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Differentiating the geographical origin of Tunisian indigenous lamb using stable isotope ratio and fatty acid content

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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, characterised by woody pasture, and Amdoun and Joumine, characterised 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 five main bio-elements in the protein and fat fractions of lamb and for fatty acid (FA) determination. Using partial least squares discriminant analysis (PLS-DA) the IR profiles of Tunisian lamb types allowed correct assignment of the training meat samples to the area of origin. Inclusion of the FA profile in the classification model slightly improved its evaluation performance for the individual farming systems; the average accuracy increased by 2%–94%. However, the differences between samples were not sufficiently wide to be validated by an external set of samples including 10 Italian lamb types. A broader scale geographical signature discriminating lambs from the Amdoun area, the rest of north-west Tunisia and Italy appeared to be workable, although some foreign lamb types from neighbouring Mediterranean regions such as Sicily were misclassified as Tunisian.

1. Introduction

In Mediterranean countries, lamb is widely appreciated by consumers (Gürsoy, 2006). Meat quality appears to be strongly affected by the animal feeding system (Priolo et al., 2001; Renerre, 1990). The main reported differences are in subcutaneous fat (Prache and Theriez, 1999), meat colour (Ådnøy et al., 2005), carcass fatness (Atti and Abdouli, 2001; Joy et al., 2008) and fatty acid composition (Ådnøy et al., 2005; Aurosseau et al., 2007; Hajji et al., 2016). The three main components in eating quality – tenderness, juiciness and flavour – can also vary due to production factors (Rousset-Akrim et al., 1997; Wood et al., 2008). 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 distinguished by particular meat characteristics that consumers may identify and appreciate (Sañudo et al., 2007). The farming system could thus result in a "Protected Designation of Origin" for specific meat, associated by consumers with higher quality and healthier foods (Hermansen, 2003).

There is a growing enthusiasm among consumers for high quality food with a clear regional identity. The reasons for this vary from certain specific culinary and sensory qualities, or purported health benefits associated with regional products, to decreased confidence in the quality and safety of food produced outside their local region (Kelly et al., 2005). Authentication and objective food information are major demands from consumers (Monin, 1998). Of the analytical approaches that can be used for the authentication of meat products, stable isotope ratio analysis is well-known as an accurate method (Piasentier et al., 2003; Franke et al., 2005; Camin et al., 2007; Perini et al., 2009), although in the field of food control, there are currently no official methods for multi-element stable

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isotope analysis of meat (Camin et al., 2007). 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). Dietary components have typical isotopic "signatures" determined by climate (ratios of H and O), vegetation composition (C), feed type (C, N), crop production practices (N), and proximity to the sea (S) (Moloney et al., 2009). Isotopes can therefore be used to detect dietary differences and origin.

Due to its relationship with diet composition (Atti et al., 2005, 2013; Hajji et al., 2016), the fatty acid composition may also be regarded as an efficient method to provide information on animal feeding regime and to help in tracing samples from different farming systems.

This experiment is part of a project focusing on aspects related to the regional origin of lamb from Tunisia. The research aims to evaluate analytical techniques and chemical profiles useful for authentication and differentiating the origin of lamb, which is one of the first fresh meat products with the potential to be recognised with a Protected Designation of Origin in Tunisia. The main objective of this work was to study the traceability of meat from lambs coming from various locations in the north of Tunisia, grazing on herbaceous pasture or woody pasture and with or without concentrate and forage supplements. These objectives were achieved by developing classification models through the use of isotope ratio mass spectrometry (IRMS). The relationship between the isotope ratio (IR) profile and the fatty acid (FA) profile was also studied.

2. Materials and methods

2.1. Animals, diets and experimental design

The study was carried out at four sites in north-western Tunisia. The description of the sites, the dietary calendar and the contribution of different feedstuffs to the diet are identified in Table 1. The farming systems are characterised 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. The shrubs are represented by *Arbutus unedo*, *Calycotum villosa*, *Erica arborea*, *Myrtus communis*, *Pistacia lentiscus* and *Phillyrea angustifolia*. 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.

At all sites, lambing occurred in December and lambs of mixed breeds (Barbarine and Queue Fine de l'Ouest and their crosses) were reared with their dams until they reached weaning weight. Eight male suckling lambs from one farm per site were provided.

2.2. 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. The suckling lambs' age at slaughter was 106 days in J site, 118 in AM, 148 in F and 150 in AD. Their body weight at slaughter was 20 kg for J-HP site, 22 for AM-HP, 24 for AD-WP and 28 kg for F-WP. 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 fatty acid and IRMS analysis.

2.3. Total lipids and fatty acid analysis

Extraction of total intramuscular fat (IMF) was performed according to the procedure of Folch et al. (1957). Nonadecanoic acid (C19:0) was added as an internal standard to 1.5 g of minced

Table 1
Sites of lamb production and their dietary calendar during the follow-up period.The percentage contribution to the monthly diet of the different feedstuffs is reported in brackets.

Region	December	January	February	March
AïnDraham (AD) Lat. North: 36°46′36.35″ Long.: 8°40′49.75″ Elevation: 756 m Sea distance: 18.54 km	Forest plants (100)	Forest plants (40) Straw (15) Barley + Acorn (20) Triticale (10)	Forest plants (40) Straw (15) Barley +Acorn (25) Triticale (10)	Forest plants (70) Barley + Acorn (20) Herbaceous legumes (10)
Fernana (F) Lat. North: 36°36′46.66″ Long.: 8°36′41.28″ Elevation: 553 m Sea distance: 36.52 km	Forest plants (40) CC (20) Straw (25) Acorn (15)	Forest plants (40) Straw+ CC (20) Barley (20) Acorn (20)	Forest plants (40) Straw (15) Barley (20) Acorn (25)	Forest plants (40) Acorn + Barley (20) Herbaceous legumes (40)
Joumine (J) Lat. North: 36°55′23.21″ Long.: 9°23′13.49″ Elevation: 335 m Sea distance: 58.97 km	FC (55) Clover hay + oat straw (25) Eucalyptus + olive twig (20)	FC (45) Clover hay +oat straw (50) Eucalyptus+ olive twig (5)	FC (40) Clover hay + oat straw (50) Grass (10)	Grain barley (15) Green barley (10) Green oats (40) Clover hay (35)
Amdoun (AM) Lat. North: 36°45′54.88″ Long.: 9°05′59.52″ Elevation: 315 m Sea distance: 41.37 km	FC (45) Grain barley (35) Hay-oat (20)	FC (35) Grain barley (25) Oat straw (40)	FC (35) Grain barley (35) Ryegrass + oat straw (30)	Grain barley (20) Green barley (30) Green oats (30) Ryegrass (20)

FC: farmer concentrate = barley + wheat bran + faba beans. CC: commercial concentrate = corn+ wheat bran + soy + faba beans + barley.