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^{1}H-NMR as a tool to determine the type of feeding of Iberian pigs

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Abstract. The fact that animals are often given feed in an attempt to achieve similar carcass characteristics to those of animals exclusively fed with "montanera", has obliged us to develop methods to differentiate and determine the type of feed consumed by these animals. One of these methods is Nuclear Magnetic Resonance (^{1}H-NMR). Iberian pig fat samples were taken in the rump at 10 cm from the tail insertion of Torbiscal pure Iberian pigs fattened with montanera (batch B1; n=44) and with feedcompound (batch P1; n=16). Similar samples were also taken from diverse genetic types of pure Iberian pigs fattened with montanera (batch B2; n=10) or with special feedcompound (batch P2; n=10). The ^{1}H-NMR spectra of these fat samples were obtained according to routine methods at the NMR Unit of the Central Service for Reseach Support (SCAI) of the University of Cordoba. The Principal Component Analysis (PCA) of ^{1}H-NMR spectral data show the possibility to determine the type of feeding of Iberian pigs by using this information.

Keywords. Iberian pig – Fat – PCA – ^{1}H-NMR.

^{1}H-RMN comme outil pour déterminer le type d'alimentation des porcs Ibériques

Résumé. *Nourrir les animaux avec des aliments composés pour obtenir des carcasses dont les caractéristiques soient semblables à celles des animaux recevant exclusivement une alimentation de type «montanera», nécessite de rechercher des techniques qui permettent de différencier et de reconnaître le type de nourriture qu'ont reçue les animaux. La Résonance Magnétique Nucléaire (^{1}H-RMN) est une de ces techniques. Des échantillons de graisse ont été prélevés dans la croupe (à 10 cm de l'insertion de la queue) de porcs Torbiscal purs engraissés selon le mode «montanera» (lot P1, n = 44) ou recevant un aliment composé (lot B1,n = 16). Des échantillons similaires ont été prélevés sur des porcs Ibériques purs de différents types génétiques engraissés selon le mode «montanera» (lot B2, n =10) ou recevant des aliments spéciaux (lot P2, n =10). Des spectres ^{1}H-RMN de ces échantillons de graisse ont été obtenus selon les protocoles d'analyse de l'Unité de RMN du Service Central d'Appui à la Recherche (SCAI), de l'Université de Cordoue. L'analyse en composantes principales (ACP) des données ^{1}H-RMN montre la possibilité de déterminer le régime alimentaire des porcs de race Ibérique à partir uniquement de ces informations spectroscopiques.*

Mots-clés. *Porc Ibérique – Graisse – PCA – ^{1}H-NMR.*

I – 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 (named *montanera*) 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. Proton Nuclear Magnetic Resonance (^{1}H-NMR) spectroscopy is one of the more powerful spectroscopic tools for the investigation of the chemistry and physical properties in samples. The use of ^{1}H-NMR is becoming universal for a wide range of fields including biochemical, agricultural, medical, materials, chemical, industrial, environmental, and pharmaceutical (Alan and Alan, 2005; Larsen et *al*., 2006; Hong-Seok et *al*., 2009; Alonso-Salces et *al*., 2010). One of the benefits of ^{1}H-NMR spectroscopy is the ability to probe complex systems without necessarily requiring a separation of individual components prior to analysis. With the continued development of ^{1}H-NMR spectroscopy as an analysis tool, the size and complexity of NMR data sets make them more difficult to analyze simply through operator interaction (Alan and Alan, 2005). Multivariate methods like Principal Components Analysis (PCA) are routinely utilized in other forms of spectroscopy for the analysis of complex mixtures. The use of chemometrics in NMR is more limited, but has quickly become an important tool for the NMR spectroscopist (Alan and Alan, 2005). Hence the aim of this study is to assess how the feeding regime given to Iberian pigs affects their ^{1}H-NMR spectra and hence the possibility of using this technique to identify the animals' feeding regime, and therefore, the quality of their carcasses and their products.

II – Materials 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 (P1; 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 (P2; 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. A liquid fat sample were extracted from each subcutaneous fat sample by using a microwave oven following the methodology explained by De Pedro *et al*., (1996). The ^{1}H-NMR spectra of liquid fat samples were obtained according to routine methods at the NMR Unit of the Central Service for Reseach Support (SCAI) of the University of Cordoba.

