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SUMMARY – Twenty-three Merino Branco (MB) breed and rotational crossbred lambs with the breeds Serra da Estrela (SE) × "Romanov" (RO) × "Ile de France" (IF), were used to evaluate the effect of the genotype on the intramuscular fatty acid (FA) profile, determined by gas chromatography. All lambs were male, single-born and reared in the same grazing conditions (maternal milk and grass only), until slaughtered at 25 kg live-weight. The carcasses left sides were dissected and samples taken from the *longissimus dorsi* (LD) muscle and from the remaining muscles after mixing. Genotype significantly affected total intramuscular FA content and intramuscular FA profiles, distinguishing crossbred RO×(SE×IF×RO) lambs. Muscle sampling type did not present significant differences, showing that sampling only LD is an acceptable method that simplifies the collection of samples. Fatty acid profiles, using C16:0, C18:0, C22:6*n*-3, C10:0, C12:0 FA, were effective in discriminating between genotypes.

Key words: Lamb, fatty acid profile, intramuscular fat, genotypes.

RESUME – *"Composition en acides gras de la graisse intramusculaire des agneaux de différents génotypes abattus à 25 kg de poids vif". Vingt-trois agneaux de la race Merino Branco (MB) et d'un croisement rotatif des races Serra da Estrela (SE) × "Romanov" (RO) × "Ile de France" (IF), ont été utilisés pour étudier l'effet du génotype sur le profil en acides gras (AG) de la graisse intramusculaire, déterminé par chromatographie gazeuse. Tous les agneaux étaient de sexe masculin, nés simples et ont été élevés dans les mêmes conditions (lait maternel et pâturage) jusqu'à l'abattage à 25 kg de poids vif. Après la dissection de la moitié gauche de chaque carcasse, des échantillons du muscle* longissimus dorsi *(LD) ont été prélévés. Le reste des muscles a été mélangé et un échantillon moyen a été prélevé. Le contenu total aussi bien que les profils d'AG intramusculaires ont été affectés de manière significative par le génotype, ce qui permet de distinguer les agneaux du croisement RO×(SE×IF×RO). Le type de prélèvement des échantillons de muscle n'a pas montré de différences significatives, ce qui prouve que l'utilisation du LD est une méthode acceptable pour ce type d'analyse. La discrimination entre les génotypes, basée sur les AG C16:0, C18:0, C22:6*n*-3, C10:0, C12:0, s'est avérée une technique efficace pour les distinguer.*

Mots-clés : *Agneaux, acides gras, graisse intramusculaire, génotypes.*

Introduction

The perception of quality has increasingly become one of the main factors affecting the consumers' decision to purchase meat. This is largely related to its fat content and its fatty acid (FA) composition, since excessive consumption of fat and particularly saturated FA (SFA) is a major risk factor for coronary heart disease (Kritchevsky, 1998). However, meat and its derivatives products may be the main source of dietary polyunsaturated FA (PUFA) for certain people due to the low consumption of fish. These PUFA consist not only of the essential FA, linoleic (18:2*n*-6) and linolenic (18:3*n*-3), but also C20 and C22 PUFA present in the tissue phospholipids. Nutritional evaluation of fat relies on the ratios between PUFA and SFA and between *n*-6 and *n*-3 FA (Department of Health, 1994). Although meat from lambs presents a considerable proportion of saturated fat (about 50% of total FA), recent FA nutritional classification shows that only about 35% of total fat is hypercholesterolaemic (Bessa, 1999). Another aspect is the possible effect of breed on meat taste resulting from a variation in FA composition (Fisher *et al.*, 2000). Three genotypes were compared in this trial: Merino Branco (MB) (a meat breed) and two from a rotative crossbreeding designed to increase meat productivity in the system where MB is usually raised. The effect of genotype on meat characteristics, namely the intramuscular fatty acid profile, was evaluated from lambs reared in the same grazing conditions. Also, two different types of muscle sampling were tested.

