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Abstract. Iberian pigs fattened at *montanera* (free-range fattening phase, with diet based on grass and *Quercus* acorns) products are the most recognized from *dehesa*, because their high contents in unsaturated fatty acids. Spanish regulation establishes an official method to separate commercial categories depending of fattening period feed. Fatty acid profile evolution during fattening period helps stockmen to organize grazing and to decide slaughter date. In this study 120 pure breed Iberian pigs have been studied using the "Spring loaded biopsy" method for *in vivo* biopsy; obtaining 1.45 ± 0.28 g of tissue at the beginning of *montanera* (1st day) and 1.26 ± 0.68 g one month later. Fat was extracted by microwave to obtain 0.83 ± 0.17 g and 0.73 ± 0.23 g from the first and second biopsy respectively. A regression model has been obtained to predict the amount of extracted fat knowing biopsies weigh (EF=-0.218+0.767*BW first biopsy and EF=0.046+533*BW in the second). The amounts of fat obtained are enough for individual fatty profile analysis following the official methods of analysis.

Keywords. Iberian pig – Montanera – Back fat.

Rendement en gras des biopsies sous-cutanées du porc Ibérique en début et après un mois de montanera

Résumé. *Les produits des porcs Ibériques engraissés en montanera sont ceux qui atteignent une valeur commerciale plus élevée, ceci est dû, principalement, à leur teneur en acides gras insaturés. Actuellement, pour déterminer les catégories commerciales, la législation espagnole préconise la méthode de l'analyse du profil en acides gras de la carcasse. La connaissance de l'évolution du profil en acides gras pendant l'engraissement permet d'organiser le pâturage et de programmer les abattages. Dans ce travail, à partir de 120 porcs Ibériques purs, on étudie la quantité de tissu sous-cutané obtenu grâce à la méthode de biopsie* in vivo *"Spring loaded biopsy" (1,45 ± 0,28 g à l'arrivée en montanera et 1,26 ± 0,68 g après un mois), et la quantité de graisse extraite par micro-ondes (0,83 ± 0,17 g dans la première biopsie, et 0,73 ± 0,23 g dans la deuxième). On propose des modèles de régression qui nous permettent de connaître la quantité de graisse qu'on obtiendrait à partir du poids de la biopsie (EF=-0,218+0,767*BW dans la première biopsie et EF=0,046+0,533*BW dans la deuxième). Les résultats indiquent que ces quantités de graisse sont suffisantes pour l'analyse individuelle du profil en acides gras.*

Mots-clés. *Porc Ibérique – Montanera – Graisse dorsale.*

I – Introduction

The Iberian pig constitutes a breed of great economic importance in Spain and Portugal. In the traditional rearing system, pigs are free-reared in an expanse of land of variable area, using natural resources [mainly grass (*Quercus ilex*) and acorns (*Quercus suber*)] only. Cured ham obtained from free-range pigs has gained widespread consumer acceptance and a high commercial value by virtue of it characteristic flavour; also, the high content in unsaturated fats of the ham has increased its appreciation as a healthy food. The free-range rearing system departs considerably from the intensive farming regime, where pigs are confined and fattened with commercial feed. The Spanish official method for discriminating between pig feeding and

rearing regimes determines the fatty acid composition of pig fat in terms of four fatty acids (oleic, linoleic, palmitic and stearic).

The traditional husbandry system of the Iberian pig in the *dehesa* (cleared Mediterranean forest like savannah) is linked to the sustained use of the pasturelands, finishing pigs during the acorn mast-feeding (called *montanera*). Rodríguez-Estévez *et al*. (2009) suggest a daily DM intake of 3.1-3.6 kg acorn kernel and 0.38-0.49 kg grass, which is achieved thanks to the functional characteristics of this breed. Free range Iberian pigs fattened with acorns give the most recognized products from the *dehesa*; however this production is geographically reduced to the South West of the Iberian Peninsula Spain, and acorn production is limited to fall and winter seasons.

