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Short communication. Fatty acid composition of lamb fat depots as an origin discriminator

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Abstract

The development of quality labels for different lamb meats makes necessary to implement analytical methodologies to guarantee consumers the origin of the products. Eighty single birth male lambs from five Southern Spain sheep breeds (Grazalema Merino, Churra Lebrijana, Spanish Merino, Montesina and Segureña) were selected for the study and fed according to their traditional production system (with or without weaning). The fatty acid composition of five different depots (intramuscular, intermuscular, subcutaneous, omental and kidney knob) was analyzed by gas chromatography, showing a high variability among lamb types. The lipid profile of single fat depots was not able to assign 100% of the carcasses to their origin, but using the information from two depots (including intramuscular, or combining an external and an internal fat depot) led to a reliability of 100%. Any combination of 3, 4 or 5 depots also obtained 100% correct discrimination.

Additional key words: breed; lipids; PGI; sheep.

Resumen

Comunicación corta. Composición de ácidos grasos de los depósitos grasos de cordero como discriminador del origen

La aparición de distintivos de calidad para carnes de cordero hace necesario el desarrollo de métodos analíticos que garanticen al consumidor el origen de dichos productos. Se seleccionaron para este estudio ochenta corderos machos, provenientes de un parto simple, de cinco razas andaluzas (Merino de Grazalema, Churra Lebrijana, Merino Español, Montesina y Segureña) y se alimentaron de acuerdo a su sistema tradicional (con o sin destete). Se analizó la composición de ácidos grasos de cinco depósitos grasos (intramuscular, intermuscular, subcutáneo, omental y perirenal) con cromatografía de gases. La grasa de estos depósitos presentó gran variabilidad. El perfil lipídico de un único depósito no fue suficiente para asignar correctamente el origen del 100% de las canales, pero usando la información de dos depósitos (incluyendo el intramuscular, o combinando un depósito externo y otro interno) se obtuvo una fiabilidad del 100%. Cualquier combinación de 3, 4 o 5 depósitos también obtuvo una fiabilidad del 100% respecto a la proveniencia de la canal.

Palabras clave adicionales: IGP; lípidos; ovino; raza.

In Southern Spain, various sheep breeds have been grazed for centuries due to their adaptability to specific environments as well as to the quality of their products (Juárez *et al*., 2007). Nowadays, lamb producers are trying to adapt their product to consumer preferences. Moreover, labelled meats are perceived by consumers as healthy food, particularly if they are able to associate them to the breed and the production system (Dias *et*

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Abbreviations used: CL (Churra Lebrijana), FA (fatty acid), GM (Grazalema Merino), IM (intramuscular), IN (intermuscular), KK (kidney knob), MO (Montesina), OM (omental), PGI (Protected Geographical Indicator), SC (subcutaneous), SE (Segureña), SM (Spanish Merino).

al., 2008). In Southern Spain, purebred sheep breeders have developed «Protected Geographical Indicators» (PGIs) for Segureña (SE) and Spanish Merino (SM) sheep breeds (BOE A-2008-14080 and A-2009-3661, respectively). On the other hand, three local breeds (Grazalema Merino, GM; Churra Lebrijana, CL; Montesina, MO) have become endangered due to cross-breeding with other sheep breeds. Local authorities and sheep breeders' associations are collaborating to promote the breeding of these sheep breeds and the trade of their products. The development of quality labels for their meat is considered a priority, since each breed has specific genetic characteristics and is produced in well defined geographic regions with traditional production systems (Juárez *et al.*, 2007).

In this context, it is necessary to implement analytical methodologies to assure consumers about the products they purchase. Hence, methodologies based on the fatty acid (FA) profiles have been reported to be effective in order to differentiate meat production systems and breeds (Dias *et al.*, 2008). Santos-Silva *et al*. (2002) concluded that FA profile from intramuscular (IM) fat depot was an effective method for discriminating between lamb feeding systems with accuracy around 90%. And, in a recent study, Mellado-González *et al.* (2009) used kidney knob fat FA profile to differentiate between fattening diets of goat kids. Moreover, when the contribution of several factors to FA variations was evaluated, anatomical depot location was reported to be the most significant factor for several FAs and indices (Juárez *et al.*, 2008). However the effectiveness of using FA composition from different fat depots as an origin discriminator has not been tested.

