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The truth is in the isotopes: Authenticating regionally unique South African lamb

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ABSTRACT

Stable isotope ratios ($^{13}C/^{12}C$ and $^{15}N/^{14}N$) of South African lambs from different regions were measured by isotope ratio mass spectrometry (IRMS). Homogenised and defatted meat of the *Longissimus lumborum* muscle was assessed. The Rûens and Hantam Karoo regions had the lowest ($P \leq 0.05$) $\delta^{13}C$ values related to the presence of C_3 plants (lucerne and Karoo bushes, respectively). The Northern Karoo, Namibia and Bushmanland had the highest $\delta^{13}C$ values likely due to a high proportion of dietary C_4 grass species. The $\delta^{15}N$ values were highest for Central Karoo, Semi-extensive, Namibia and Hantam Karoo, while Rûens and Feedlot had the lowest nitrogen isotope values ($P \leq 0.05$). Classification of origin (Karoo vs. Non-Karoo) using discriminant analysis allowed 95% and 90% correct classification of the samples for the estimation model and validation models, respectively. The results confirm that IRMS provides sufficient discriminative power to classify lamb meat of varying origin.

1. Introduction

The multi-element approach, using ratios of stable isotopes to verify the origin and authenticity of food products, have been used in various studies ranging from coffee (Rodrigues et al., 2009) to olive oil (Chiocchini, Portarena, Ciolfi, Brugnoli, & Lauteri, 2016), wheat (Luo et al., 2015), poultry (Rees et al., 2016), beef (Heaton, Kelly, Hoogewerff, & Woolfe, 2008; Horacek & Min, 2010) and lamb meat (Camin et al., 2007; Erasmus, Muller, Van der Rijst, & Hoffman, 2016b; Perini, Camin, Bontempo, Rossmann, & Piasentier, 2009; Piasentier, Valusso, Camin, & Versini, 2003). This is due to the fact that the isotopes can be linked to the origin through the diet (in the case of animal products), environmental conditions (i.e. altitude, precipitation, etc.) or the ingredients used for certain food products (e.g. water added to fruit juice) (Rossmann, 2001). Part of the need for verifying the origin of food products comes from the fact that consumers want to know where products come from, as well as the fact that no commercial fraud is involved. Products with higher commercial value is usually as a result of typical quality characteristics associated with region of origin, giving products an authentic nature and the ability to stand out when compared to similar products without a geographical link. Some of these characteristic qualities may arise through its distinct or traditional production method.

In South Africa, most of the lamb produced in the Northern parts or Karoo region of the country is recognised as Karoo lamb (Weissnar & Du Rand, 2012). The meat is particularly known for its unique sensory quality (e.g. herbaceous aroma and flavour attributes) due to the sheep's diet, mainly consisting of indigenous, herbaceous Karoo bushes and shrubs (Erasmus, Hoffman, Muller, & Van der Rijst, 2016a; Estler, Milton, & Dean, 2006). However, given the quality and value associated with Karoo lamb, there is a risk that the name may be misused by retailers selling lamb meat as Karoo lamb when in actual fact it has been produced in a feedlot or different region of origin. Similar to Karoo lamb, but less well-known, other characteristic South African lamb also exists, e.g. "Rûens lamb" of the Overberg region of the Western Cape. This lamb is raised on lucerne/alfalfa (*Medicago sativa)*, typically cultivated in the region, while small grain stubble may also form part of the diet (Cloete & Olivier, 2010). It is believed that the characteristic diet of the sheep associated with a region and traditional farming practises provides the lamb meat with its unique sensory qualities (Erasmus, Muller, & Hoffman, 2017). It is thus vital that an analytical method for the authentication of South African lamb is developed. Not only would such a method be able to distinguish lamb of Karoo from Non-Karoo origin, but it would also provide scientific evidence to verify the unique nature of the product.

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Previous research using stable isotope ratio analysis revealed distinct isotopic differences of lamb meat obtained from different farms within the mentioned regions (Erasmus, Muller, et al., 2016b). The differences found were related to diet linked to the origin. However, the classification was made at farm level. This raised questions in terms of whether a larger samples size, including more farms and regions with varying factors (i.e. breed, age and diet), would produce the same results. Also, the use of a larger sample set would be more representative of the different regions. Therefore, it was essential to perform stable isotope ratio analysis to extend and strengthen the baseline data and promote the development of robust classification models for region of origin authentication of South African sheep meat. In effect, this research forms part of the first scientific evidence, using carbon and nitrogen isotope ratios, to verify the authentic nature of South African lamb. The larger sample size provides sufficient data from authentic samples to ensure that the interpretations are meaningful and representative of the origin. Given that other authentic sheep meat in the European Union also requires additional scientific evidence to validate its unique quality (Erasmus et al., 2017), this manuscript aims to demonstrate how it can be achieved. For the study, lambs from regions within different provinces of South Africa were sourced to investigate stable isotope ratio analysis as an analytical tool for the classification of lamb meat.