Each liquid fat sample was dissolved in 1 ml of deuterated chloroform and placed in a 5 mm NMR tube. The ^{1}H-NMR experiments were performed on a Bruker (Rheinstetten, Germany) Avance 400 WB spectrometer. The spectra were recorded using a 6.5 µs pulse, an acquisition time of 4.0 s (24k data points) and a total recycling time of 3.0 s, a spectral width of 3000 Hz (7.6 ppm), 16 scans. Prior to Fourier transformation, the free induction decays (FIDs) were zero-filled to 32k and a 0.3 Hz line-broadening factor was applied. The chemical shifts are expressed in d scale (ppm), referenced to the residual signal of chloroform (7.24 ppm). XWINNMR were used to perform the processing of the spectra.

The normalized spectral data were analyzed by multivariate technique like PCA with the statistical software package Unscrambler 9.2 (Camo Process AS, Oslo, Norway, 2005).

III – Results

^{1}H-NMR spectra of the 80 liquid Iberian pig fat samples were recorded. Figure 1 shows the full ^{1}H-NMR spectral range used in this work (0.5-6.0 ppm). According to this figure, a wide range of highly specific information is obtained by ^{1}H-NMR spectroscopy. The spectra include the chemical shifts of the ^{1}H signals of the different functional groups of major (triglycerides) and minor components of Iberian pig fat. Figure 2 show the score plot of the sample scores in the space defined by the two first principal components (PC1 and PC2). It can be seen that samples were grouped according to their feeding regime but some clusters were partially overlapped. It occurs with batches fed with similar type of feeding: with acorn (B1 and B2) or with commercial feedcompound (P1 and P2). However, there are more differences between batches fed with different type of feeding (B1 or B2 versus P1 or P2). To confirm these results it is suggested to test a supervised algorithm like SIMCA or Discriminant Analysis to classify these spectra from animals with different feeding. Therefore, it can be concluded that ^{1}H-NMR spectroscopy could be a useful technique to verify the animals´ feeding regime during the final fattening stage.

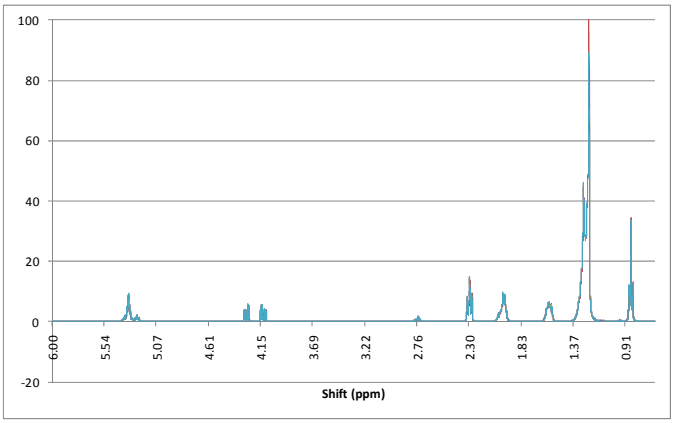

Fig. 1. ^{1}H NMR spectra of Iberian pig fat.

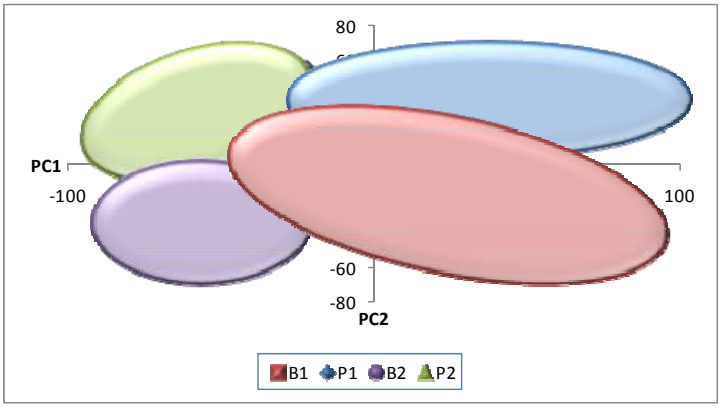

Fig. 2. Score plot.

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