0	8.8	7.9	9.5	56.1	53.4	58.2	8.9	7.7	9.6
C2	22.3	21.4	23.8	8.9	6.9	10.3	53.9	51.6	55.8	9.1	8.4	10.3

Table 2 shows the isotope ratio $^{15}N/^{14}N$ values (average, minimum and maximum) of the loin meat taken from the experimental animals.

Table 2. Isotope ratio $^{15}N/^{14}N$ values for pork loins from pigs fattened using different feeding regimes

Batch	Isotope ratio $^{15}N/^{14}N$ values		
	Mean	Minimum	Maximum
B1	4.57	4.17	5.10
C1	5.34	4.60	5.65
B2	3.78	3.40	4.89
C2	5.93	5.74	6.24

As we can see in the above table, the average isotope ratio $^{15}N/^{14}N$ of batches B1 and B2 was lower than batches C1 and C2. When the animals have consumed compound feed, the average isotope ratio $^{15}N/^{14}N$ values are above 5, and for animals that have been fattened on *montanera* this ratio is under 5. The only exceptions were in batch C1, where one animal obtained a value higher than 5 (5.10) and in batch B1, where one animal obtained a value lower than 5 (4.17).

Since the differences are so clear between the batches and bearing in mind that these animals were slaughtered on the same day and in the same slaughter house, it is possible that a mistake may have occurred in identifying the animals or in the sample that was taken and therefore this may be the reason why the ratios $^{15}N/^{14}N$ for these samples would classify them in a different category.

Therefore, we can conclude that the isotope ratio $^{15}N/^{14}N$ could be an indicator that verifies the animals' feeding regime during the final fattening stage prior to slaughter.

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