Materials and methods

Twenty-three lambs were used in this study: 8 from MB breed; and 15 from the rotative crossbreeding Serra da Estrela (SE) × "Romanov" (RO) × "Ile de France" (IF) – 8 RO×(SE×IF×RO) and 7 SE×(IF×RO×SE). All lambs were male, single-born and fed with maternal milk and grass (*Dactylis glomerata*, *Medicago* spp., *Trifolium subterraneum*) only, until slaughtered at 25 kg live-weight. The crossbreeding IF×(RO×SE×IF) did not produce single-born lambs thus, this genotype was not included.

Carcasses were chilled at 4°C after 24 h at room temperature. Forty-eight hours after slaughter, carcasses were split along the spine and left sides were dissected separating muscle from bone, subcutaneous and intermuscular fat and epimysium. Samples were taken from the *longissimus dorsi* (LD) muscle and a total muscle sample (TM) was prepared from the remaining muscles, after mincing. Samples were frozen at -20°C, freeze-dried, minced and stored at -20°C pending analysis. FAs composition of intramuscular fat was determined after extraction and methylation (Rule, 1997) and analysis by GLC using a 30 m × 0.25 mm ID × 0.20 µm film thickness SP-2380 fused-silica capillary column (Supelco, Bellefonte, PA, USA) with a flame ionisation detector and split injection. Helium served as carrier gas at a flow rate of 0.7 ml/min. The injector and detector temperatures were 250°C and 260°C, respectively. Initial oven temperature was 95°C, held for 3 min, subsequently increased to 165°C at a rate of 5°C/min, then raised to 190°C at 2.5°C/min, then to 250°C at 5°C/min and held for 6 min. Quantification of fatty acids methyl esthers (FAME) was done using 2 mg of nonadecanoic acid (C19:0) as internal standard (Velasco *et al.*, 2001). Peaks were identified using co-chromatography with standards (Fluka, Sigma-Aldrich, Bucks, SW). The C18:1 isomers are reported as one value. All data are expressed as least square mean ± SE. Differences between genotypes and muscle sampling type were analysed by the least squares method using GLM procedures (SAS, 1989). The possibility of discriminating the different genotypes from the fatty acids composition of intramuscular fat was tested using a canonical discriminating analysis by a forward stepwise method of Statistica software (Statsoft, 1995).

Results and discussion

Average values and standard errors for total FA content [mg/100 g of muscle dry matter (DM)] and intramuscular FA composition (% weight) are given in Table 1.

Muscle sampling type did not present significant differences for the FA composition profile, showing that sampling only LD is an acceptable method to simplify sample collection.

On the other hand, genotype affected total FA content with a significantly higher value for RO×(SE×IF×RO) crossbred lambs, 9.32 mg/100 g muscle DM. However, the values obtained were not different from those reported in the literature, such as the Spanish Merino (2.22 mg/100 g muscle) and Rasa Aragonesa (1.99 mg/100 g muscle) (Sañudo *et al.*, 2000), grass-fed Suffolk (2.8 mg/100 g muscle) (Fisher *et al.*, 2000) or MB and IF×MB lambs (1.9-2.1 mg/100 g muscle) (Santos-Silva *et al.*, 2002), considering 25% DM in muscle.

The intramuscular FA profiles differed with genotype for a number of FA concentrations. Lambs from RO×(SE×IF×RO) crossbreeding presented a significantly different profile from the other lambs, with higher values for C10:0, C14:0, C16:0, C16:1*cis-9,* C18:3*n*-3 and C22:6*n*-3 and lower for C18:0. While other authors (Fisher *et al.*, 2000; Sañudo *et al.*, 2000) found also differences in FA composition between sheep breeds, Santos-Silva *et al.* (2002), using MB and the crossbred IF×MB, did not find such differences, basing the contradiction on the similar origin and/or aptitude of the genotypes.

Fisher *et al.* (2000) obtained effects of breed, although in combination to feeding regime, on the FA composition and sensory characteristics of lamb. Panel test, allowed to identify positive high correlation between flavour intensity and C18:3*n*-3, but this correlation was negative for C18:2*n*-6. The effect on flavour results from a different development of flavour volatiles during cooking, through lipid oxidation, due to the variations in the relative proportions of different fatty acids.

