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

Sara Wilhelmina Erasmus [a,b], Magdalena Muller [a], Marieta van der Rijst [c], Louwrens Christiaan Hoffman [b,*]

[a] Department of Food Science, Stellenbosch University, Private Bag X1, Matieland (Stellenbosch) 7602, South Africa
[b] Department of Animal Sciences, Stellenbosch University, Private Bag X1, Matieland (Stellenbosch) 7602, South Africa
[c] ARC Infruitec-Nietvoorbij, Private Bag X5026, Stellenbosch 7599, South Africa

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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

* Corresponding author.
E-mail address: lch@sun.ac.za (L.C. Hoffman).

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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 $\delta^{15}N$ values, while the application of organic fertilisers, domestic grazing and cultivation may result in an increase of $\delta^{15}N$ 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 $\delta^{15}N$ values, since *Senegalia nigrescens* and *Colophospermum mopane* (common leguminous tree taxa of the savanna) have high $\delta^{15}N$ 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).

Table 1
Lamb farms selected for this study and the plant species collected from the farms.

Farm	Area of origin	Code	Carcass classification (no. of samples)	Plant species collected from area	Number of species with pathway[a]		
					C_3	CAM	C_4
Carnarvon	Central Karoo	CK	A2 (n = 10)	*Galenia sarcophylla*; *Lycium cinereum*; *Pentzia incana*; *Plinthus karrooicus*; *Ruschia intricata*; *Salsola glabrescens*; *Stipagrostis ciliata*; *Stipagrostis obtusa*	4	1	3
Prieska	Northern Karoo	NK	A2 (n = 10)	*Eriocephalus ericoides*; *Felicia muricata*; *Fingerhuthia africana*; *Lycium cinereum*; *Pentzia incana*; *Phaeoptilum spinosum*; *Psilocaulon absimile*; *Pteronia glauca*; *Rhigozum trichotomum*; *Rosenia humilis*; *Ruschia intricata*; *Salsola aphylla*; *Salsola calluna*; *Salsola tuberculata*; *Stipagrostis ciliata*; *Stipagrostis obtusa*; *Zygophyllum gilfillanii*	8	2	7
Loeriesfontein	Hantam Karoo	HK/LO	A2 (n = 10)	*Chrysanthemoides incana*; *Pentzia incana*; *Pteronia sordida*; *Salsola tuberculata*; *Stoeberia beetzii*; *Tetragonia fruticosa*; *Zygophyllum lichtensteinianum*	3	2	2
Nieuwoudtville	Knersvlakte	KV	A2 (n = 10)	*Chaetobromus dregeanus*; *Ereiodium moschatum*; *Eriocephalus punctulatus*; *Galenia africana*; *Lebeckia leipoldtiana*; *Medicago polymorpha*; *Nylandtia spinosa*; *Pentzia incana*; *Wiborgia monoptera*; *Wiborgia sericea*	9	0	1
Calvinia	Hantam Karoo	HK/CAL	A2 (n = 5) A3 (n = 5)	*Chrysocoma ciliata*; *Drosanthemum hispidum*; *Eberlanzia ferox*; *Eriocephalus ericoides*; *Justica orchioides*; *Lycium* spp.; *Mesembryanthemum vaginatum* (*Brownanthus* or *Ruschia vaginatum*); *Pentzia incana*; *Pentzia sphaerocephala*; *Salsola calluna*; *Zygophyllum lichtensteinianum*	6	3	2
Swellendam	Rûens	RU	A2 (n = 10)	*Cynodon dactylon*; *Medicago sativa*	1	0	1
Boshof	Free State	FS	A2 (n = 10)	*Aristida congesta* subsp. *congesta*; *Cynodon dactylon*; *Eragrostis lehmanniana*; *Eragrostis superba*; *Fingerhuthia africana*; *Heteropogon contortus*; *Schmidtia kalihariensis*; *Themeda triandra*	0	0	8

(No.) Number; A2: (A) no permanent incisor teeth, (2) fat depth measures 1.0–4.0 mm (lean); A3: (A) no permanent incisor teeth, (3) fat depth measures 4.1–7.0 mm (medium); The fat depth is measured between the 3rd and 4th lumbar vertebrae, 25 mm from the midline in sheep (National Department of Agriculture (NDA), 1990).
[a] See Table S1 (supplementary material) for specific details; (CAM) crassulacean acid metabolism.