Therefore, the aim of the present study was to assess the reliability of using FA profiles from five fat depots to discriminate the origin (breed × production system) of lamb carcasses.

Eighty single birth male lambs from five Southern Spain (Andalusia) sheep breeds were selected for the study. Eight SM, 16 GM and 16 CL were not weaned, but had access to concentrate from 45 days after birth. Eight SM, 16 SE and 16 MO lambs were weaned 45 days after birth and fed with concentrate *ad libitum* until slaughter. The lambs were slaughtered in an EU accredited abattoir (slaughter weight: 21 ± 1.3 kg). Samples from IM, omental (OM), kidney knob (KK), subcutaneous (SC) and intermuscular (IN) depots were collected within the first hour *post-mortem*, vacuum packed and frozen at –20°C (Juárez *et al*., 2008). FAs were analyzed following the method described by Aldai *et al.* (2006) based on previous saponification, double extraction and methylation by trimethylsilyldiazomethane. Separation and quantification of the fatty acid methyl esters was carried out using a gas chromatograph (GC, Agilent 6890N, Agilent Technologies S.L., Madrid, Spain) equipped with a flame ionisation detector automatic sample injector HP 7683, and using a HP-88 J&W fused silica capillary column (100 m, 0.25 mm i.d., 0.2 μm film thickness, Agilent Technologies Spain). Nonadecanoic acid methyl ester (C19:0 ME) at 10 mg mL^{-1} was used as an internal standard. FAs were expressed as a percentage of total FAs identified.

Statistical analyses were performed using Statistica 7.0 for Windows (StatSoft Inc., 2006). Descriptive statistical parameters were calculated and the type effect was studied by analysis of variance (ANOVA) and Tukey's test at a significance level of $p < 0.05$. The possibility of classifying lamb types (breeds × feeding systems) using information from the FA profile of five fat depots (individually or grouped) was tested using a canonical discriminant analysis to assess the reliability of using the FA profile of an animal to assign it to a given breed and feeding system. Ten FAs (> 95% total identified FAs) were selected based on their discriminant power. The discriminant analysis was conducted using stepwise model analysis, which performed the best-subset selection of the quantitative predictor by a procedure of entrance-remove of variables in the model. The significant level of a variable to enter in the model was 0.05.

The FA composition of the different depots from each lamb type was compared to analyze the differences among groups (Table 1). FA profiles were in line with those reported by other authors who studied lamb fat depots within the same live weight range and similar feeding systems (Sañudo *et al*., 1997; Arsenos *et al*., 2006). Fat depots from SE, followed by MO lambs, had the lowest 14:0 ($p < 0.001$) content. The highest IM 16:0 level was observed in fat from MO lambs ($p < 0.05$). For the rest of the depots, MO lambs always presented higher 16:0 levels than SMuw lambs ($p < 0.05$). In general, SMw presented the lowest 18:0 content ($p < 0.01$). The highest 16:1 levels were observed in IM ($p < 0.001$), IN ($p < 0.001$), SC ($p < 0.001$) and OM ($p < 0.01$) fat depots from SMw lambs and in KK fat from MO lambs ($p < 0.001$). Total *trans* 18:1 content was higher in fat from MO and SE lambs than in fat from CL and GM lambs ($p < 0.001$). The lowest

