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Stable isotope ratio analysis: A potential analytical tool for the authentication of South African lamb meat

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ABSTRACT

Stable isotope ratios ($^{13}C/^{12}C$ and $^{15}N/^{14}N$) of South African Dorper lambs from farms with different vegetation types were measured by isotope ratio mass spectrometry (IRMS), to evaluate it as a tool for the authentication of origin and feeding regime. Homogenised and defatted meat of the *Longissimus lumborum* (LL) muscle of lambs from seven different farms was assessed. The $\delta^{13}C$ values were affected by the origin of the meat, mainly reflecting the diet. The Rûens and Free State farms had the lowest ($p \leqslant 0.05$) $\delta^{15}N$ values, followed by the Northern Cape farms, with Hantam Karoo/Calvinia having the highest $\delta^{15}N$ values. Discriminant analysis showed $\delta^{13}C$ and $\delta^{15}N$ differences as promising results for the use of IRMS as a reliable analytical tool for lamb meat authentication. The results suggest that diet, linked to origin, is an important factor to consider regarding region of origin classification for South African lamb.

1. Introduction

Stable isotope ratio analysis can be an important analytical tool when determining geographic origin. It is a well-known and accurate method than can be used for the authentication of meat products (Camin et al., 2007; Franke, Gremaud, Hadorn, & Kreuzer, 2005). Recent studies have focused on the use of isotopic measurements to determine the geographical origin of beef and lamb (Kelly, Heaton, & Hoogewerff, 2005). Piasentier, Valusso, Camin, and Versini (2003) draws attention to the use of stable isotope ratio analysis as a tool for the characterisation of animal diet by tracing the feeding system used in lamb meat production. The determination of stable isotope ratios is used as an analytical tool to confirm the origin of meat, as specific isotopic patterns may subsist in the region of origin (Crawford, McDonald, & Bearhop, 2008; Franke et al., 2005). Isotopes are incorporated in local feeds and consequently taken up through the diet of animals during their lifetime (DeNiro & Epstein, 1978). Carbon (^{13}C) and nitrogen (^{15}N) isotope enrichment of animal products depends largely on the diet (Camin et al., 2007; Codron, Codron, Lee-Thorp, Sponheimer, & De Ruiter, 2005a; DeNiro & Epstein, 1978, 1981; Franke et al., 2005; Kelly et al., 2005; Sandberg, Loudon, & Sponheimer, 2012). Hence, through isotope enrichment from one trophic level to another it is possible to link meat to its diet and if the diet is regionally unique, to its geographic origin (Sandberg et al., 2012).

By examining the $^{13}C/^{12}C$ isotope ratio it is possible to determine whether animals predominantly ate C_3, C_4 or crassulacean acid metabolism (CAM) plants (Capuano, Boerrigter-Eenling, Van der Veer, & Van Ruth, 2013; Sandberg et al., 2012). The C_4 pathway enables the plant to concentrate atmospheric CO_2 in such a way to avoid photorespiration due to the specialized Kranz anatomy (bundle sheath cells) of the leaves, which is absent in the C_3 pathway (Gibson, 2009; Vogel, Fuls, & Ellis, 1978). Essentially, C_4 and C_3 plants are distinguished based on Kranz and non-Kranz anatomy, whereas CAM plants have the ability to utilise both C_3 and C_4 modes of carbon fixation (Vogel et al., 1978). C_4 plants result in ^{13}C-enrichment (i.e. elevated carbon isotope ratios) compared to C_3 and CAM plants (Gibson, 2009; Kelly et al., 2005; Vogel et al., 1978). Previously published data for South Africa revealed average $\delta^{13}C$ values of −26.5‰ for C_3 plants and −12.6‰ for C_4 grasses (Vogel et al., 1978). C_3 plants consist of trees, bushes/shrubs (including their leaves and fruits), non-grassy herbs/forbs, most vegetables, cool-season grasses and grains such as lucerne (alfalfa), wheat, oats, barley and rice (Capuano et al., 2013; Vogel et al., 1978). C_4 plants include warm-season or tropical grasses and sedges and their seeds, leaves or storage organs such as roots and tubers (Capuano et al., 2013; Vogel et al., 1978). C_4 grains

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include maize and millet. Succulents are typical CAM plants (Vogel et al., 1978).

Nitrogen isotopes also provide some information about the animal's diet as the consumption of leguminous plants may decrease the δ^{15}N values, while the application of organic fertilisers, domestic grazing and cultivation may result in an increase of δ^{15}N values (DeNiro & Epstein, 1981; Devincenzi, Delfosse, Andueza, Nabinger, & Prache, 2014; Perini, Camin, Bontempo, Rossmann, & Piasentier, 2009; Piasentier et al., 2003; Sandberg et al., 2012). Not all leguminous plants may result in low δ^{15}N values, since *Senegalia nigrescens* and *Colophospermum mopane* (common leguminous tree taxa of the savanna) have high δ^{15}N values (Codron, Codron, Lee-Thorp, Sponheimer, & De Ruiter, 2005b). Ratios within soils and plants also increase with decreasing rainfall (i.e. arid conditions) (Perini et al., 2009; Sandberg et al., 2012). The protein fraction of young and light lamb meat, raised on ewe's milk, may have the highest nitrogen isotope ratios as a result of ^{15}N-enrichment of the milk (Perini et al., 2009). However, such trophic level enrichment can vary and depend on the combination of the specific plant species, habitat and feeding regime at hand. An important aspect of the measurement of nitrogen isotopes is that different isotope signatures may exist in sheep fed the same diet in varying spatial scales (DeNiro & Epstein, 1981; Perini et al., 2009; Piasentier et al., 2003). This enables the characterisation of meat obtained from different regions, although raised on the same diet.