Table 1. Analysis of variance and least-square means of total FA content (mg/100 g muscle DM) and FA composition (weight %) of intramuscular fat of lamb meat from three genotypes considering two types of muscle sampling

	Genotypes (G)				Muscle sampling type (MST)			Effects	
	MB	RO×(SE×IF×RO)	SE×(IF×RO×SE)	SE†	LD	TM	SE†	G	MST
Total FA	7.39 a	9.32 b	7.81 a	0.437	7.74	8.60	0.357	**	ns
C10:0	0.36 a	0.52 b	0.32 a	0.046	0.42	0.38	0.038	**	ns
C12:0	0.85	0.82	0.85	0.069	0.81	0.87	0.056	ns	ns
C14:0	5.42 a	6.42 b	6.21 b	0.238	5.94	6.10	0.194	*	ns
C16:0	21.1 a	22.5 b	22.9 b	0.34	22.3	22.1	0.28	**	ns
C16:1*cis-9*	1.40 a	1.77 b	1.58 ab	0.070	1.54	1.63	0.057	**	ns
C18:0	13.6 b	12.0 a	13.7 b	0.39	13.3	12.9	0.32	**	ns
∑C18:1	30.4	29.8	30.3	0.55	30.0	30.4	0.45	ns	ns
C18:2*n*-6	7.66	7.64	7.13	0.298	7.66	7.29	0.243	ns	ns
CLA††	0.83	0.90	0.82	0.105	0.83	0.87	0.027	ns	ns
C18:3*n*-3	2.13 a	2.56 b	2.22 a	0.034	2.33	2.28	0.086	*	ns
C20:4*n*-6	3.20	3.21	3.28	0.169	3.36	3.10	0.138	ns	ns
C20:5*n*-3	0.90	1.21	1.05	0.098	1.15	0.95	0.080	ns	ns
C22:5*n*-3	1.26	1.19	1.22	0.075	1.22	1.23	0.061	ns	ns
C22:6*n*-3	0.53 a	0.82 b	0.64 a	0.052	0.69	0.64	0.042	***	ns
n-6/*n*-3†††	2.29 b	1.89 a	2.07 a	0.072	2.08	2.09	0.059	***	ns

†SE = standard error.

††CLA: conjugated linoleic acid.

†††*n*-6/*n*-3 = (C18:2*n*-6, C20:4*n*-6)/(C18:3*n*-3, C20:5*n*-3, C22:5*n*-3, C22:6*n*-3).

*P < 0.05, **P < 0.01, ***P < 0.001, ns = non-significant.

[a,b]Means in the same row with different letters are significantly different (P < 0.05).

When considering the ratio between *n*-6/*n*-3 FA, the higher ratio *n*-6/*n*-3 FA values were obtained in the meat from MB lambs but PUFA/saturated FA (SAT) ratio was not significantly different between genotypes (about 0.40). If the *n*-6/*n*-3 ratio in the human diet should be decreased to a value of about 2-4 (Okuyama and Ikemoto, 1999), all the lambs meat obtained in this trial falls in the range, emphasizing its nutritional value. The fact that these lambs were raised on pasture probably explains the low *n*-6/*n*-3 ratios. Velasco *et al.* (2001) found that suckling lambs raised on pasture presented higher PUFA/SFA and lower *n*-6/*n*-3 ratios in intramuscular fat (0.35 and 1.39, respectively) when compared to suckling lambs raised on dry-lot. Nuernberg *et al.* (2002) found that intramuscular fat from *longissimus* muscle in steers on pasture grazing contained significantly higher level of total *n*-3 FA and lower level of *n*-6 FA as compared to steers fed concentrate resulting in a *n*-6/*n*-3 ratio of 1.2 vs 9.2, respectively. The PUFA/SAT ratio was higher than that reported by Sañudo *et al.* (2000) and similar to that found by (Fisher *et al.*, 2000) on grass-fed Suffolk and to the recommended value (0.45) for the diet (Department of Health, 1994). Grazing is related to a higher content of PUFA since chloroplasts in grasses may provide a natural protection of PUFA in the rumen as compared to FA in concentrate (Wood and Enser, 1997).

The possibility of discriminating the different genotypes from the FAs composition of intramuscular fat was tested and the analysis generated two significant uncorrelated linear combinations of 5 FA (C16:0, C18:0, C22:6*n*-3, C10:0, C12:0) (Table 2).

