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Using stable isotope ratio to differentiate the Tunisian indigenous lamb grazing two different pasture types

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Abstract. The efficacy of isotope ratio mass spectrometry (IRMS) in tracing lamb production systems was investigated for four farming systems in the Tunisian North-West: Ain Draham and Fernana, characterized by woody pasture, and Amdoun and Joumine, characterized by herbaceous pasture. Mixed breed lambs aged 3.5–5 months were reared under semi-extensive and extensive systems. Samples of Longissimus dorsi muscle were taken from eight lambs for each farming system for stable IR assessment of the three main bio-elements (C, N, S) in the protein and fat fractions of lamb. Using partial least squares discriminate analysis (PLS-DA) the IR profiles of Tunisian lamb types allowed correct assignment of the training meat samples to pasture type. A broader scale pasture type discriminating lambs from the Amdoun (herbaceous pasture), the rest of north-west Tunisia appeared to be workable.

Keywords. Tunisian farming systems – Pasture type – Lamb Meat analysis – Isotope ratio.

Utilisation du ratio isotopique stable pour différencier l'agneau indigène tunisien pâturant en deux types de pâturage différents

Résumé. *L'efficacité de spectrométrie de masse à ratio isotopique (IRMS) dans le traçage du système de production d'agneau a été examinée pour quatre régions agricoles en Tunisie: Ain Draham (AD) et Fernana (F), caractérisés par le pâturage boisé (WP) et Amdoun (AM) et Joumine (J), caractérisés par le pâturage herbacé (HP). Des agneaux de différentes race, âgés de 3,5 à 5 mois ont été élevés dans des systèmes semi-extensifs et extensifs. Les échantillons de muscle Longissimus dorsi ont été prélevés sur huit agneaux pour chaque système d'exploitation afin d'obtenir une évaluation IR stable des trois principaux bioéléments (C, N, S) dans les fractions protéique et lipidiques de la viande. En utilisant l'analyse discriminante partielle des moindres carrés (PLS-DA), les profils IR des types d'agneau tunisiens ont permis d'affecter correctement les échantillons de viande provenant de pâturage. Le type de pâturage a distingué les agneaux d'Amdoun (pâturage herbacé), le reste du nord-ouest de la Tunisie semblait être réalisable.*

Mots-clés. *Système d'élevage tunisien – Type de pâturage – Analyse de la viande d'agneau – Ratio isotopique.*

I – Introduction

Meat quality appears to be strongly affected by the animal feeding system (Priolo *et al.*, 2001; Renerre, 1990). The farming system, namely the combination of the geographical, orographic, climatic, social, historical and cultural conditions in the reference area and production factors such as feeding regime, husbandry techniques, animal breed and category, gives rise to specific lamb types dis-

tinguished by particular meat characteristics that consumers may identify and appreciate (Sañudo *et al*., 2007). Authentication and objective food information are major demands from consumers (Monin, 1998). Stable isotope ratios provide an analytical tool for confirming meat origin, as there are region-specific patterns in the environmental isotopic ratios (Piasentier *et al*., 2003; Franke *et al*., 2005). Vegetation composition (C), feed type (C, N), crop production practices (N), and the sea (S) proximity (Moloney *et al*., 2009). Isotopes can therefore be used to detect dietary differences and origin. These objectives were achieved by developing classification models through the use of isotope ratio mass spectrometry (IRMS).

II – Materials and methods

1. Animals, diets and experimental design

The study was carried out at four sites in north-western Tunisia. The farming systems are characterized by different kinds of pasture, mountainous terrain covered with woody pasture (WP) and plains dominated by herbaceous pasture (HP). In the Ain Draham (AD) and Fernana (F) systems, pasture grazing takes place virtually throughout the year, with bushes and shrubs dominated by cork oak (*Quercus suber*), while the kermes oak (*Quercus coccifera*) covers relatively small areas. Lambs are given supplements of oak acorn, some commercial concentrates, barley and oat hay. The Amdoun (AM) and Joumine (J) sites are characterised by HP, comprising a herbaceous stratum dominated by *Gramineae*, on which the lambs graze with their dams; as a supplement to pasture, the flocks receive commercial or farm concentrate, green barley, oats, hay and wheat straw.