In South Africa several extensive sheep grazing systems, varying on account of diet, exist. Karoo lamb, produced in the Karoo region and known for its specific regional qualities (Du Plessis & Du Rand, 2012; Weissnar & Du Rand, 2012), is the most well-known. Consumers appreciate Karoo lamb for its quality and unique sensory characteristics (i.e. herbaceous aroma and flavour), which are believed to be attributed to the free-range conditions and the grazing on fragrant Karoo plants (Estler, Milton, & Dean, 2006; Weissnar & Du Rand, 2012). The labelling of certified sheep meat and the associated premium price also indicate to consumers a superior quality. Sheep meat from the Karoo may be certified as *Karoo Meat Of Origin* through the certification scheme of the Karoo Development Foundation (KDF), South Africa. Certified meat is recognised by a mark placed on the packaging of the meat (*Certified Karoo Meat of Origin*). Other typical sheep grazing systems include sheep from the Swartland and Overberg regions in the Western Cape, sheep from the Kalahari, sheep raised on either the grasslands of the Free State or planted kikuyu/clover pastures of the South-Western Cape. Evaluating plant type intake is an important aspect of this research study, given the widely documented effect of diet on the sensory characteristics of sheep meat (Almela et al., 2010; Resconi et al., 2010; Young, Lane, Priolo, & Fraser, 2003). The effect of diet is commonly being used in current marketing strategies as more emphasis is being placed on product qualities linked to the origin of meat. In South Africa, sheep meat are particularly promoted through labelling according to production practice, such as "free-range lamb", "certified natural lamb", or origin, such as "Karoo lamb". However, with these developments, the probability for opportunistic behaviour and false labelling increases. To ensure enforcement and better policing, the need exists to establish analytical tools for authentication. In keeping with current methods, a reasonable approach could be through the application of stable isotope ratio analysis; although there are currently no official methods in the field of food control for multi-element stable isotope analysis of animal products (Camin et al., 2007).

There are currently no published results regarding the use of stable isotope ratios for the purpose of authenticating South African lamb. Hence, the stable isotope ratios of carbon and nitrogen were measured to provide an evaluation of the effectiveness of stable isotope ratio analysis as a potential tool for the authentication of origin and feeding regime of South African Dorper lamb from extensive grazing systems. It was important to determine whether lamb from different farms can be distinguished from one another based on its isotopic profile as dietary differences linked to the variation in vegetation within the regions is expected. It is also essential to establish the discriminative power of the isotope results. Lambs from farms within the Northern Cape, Western Cape and Free State provinces of South Africa were included in the study.

2. Materials and methods

2.1. Experimental layout and study farms

Seven farms, each unique in terms of its vegetation and the extensive grazing conditions, were selected for the purpose of the study (Table 1). Five farms were from the Northern Cape (CK, NK, HK/LO, KV, HK/CAL), one from the Western Cape (RU) and one from the Free State (FS). Ten slaughter ready Dorper lambs ($n = 10$) were sourced from each farm (Fig. 1). The selected farms are shown in Fig. 1.

2.2. Northern Cape province

The Northern Cape covers the vast Karoo ecotype and is described as arid to hyper-arid with limited cropping potential (Cloete & Olivier, 2010). Sheep farming is practised in 82.0% of the province due to the limitation on alternative farming ventures. The Karoo constitutes the largest area of the province and features a variety of different vegetation types (Bramley, Bienabe, & Kirsten, 2009; Vorster & Roux, 1983). The rainfall is low and varies from less than 200 mm or 201–400 mm to 401–600 mm per annum in some places, while droughts may also occur for several years on end (Palmer & Ainslie, 2005). During these periods of drought, the region's plant growth is greatly affected. The region has a low carrying capacity of less than one large stock unit per 40 ha, where the natural pasture for the lambs varies from grassy, dwarf shrublands (Nama-Karoo biome) to dwarf, succulent shrubs (succulent Karoo biome) (Acocks, 1988; Cloete & Olivier, 2010; Du Plessis & Du Rand, 2012; Vorster & Roux, 1983). From the five farms selected within the Northern Cape: CK and NK fall in the Nama-Karoo biome, HK/LO and HK/CAL in the succulent Karoo biome and KV mainly in the fynbos biome (Fig. 1).

2.3. Western Cape province

The South African sheep industry comprises of either extensive or fairly intensive enterprises in the pasture-cropping regions and intensive horticultural areas of South Africa (Cloete & Olivier, 2010). The Swartland (western seaboard) and Overberg (southern seaboard) regions of the Western Cape have a typical Mediterranean climate, where sheep production is coordinated with winter grain cropping (Cloete & Olivier, 2010). In the Overberg region, lucerne/alfalfa (*Medicago sativa*) is typically cultivated in the pasture phase and serves as feed for sheep. Small grain stubble is another characteristic feed of the region, which may also form part of the diet (Cloete & Olivier, 2010). The lamb produced within this region is known as "Rûens lamb" (RU), where the typical diet of the sheep associated with the region and traditional farming practises gives the lamb meat its unique sensory qualities. The sheep selected from a farm within the Overberg region was extensively raised on lucerne situated within the fynbos biome and known as the Rûens shale renosterveld (Fig. 1).