Table 1. Fatty acid profile (% total identified fatty acids) of five fat depots

Fatty acid	Fat depot[1]	CL[2]	GM[3]	SMuw[4]	SMw[5]	MO[6]	SE[7]	SEM[8]	Sig.[9]
14:0	IM	5.46^{a}	5.57^{a}	5.27^{ab}	5.72^{a}	4.51^{ab}	3.30^{b}	0.399	***
	IN	6.79^{ab}	8.57^{a}	5.61^{bc}	7.13^{ab}	4.93^{bc}	3.70^{c}	0.454	***
	SC	8.14^{b}	10.42^{a}	7.28^{b}	8.30^{ab}	7.49^{b}	3.42^{c}	0.442	***
	OM	7.69^{b}	9.87^{a}	7.30^{bc}	8.46^{ab}	5.15^{cd}	3.74^{d}	0.441	***
	KK	6.78^{ab}	7.94^{a}	6.23^{b}	6.79^{ab}	4.91^{b}	2.96^{c}	0.474	***
16:0	IM	23.5^{ab}	21.3^{b}	22.0^{ab}	22.9^{ab}	24.3^{a}	23.2^{ab}	0.597	*
	IN	24.0^{a}	23.8^{a}	19.5^{b}	22.7^{ab}	23.9^{a}	22.5^{ab}	0.717	**
	SC	25.1^{ab}	25.6^{a}	22.5^{b}	24.6^{ab}	25.7^{a}	22.1^{b}	0.579	***
	OM	25.7^{a}	25.4^{a}	22.7^{b}	25.5^{a}	24.3^{a}	23.4^{ab}	0.529	*
	KK	24.1^{a}	21.2^{b}	19.1^{b}	20.8^{b}	24.2^{a}	19.1^{b}	0.608	***
16:1	IM	1.66^{c}	1.70^{c}	1.92^{ab}	2.23^{a}	1.99^{ab}	1.84^{b}	0.075	***
	IN	1.57^{c}	1.70^{bc}	1.48^{c}	2.02^{a}	1.85^{ab}	1.60^{bc}	0.069	***
	SC	1.95^{c}	2.02^{bc}	2.56^{ab}	3.00^{a}	2.28^{bc}	2.25^{bc}	0.130	***
	OM	1.50^{b}	1.55^{b}	1.67^{ab}	1.82^{a}	1.59^{ab}	1.48^{b}	0.053	**
	KK	1.36^{b}	1.18^{bc}	1.30^{bc}	1.55^{ab}	1.73^{a}	1.13^{c}	0.046	***
18:0	IM	15.4^{ab}	16.2^{a}	15.0^{ab}	13.4^{b}	15.1^{ab}	16.2^{a}	0.420	**
	IN	19.0^{ab}	17.7^{bc}	19.6^{ab}	15.3^{c}	20.3^{ab}	20.6^{a}	0.653	***
	SC	16.5^{a}	15.5^{ab}	13.1^{bc}	11.4^{c}	15.0^{ab}	13.0^{bc}	0.689	***
	OM	20.8^{ab}	18.4^{ab}	16.9^{b}	16.7^{b}	21.5^{a}	21.8^{a}	0.775	***
	KK	22.9^{b}	23.6^{b}	21.9^{bc}	20.2^{c}	21.6^{bc}	27.5^{a}	0.787	***
All *trans* 18:1	IM	2.72^{b}	2.33^{b}	4.73^{a}	4.46^{a}	5.17^{a}	4.16^{a}	0.386	***
	IN	2.97^{b}	2.72^{b}	7.56^{a}	6.02^{a}	8.10^{a}	6.33^{a}	0.637	***
	SC	3.90^{b}	3.26^{b}	4.67^{b}	3.25^{b}	9.16^{a}	8.84^{a}	0.492	***
	OM	4.28^{cd}	2.84^{d}	6.63^{bc}	3.66^{cd}	9.72^{a}	9.26^{ab}	0.462	***