2. Slaughter and sampling procedures

The lambs were transported to a commercial slaughterhouse located 120 km from the farms and then slaughtered after an overnight period without feed but with free access to water. All the procedures employed in this study (transport and slaughtering) meet ethical guidelines and adhere to Tunisian legal requirements in accordance with Law no. 2005-95 (18 October 2005).

The *Longissimus dorsi* (thoracis + lumborum) (LD) muscles of both sides of the lamb carcasses were separated for meat quality analysis. Two equivalent samples from the left and right muscles from each lamb were frozen at –20°C for IRMS analysis.

3. Sample preparation and isotope analysis

The LD meat samples were minced and freeze-dried in a lyophilizer (freeze-drier) then homogenized with a suitable grinder and freeze-dried again. The resulting dry powder was fractionated into crude fat (Fat), by extraction with petroleum ether for 6 h in a Soxhlet apparatus, and defatted dry matter (Protein), essentially protein. Afterwards the protein and fat fractions were stored in an appropriate container until measurement. Measurement of the $^{13}C/^{12}C$, $^{15}N/^{14}N$, ratios of protein and fat fractions was carried out as described by Perini *et al*. (2009). For $^{13}C/^{12}C$ and $^{15}N/^{14}N$ ratios we used an Isotope Ratio Mass Spectrometer (DELTA V, Thermo Scientific, Germany) following total combustion in an elemental analyser (EA Flash 1112, Thermo Scientific). $^{34}S/^{32}S$ was determined using IRMS (Vario Isotope Cube, Elementar Analysensysteme GmbH, Germany) The values were expressed in δ‰ against international standards, calculated against working in-house standards and calibrated against international reference materials, as reported by the same authors. For measurement of the $^{34}S/^{32}S$ ratios, we used an elemental analyser (EA Flash 1112 Thermo Finnigan, Bremen, Germany) connected to an isotope ratio mass spectrometer (Delta plus XP mass spectrometer, Thermo Finnigan). The protein sample (2.5 mg) was burned at 1000 °C in a quartz tube filled from the bottom with quartz wool (2 cm), elemental copper (14 cm), copper

oxide (5 cm) and quartz wool (1 cm). The water was removed using a glass trap filled with Mg $(ClO_4)_2$. The isotopic values were calculated against international reference materials: IAEA-SO-5 ($\delta^{34}S$ = +0.5‰) and NBS 127 ($\delta^{34}S$ = +20.3‰), through the creation of a linear equation.

4. Statistical analysis

The four farming systems located in four areas of Northwest Tunisia were first compared by univariate analysis. Particularly, the effect of lamb farming system on each individual stable isotope ratio in meat was investigated using ANOVA, followed by Fishers Least Significant Difference (LSD)

III – Results and discussion

The IR profiles of lamb produced at the four locations in north-west Tunisia are shown in Table 1. All the IRs were significantly influenced by the geographical origin of the meat. However, only in the case of $\delta^{34}S_{Protein}$ did the pasture type significantly affect the IR value (9.7‰ vs. 6.9‰ for WP and HP respectively; p<0.001). This means that IR variability between sites is higher than that between pasture types.

A clear example of this can be seen in the $\delta^{13}C$ values of both protein and the more depleted fat fractions (DeNiro and Epstein, 1977), which did not differ for the pasture systems. $\delta^{13}C$ values instead showed statistically significant differences between sites. Indeed, the maximum $\delta^{13}C$ values for protein and fat fractions, recorded in Fernana and Amdoun lamb meat (-25.1‰ and –31.3‰ respectively), only differed by 1.1-1.4‰ from the minimum $\delta^{13}C$ values that were respectively observed in Joumine (-26.2‰ for protein) and Ain Draham (-32.5‰ for fat). These differences are probably linked to the forage/concentrate ratio in the lamb diet and the acorn intake (González-Martin*et al*., 2001; Perini *et al*., 2013). As expected, the $\delta^{13}C$ values were low in comparison with those observed by Perini *et al*. (2009) in Italian lamb types, because of the absence of C4 plants in their diet (Camin *et al*., 2007).