Montanera products attain the highest prices in the market because of preference by consumers. The main reason for this preference is the high oleic acid content in the fat of these pigs free-range fattened (Daza *et al*., 2007), mostly due to the high oleic acid content of acorns. This high proportion of oleic acid in the carcass strongly influences the properties of fat, leading a soft and oily lard, which is highly appreciated by consumer, who select these products for special occasions and pays a high price for them (López-Bote, 1998). Moreover, the higher oxidative stability of this pork, because of high α-tocopherol content of grass and γ-tocopherol of acorns improves technological and sensorial quality of Iberian pigs meat and products (Daza *et al*., 2007).

To prevent fraud the Iberian pig Spanish regulations establish an official method to evaluate the finishing diet regime (MAPA, 2007), based on the analysis of the subcutaneous backfat tissue fatty acid profile.

The aim of this study is to know the lipid performance of biopsies from subcutaneous back tissue as source of fat to analyze the fatty acid profile evolution during the *montanera* fattening.

II – Materials and methods

The experimental procedures and animal care conditions were approved by the Animal Experimentation Ethical Committee of the University of Córdoba, Spain.

1. Animals and handling

The study was conducted at a *dehesa* of evergreen oaks (*Quercus ilex rotundifolia*) with 120 purebred Iberian fattening pigs (male and female) of the Silvela variety. Pigs were on average 111.8±0.7 kg of LW at the start of the study and 157.9±1.7 kg at the end, after ≥ 2 months. All pigs were castrated following the Spanish regulations, to work with the same kind of pigs of the traditional montanera system. The stocking rate (0.76 pigs/ha) was established with margins that guaranteed that the acorns would not run out before the fattening was completed (Rodríguez-Estévez, 2007; Rodríguez-Estévez *et al*., 2008)

2. Weighing of the pigs

To calculate body weigh (BW), all the pigs of the herd were weighed individually at the beginning of *montanera*, after a month and the day previous slaughtering. An electronic scale (precision of 100 g) was used for weighing.

3. Biopsy collection

The biopsies were taken the first day of montanera and one month later. The samples were collected *in vivo* with a *Spring loaded biopsy* instrument, following the method described by (Bosch Puig *et al*., 2008). Once the sample was extracted, these samples were collected from the cannula and introduced in individual plastic tubes with the animal reference number, cleaning properly the cannula before taking the next sample.

All biopsies were transported at ≈ 10ºC to the laboratory, where these were stored at –25ºC, until their processing.

4. Lipid extraction

After completely defrosting each sample was weighed before using the technique of microwave oven described by De Pedro *et al*., 1997 to extract the fat from these tissue biopsies. Later the fat obtained from each biopsy was weighed and frozen to store it for future analysis of fatty acid composition.

III – Results and discussion

Descriptive statistics of weights of first and second biopsies (first day and one month later respectively) are showed in Fig. 1 and Table 1.