	KK	3.92^{bc}	3.06^{c}	5.00^{b}	3.86^{bc}	9.22^{a}	8.92^{a}	0.496	***
18:1n-9	IM	35.3^{ab}	33.5^{b}	36.1^{ab}	37.9^{a}	35.0^{ab}	38.2^{a}	0.914	**
	IN	37.8^{a}	37.4^{a}	36.5^{a}	39.2^{a}	31.9^{b}	35.4^{ab}	0.920	***
	SC	35.5^{b}	34.8^{b}	39.9^{a}	41.4^{a}	30.9^{c}	39.4^{a}	0.944	***
	OM	31.4^{b}	33.7^{b}	34.9^{ab}	35.8^{a}	29.0^{c}	30.7^{c}	0.876	***
	KK	32.5^{c}	35.5^{b}	36.9^{ab}	38.4^{a}	29.8^{d}	31.0^{cd}	1.052	***
18:2n-6	IM	8.38^{ab}	9.54^{a}	6.49^{b}	6.61^{b}	7.48^{ab}	6.99^{ab}	0.615	*
	IN	3.20^{bc}	2.27^{c}	2.78^{c}	2.94^{bc}	4.19^{ab}	4.69^{a}	0.229	***
	SC	3.43^{b}	2.11^{c}	2.56^{bc}	2.98^{bc}	3.83^{ab}	4.65^{a}	0.210	***
	OM	3.46^{ab}	2.07^{c}	2.62^{bc}	2.98^{bc}	3.94^{a}	4.49^{a}	0.187	***
	KK	3.61^{b}	2.14^{c}	2.72^{c}	3.75^{b}	3.68^{b}	4.80^{a}	0.257	***
18:3n-3	IM	0.47^{c}	0.76^{a}	0.67^{ab}	0.62^{ab}	0.37^{cd}	0.25^{d}	0.051	***
	IN	0.40^{c}	0.63^{b}	0.94^{a}	0.44^{bc}	0.43^{bc}	0.26^{c}	0.059	***
	SC	0.34^{cd}	0.53^{b}	0.82^{a}	0.44^{bc}	0.37^{cd}	0.26^{d}	0.051	***
	OM	0.34^{cd}	0.56^{b}	0.88^{a}	0.44^{c}	0.35^{cd}	0.24^{d}	0.056	***
	KK	0.33^{bc}	0.48^{ab}	0.60^{a}	0.42^{b}	0.37^{bc}	0.25^{c}	0.042	***
Total CLA	IM	0.82^{bc}	0.99^{b}	1.65^{a}	0.76^{bc}	0.40^{d}	0.53^{cd}	0.093	***
	IN	0.96^{bc}	1.23^{b}	1.92^{a}	0.80^{bc}	0.51^{d}	0.62^{cd}	0.109	***
	SC	1.05^{b}	1.26^{b}	1.98^{a}	0.93^{b}	0.50^{c}	0.65^{c}	0.110	***
	OM	0.92^{b}	1.17^{b}	1.99^{a}	0.77^{bc}	0.39^{d}	0.49^{cd}	0.102	***
	KK	0.86^{b}	0.92^{b}	2.01^{a}	0.71^{bc}	0.39^{c}	0.42^{c}	0.088	***
20:4n-6	IM	3.50^{b}	5.15^{a}	2.54^{b}	2.56^{b}	2.54^{b}	2.35^{b}	0.375	***
	IN	0.14^{a}	0.10^{b}	0.12^{ab}	0.14^{ab}	0.15^{a}	0.14^{ab}	0.009	*
	SC	0.13^{bc}	0.10^{c}	0.14^{bc}	0.15^{bc}	0.19^{ab}	0.21^{a}	0.018	***
	OM	0.13^{b}	0.08^{c}	0.12^{bc}	0.15^{a}	0.14^{a}	0.14^{a}	0.011	**
	KK	0.09	0.07	0.10	0.08	0.09	0.09	0.007	ns