2. Materials and methods

2.1. Experimental layout and study regions

Six regions, each unique in terms of vegetation and extensive grazing conditions, and three additional lamb types were selected for the purpose of the study. The six selected regions and one lamb type are shown in Fig. S1 (Supplementary material). Four regions were from the Northern Cape province, one from the Eastern Cape province and one from the Western Cape province. Lambs obtained from Namibia (NAM) (neighbour country), feedlot (FL) and semi-extensive (SE) grazing conditions were also included (deemed types). Three slaughter ready lambs were sourced from each farm, where the number of farms per region ranged from 2 to 15 (Table 1).

2.2. Selected regions

The Northern Cape province is largely composed of the Karoo ecotype which features a variety of different biomes (Acocks, 1988; Estler et al., 2006). The regions selected for this study are based on these biomes as they vary according to vegetation type. Due to this variation, a difference in diet is also expected as the sheep are mostly raised extensively. The Northern Cape/Karoo regions selected were the Central Karoo (CK), Hantam Karoo (HK), Northern Karoo (NK) and Bushmanland (BL). A "Karoo" region from the Eastern Cape was also included, the Eastern Karoo (EK). The borders of the Karoo are still widely disputed as some regions contain little and others none of the typical Karoo plants. For this reason, EK was also included as a Karoo region given that Karoo bushes are part of the vegetation. The HK is largely covered by the unique Karoo bushes and fall within the succulent Karoo biome, while CK, NK and BL fall in the Nama-Karoo biome, comprising a combination of Karoo bush and savanna-type or bushman grasses (Estler et al., 2006). The "Non-Karoo" selected region is in the Western Cape and known as the Rûens (RU). Here lambs are typically raised on lucerne/alfalfa (*Medicago sativa*) pastures though, depending on season, they may also be raised on stubble after the grain harvesting period (usually from December to February) (Cloete & Olivier, 2010). For this study lambs were raised on lucerne.

Apart from the above-mentioned extensive enterprises, semi-extensive sheep farming is also practised and animals are provided with additional feed (i.e. concentrates) when they are not grazing in the veld. This system has less impact on the veld, allowing more time for the plants to recover from grazing, reducing the chances of over-grazing and erosion. In times of drought this system is largely used by Karoo farmers. For this reason, semi-extensive lambs were also included in the study.

2.3. Sample collection

Animals were slaughtered according to standard procedures and regulations in three registered abattoirs in South Africa (Department of Agriculture & Fisheries (DAFF), 2000). Extensively raised lambs (three per farm), classed A2 (A, no permanent incisor teeth; 2, degree of fatness) of any breed and gender, and a carcass weight of approximately 18 kg were sourced. Carcasses are classified in South Africa according to different classes relating to the age of the animal, amount of fat, its body conformation and visible damage (Department of Agriculture & Fisheries (DAFF), 1990, Department of Agriculture & Fisheries (DAFF), 2006). The degree of fatness is classified according to the amount of fat measured between the 3rd and 4th lumbar vertebrae, where class 2 carcasses measures 1.0–4.0 mm (lean). In total, 201 lambs sourced from 67 farms were used for the study. Twenty-four hours after slaughter and refrigeration at 4 °C, meat steaks (1.5–2 cm thick) were cut perpendicular to the grain of the left *Longissimus lumborum* muscle of the carcass at the $L_{4\text{-}5}$ position (4th to 5th lumbar vertebrae). The steaks were vacuum packed and stored at −20 °C in absence of light until the analyses were conducted.

2.4. Sample preparation

The fat of a 5 g homogenised meat sample was extracted (Lee, Trevino, & Chaiyawat, 1996). As a result of the effect of the biochemical isotopic fractionation, the fat was removed to correct for variation in isotopic ratios between proteins and lipids (Camin et al., 2007). The resultant protein residue was freeze-dried and finely ground into a homogenous powder using a pestle and mortar. Powdered meat samples were then vacuum sealed and stored at −20 °C until analysis.

2.5. Isotope ratio analysis

For the isotope analysis ~0.5 mg powdered meat samples were weighed into tin (for $^{13}C/^{12}C$ and $^{15}N/^{14}N$) capsules and combusted individually in a Flash HT Plus elemental analyser (Thermo Fisher Scientific, Bremen, Germany). The resultant CO_2 and N_2 gas was introduced to an isotopic mass spectrometer (DELTA V Advantage) using a Continuous Flow Interface (ConFlo IV) (Thermo Fisher Scientific, Bremen, Germany). Isotope ratios are expressed in the conventional delta (δ) notation in parts per mil (‰), according to the following general formula:

$$\delta‰ = \frac{\mathbf{R}\ \text{sample} - \mathbf{R}\ \text{standard}}{\mathbf{R}\ \text{standard}} \times 1000$$

where **R** represents the ratio between the abundant isotopes i.e. $^{13}C/^{12}C$ and $^{15}N/^{14}N$. Standard refers to an international standard: V-PDB (Vienna PeeDee Belemnite) for $\delta^{13}C$ and nitrogen air (N_2) for $\delta^{15}N$. The negative $\delta^{13}C$ values of plant and animal tissues are attributed to the fact that V-PDB has relatively more ^{13}C than most of the terrestrial biosphere (Sandberg, Loudon, & Sponheimer, 2012). Standard deviations of repeated measurements (n = 38) of in-house standard were 0.41‰ for $\delta^{13}C$ and 0.42‰ for $\delta^{15}N$. The