Discriminant function 1 had a higher discriminant power than function 2, accounting for 80.7% of the total variation between groups. The discriminant analysis allowed allocating properly 91.3% of samples (Fig. 1). The percentage of correct allocation was 87.5, 100 and 85.7%, for genotypes MB, RO×(SE×IF×RO) and SE×(IF×RO×SE), respectively.

This statistical tool also generated classification functions, which can be useful to determine the genotype where an individual sample most likely belongs (Table 3), emphasizing the importance of genotype on chemical characteristics of the intramuscular fat.

Table 2. Standardized coefficients for discriminant functions and statistics

	Discriminant function 1	Discriminant function 2
C16:0	-0.559	0.991
C18:0	0.616	0.592
C22:6*n*-3	-1.021	0.168
C10:0	-1.169	-0.162
C12:0	0.974	-0.037
Eigenvalue	3.623	0.868
Cumulative proportion	0.807	1.000
Canonical R	0.885	0.682
Wilk's Lambda	0.116	0.535
p-level	<0.0001	<0.05

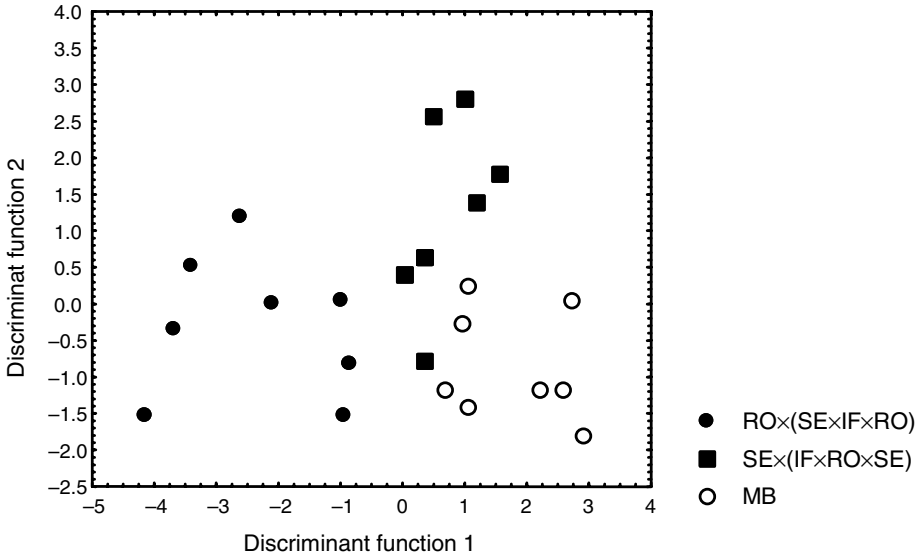

Fig. 1. Spatial distribution of intramuscular fatty acid profile of LD from 3 genotypes according to the two discriminant functions.

Table 3. Classification functions for allocating lambs to the 3 genotypes

	MB	RO×(SE×IF×RO)	SE×(IF×RO×SE)
Constant	-314.80	-355.20	-370.13
C16:0	19.97	22.16	22.03
C18:0	13.64	11.86	14.11
C22:6*n*-3	39.52	63.46	47.50
C10:0	62.63	88.94	67.61
C12:0	-30.27	-46.64	-34.76

Conclusions

Sampling LD could be considered as an effective method to simplify meat sample collection. Genotype affected total intramuscular FA content as well as the FA profiles. Lambs from

RO×(SE×IF×RO) crossbreeding had a significantly higher value of total FA content, 9.32 mg/100 g muscle DM, and percentual higher values for C10:0, C14:0, C16:0, C16:1*cis-9,* C18:3*n*-3 and C22:6*n*-3 and lower for C18:0. All lambs meat obtained in this trial presented low *n*-6/*n*-3 ratios and PUFA/SAT ratio averaging 0.4, probably as a consequence of the rearing grazing conditions. This causes a re-evaluation of consumers interest towards meat from ruminants and, as Nuernberg *et al.* (2002) refer, red meat enriched with long-chain *n*-3 FA by feeding grass can make an important contribution to increase these FA content in the human diet. The application of FA profiles of intramuscular fat to discriminate the origin of lambs (genotype and/or association to production system) seems to be a valuable tool in view of the consumers' increasing concern on tracebility of food products.

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