[1] IM: intramuscular. IN: intermuscular. SC: subcutaneous. OM: omental. KK: kidney knob) from six Southern Spain lamb types (breed × production system). [2] CL: Churra Lebrijana. [3] GM: Grazalema Merino. [4] SMuw: Spanish Merino unweaned. [5] SMw: Spanish Merino weaned. [6] MO: Montesina. [7] SE: Segureña. [8] SEM: standard error of the mean. [9] Sig.: significant differences. ns: $p > 0.05$; *: $p < 0.05$; **: $p < 0.01$; ***: $p < 0.001$. [a, b, c, d]: values in the same row with different superscripts are significantly different ($p < 0.05$).

18:1n-9 levels were found in GM IM fat ($p < 0.05$) and in MO IN, SC, OM and KK fat depots ($p < 0.001$). The highest 18:2n-6 levels in IM fat were those from GM lambs ($p < 0.05$). However that same lamb type had the lowest 18:2n-6 content in the other four fat depots, while SE had the highest, followed by MO lambs ($p < 0.001$). IM fat from GM lambs had the highest 18:3n-3 levels ($p < 0.001$), while SMuw presented the highest values for the other four fat depots ($p < 0.001$). For the five fat depots, the Total CLA levels were always highest ($p < 0.001$) for SMuw lambs, and lowest for MO and SE, followed by SMw lambs. The highest IM 20:4n-6 relative content was observed for GM lambs ($p < 0.001$). Weaning effect has been studied by several authors, *e.g.,* Sañudo *et al*. (1998) and Cañeque *et al*. (2001) in Rasa Aragonesa and Talaverana breed, respectively, reporting an increase in saturated fatty acids of less than C16 chain length, as well as an increase in 18:3n-3 and CLA in intramuscular fat of unweaned lambs, as observed in Spanish Merino in the present study. Hence, the differences in FA composition among lamb types, due to the combination of genotype and production system, were evident and could be potentially used as an origin discriminator.

Table 2 shows the results from the discriminant analysis using the FA profiles from each fat depot, as well as all their possible two-by-two combinations. The values represent the reliability of predicting the origin (breed × feeding system) of a carcass using the information from the FA profile/s. Thus, individual depots were not able to assign 100% of the carcasses to their origin. The greatest and lowest discrimination rates were obtained when individual OM (96.25%) and SC (88.75%) FA profiles were used, respectively. According to Kirton *et al.* (1972), OM is the last fat depot to mature in sheep. Therefore, in young animals, as suckling lambs, variations in growth rate and dietary treatment among breeds would lead to greater modifications of OM-FA composition than in other fat depots. The lower discrimination rate obtained using SC-FA profile could be explained by a higher desaturase enzyme activity in that depot, as suggested by Moibi and Christopherson (2001) and Juárez *et al.* (2008), what would dilute the variability due to dietary differences. Combining the information obtained from the FA profile of IM with IN, OM or KK fat depots, all of the carcasses (100%) were correctly assigned to their lamb type. That same result was obtained by combining an external (IN) and an internal (KK) fat depot. The highest discrimination is obtained when the correlation between depots is lowest, and the lowest discrimination is obtained with similar fat depots, as observed for the combination OM-KK (87.50%). Thus, the IM-FA composition, richer in phospholipids and polyunsaturated FA than any other fat depot (Wood *et al*., 2008), combined with an external (IN) or internal (OM, KK) depot, obtained a high discrimination rate. For that same reason, the combination of two different depots, IN (external) and KK (internal), was able to correctly assign 100% of the carcasses to their origin. Thus, any combination of FA profiles from 3, 4 or 5 fat depots, always including the IM fat depot in combination with other two, or an internal and external depot, led to a correct discrimination of 100% of the carcasses.

Table 2. Reliability of using the fatty acid profile of one or two depots to assign an animal to a given type (breed × feeding system)

Fat depot/s[1]	% reliability
IM	95.00
IN	93.75
SC	88.75
OM	96.25
KK	93.75
IM-IN	100
IM-SC	97.50
IM-OM	100
IM-KK	100
IN-SC	96.25
IN-OM	97.50
IN-KK	100
SC-OM	96.25
SC-KK	97.50
OM-KK	87.50

[1] IM: intramuscular. IN: intermuscular. SC: subcutaneous. OM: omental. KK: kidney knob.

Previous studies have shown that FA profiles from IM (Dias *et al.*, 2008). Santos-Silva *et al*. (2002) and KK fat depots Mellado-González *et al.* (2009) were able to discriminate between groups with different production systems, even if gender, age and slaughter were not taken into account. However, in the present study, when different breeds and production systems were compared, the information obtained from single fat depots was not enough to discriminate the groups with 100% reliability. Using the IM-FA profile in combination with other fat depot seems to get a higher reliability, but it needs to obtain expensive samples (*longissimus* muscle). The use of an internal and an external fat depot, easy to access and analyze, could be a cheaper option. Moreover, the ten chosen FAs (ex-

pressed as % of total fat) can be analysed by using fast methods, such as Near Infrared Spectroscopy (NIRS, Prieto *et al*., 2009), what could be adapted as an on-line method at the abattoir.

In conclusion, FA profiles from individual fat depots were not able to completely discriminate the six lamb types present in Southern Spain. However, combining one fat depot with IM-FA profile, or using the FA compositions of an internal and an external fat depots, resulted in a correct assignation of 100% of the carcasses to their origin.

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