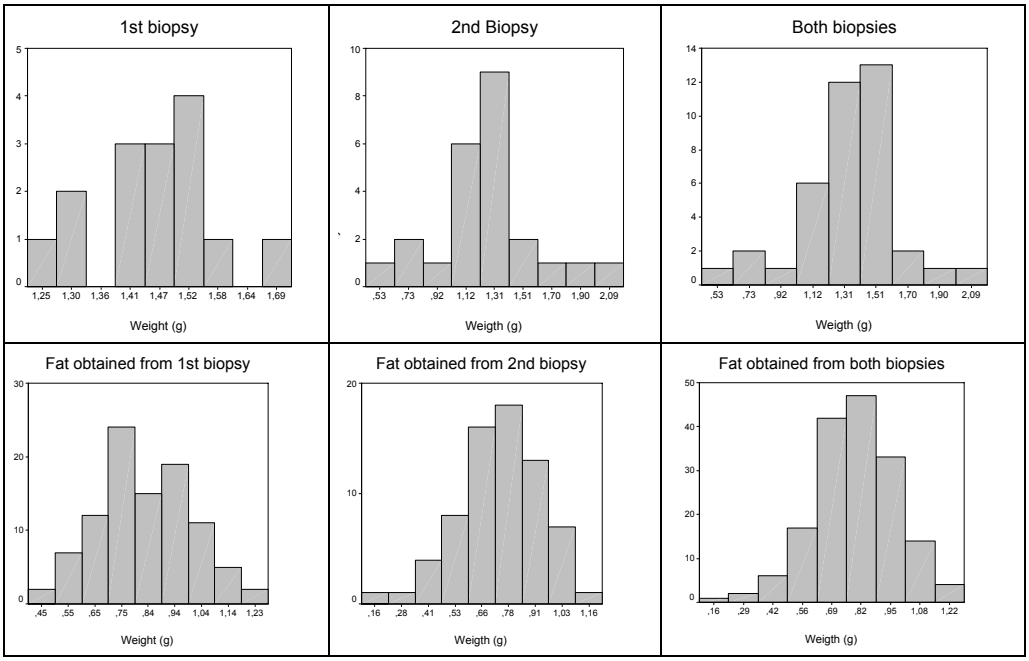

Fig. 1. Weigh of first, second and both biopsies and of fat obtained by microwave extraction.

Table 1. Biopsy weight and fat obtained in the 1st and 2nd biopsies

	1st Biopsy Weight	Fat obtained	2nd Biopsy Weight	Fat obtained	Both Biopsies Weight	Fat obtained
Mean±SE	1.45±0.28	0.83±0.17	1.26±0.68	0.73 ± 0. 23	1.33 ± 0.45	0.79 ± 0. 14
Minimum value	1.27	0.42	0.54	0.15	0.54	0.15
Maximum value	1.67	1.24	2.10	1.17	2.10	1.24
Percentile 25	1.42	0.74	1.08	1.08	1.21	0.68
Percentile 50	1.45	0.83	1.24	1.24	1.34	0.79
Percentile 75	1.52	0.93	1.38	1.38	1.51	0.91

Table 2. Linear regression models for the weight of fat obtained from biopsies (BW = body weight)

Effect variable	Linear regression	R^2	Standard Error	Sig.
Fat obtained (first biopsy)	=-0,218+0,767*BW	0,419	0,102	0,009
Fat obtained (second biopsy)	=0,046+533*BW	0,665	0,128	0,009

As Table 2 shows, it is possible to determine the final fat amount through linear regressions either in the first biopsy (fat obtained = -0,218+0,767*BW) or the second one (fat obtained = 0.046+533*BW).

Results show that it is possible to use this method of fat extraction, because it allows to obtain the necessary amount of fat to determine the fatty acid profile trough different methods of analysis, as it is shown in Table 3.

Table 3. Comparison of different analytical methods to determine the fatty acid profile

Reference	Method of extraction of fat	Method of analysis	Needed amount of fat extracted
(Carrapiso *et al*., 2001)	Dissection	Electronic nose	5 g (adipose tissue)
ISO 15304			250 mg
(Presidencia, 2004) (Oficial method)	Dissection and solvent or microwave extraction.	Gas Chromatography	0.2 g
(Alonso *et al*., 2008)	Not specified	Ion Mobility Spectrometry	1 g
(López-Vidal *et al*., 2008)	Microwave	Gas Chromatography - Mass Spectrometry and Chemometrics	0.2 g
(Arce *et al*., 2009)	Microwave	Infrared spectroscopy	A few microlitres
(Pascual *et al*., 2006)	Folch, Lee and Stanley (1957)	Gas Chromatography	0.5 g
(Regueiro *et al*., 2006)	ISO-1443 (Soxhlet) Folch, Lee and Stanley (1957) Bligh and Dyer (1959) Accelerated extraction methods Hexano-2, propanol 2:1	Gas Chromatography (FID) -Mass Spectrometry and Chemometrics	

IV –Conclusions

The combination of the *Spring loaded biopsy* and microwave fat extraction could be used to predict and to categorize animals before finishing and during the *montanera* in order to determine their handling and their slaughter date. It is possible to obtain *in vivo* biopsies and the amounts of fat obtained are enough for individual fatty acid profile analysis following the official methods of analysis.

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