Meat from Amdoun, produced from lambs grazing on herbaceous pasture with their dams, was characterised by the highest $\delta^{15}N_{Protein}$. The WP systems showed intermediate values. The differences between Amdoun and Joumine, characterised by a similar humid climate and HP, may result from differences in agricultural practices, particularly the organic fertilisation largely used in Amdoun, which increases the $\delta^{15}N$ of forage (Laursen *et al*., 2013). Moreover, in comparison with AM-HP (Table 1), J-HP breeders feed their flocks with significant amounts of legumes, namely clover, which is known to have a low $\delta^{15}N$ level in its nitrogen, compounds (Laursen *et al*., 2013). Furthermore, they usually wean their lambs earlier than AM lambs, which are kept with the ewes until slaughter, and higher $\delta^{15}N$ is expected in suckling animals (Perini *et al*., 2009). The higher level of deuterium in the fat fraction of J-HP meat was in line with the rougher diet of J-HP than AM-HP sheep that receive higher amounts of concentrates and barley (Table 1).

The $\delta^{34}S$ examined, sulphur provided the clearest lamb type discrimination. Similar results were obtained in a study carried out on Italian lamb types (Perini *et al*., 2009). The basis of Italian-lamb-type variability was not feed dependent. The upper end of the $\delta^{34}S_{Protein}$ range was represented by two lamb types (9.5‰ and 9.2‰), reared on the Mediterranean islands of Sicily and Sardinia at 5 and 10 km from the sea respectively. The two WP systems in Tunisia, Fernana and Ain Draham in particular, are also the closest to the sea. Thus, the high $\delta^{34}S$ content would seem to be a “coastal” signal, resulting from the influence of so-called sea-spray. Sea-spray sulphate is deposited as an aerosol over pasture and crops, in decreasing amounts with increasing distance from the sea.

Table 1. Isotopic ratio values (‰) of protein and fat of lamb meat from four farming systems located in four areas of Northwest Tunisia, Ain Draham (AD) and Fernana (F) characterized by woody pasture (WP), Amdoun (AM) and Joumine (J) characterized by herbaceous pasture (HP)

	AD-WP	F-WP	J-HP	AM-HP	*p*	SEM
$\delta^{13}C_{Protein}$	-26.1^{c}	-25.1^{a}	-26.2^{c}	-25.6^{b}	.000	.08
$\delta^{15}N_{Protein}$	7.1^{b}	6.3^{bc}	5.6^{c}	10.2^{a}	.000	.15
$\delta^{34}S_{Protein}$	10.0^{a}	9.4^{a}	7.5^{b}	6.3^{b}	.000	.21
$\delta^{13}C_{Fat}$	-32.5^{b}	-31.3^{a}	-32.3^{b}	-31.1^{a}	.000	.10

^{a,b,c}: $p<0.05$.

IV – Conclusions

Initial application of IRMS to trace farming systems in north-west Tunisia provided promising results for both large-scale discrimination of north-west Tunisia as an overall lamb-producing geographical region and small-scale classification of individual regional farming systems. In particular, it appeared to be reliable in distinguishing lamb produced in the Amdoun area, characterised by a humid climate, extensive use of organic manure to fertilise forage crops and fallows grazed by flocks comprising lambs suckled by their dams until slaughtering, on the basis of the IR profile. The use of meat from other Tunisian production systems, such as naturally reared prairie or feedlot lambs, may be interesting to discriminate the mountainous meat production system.

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