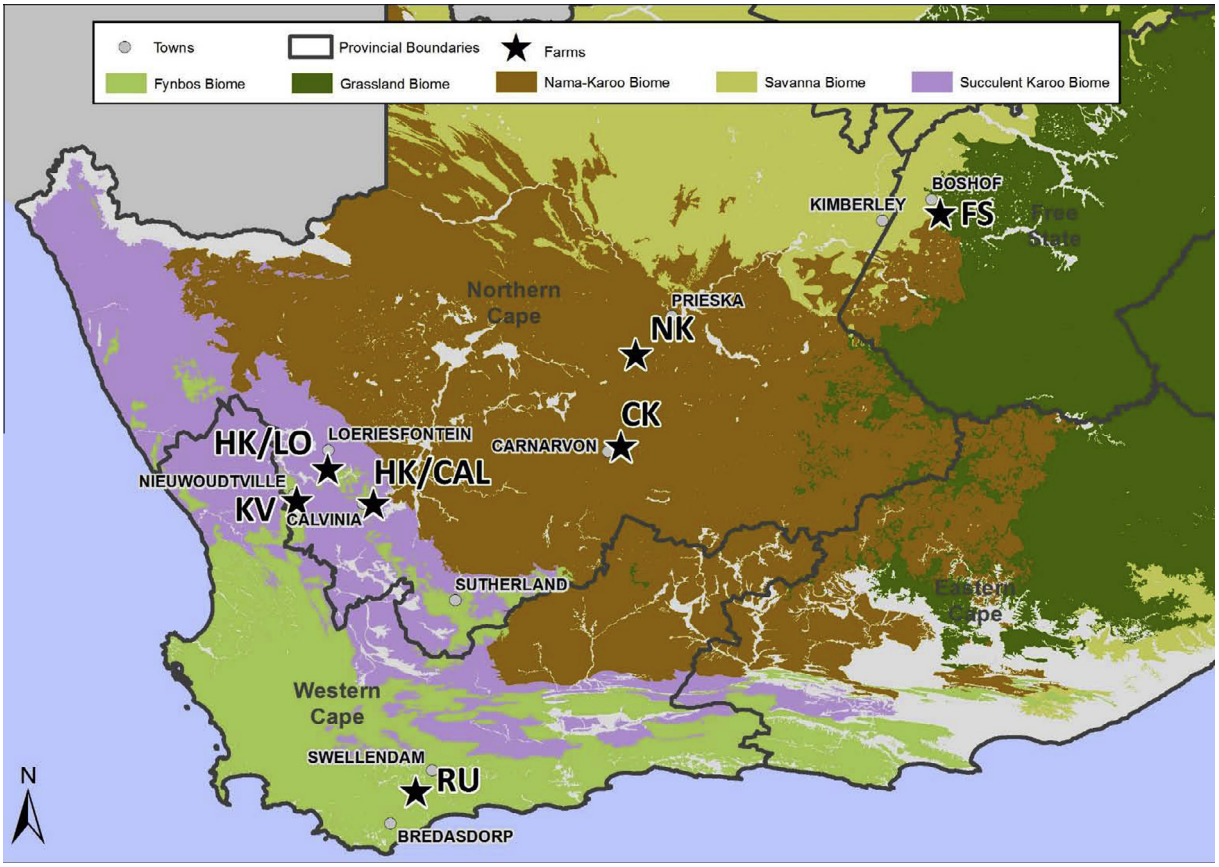


Fig. 1. Map of the different biomes related to the seven farms selected. (CK) Central Karoo; (NK) Northern Karoo; (HK/LO) Hantam Karoo/Loeriesfontein; (KV) Knersvlakte; (HK/CAL) Hantam Karoo/Calvinia; (RU) Rûens; (FS) Free State. Map supplied by Western Cape Department of Agriculture, South Africa (Mucina & Rutherford, 2006).

2.4. Free State province

Sheep farming is also practised extensively in the Free State grasslands region. This region consists of plains with summer rainfall (Cloete & Olivier, 2010). The vegetation of the farm selected (FS) mainly consisted of Kimberley thornveld (Savanna biome) and to a lesser extent that of the Western Free State clay grassland (grassland biome) (Cloete & Olivier, 2010) (Fig. 1).

2.5. Plants

Lambs were raised extensively on natural vegetation within the vicinity of the farm. Plant samples were collected from the farms after the lambs were slaughtered. Plants were either collected from the field last grazed before slaughter or, in cases where the field was depleted, from a similar field (less than 1 km away). Sampling of plants from the vicinity grazed provides an indication of the diet of the animal, as the latter is largely dependent on animal behaviour and the availability of forage (Radloff, Van der Waal, & Bond, 2013). The farmers confirmed the typical diet of the sheep. Within all farms the lambs were born and raised extensively within the borders of the farm. Plant species collected from the farms are listed in Table 1 and a more detailed description is provided as Supplementary data (Table S1). The number of species from the group with a specific pathway (i.e. C_3, CAM or C_4) is also shown in Table 1.

2.6. Sample collection

The day before slaughter, lambs from each farm were transported to the nearest abattoir. Animals were slaughtered according to standard South African procedures and regulations (National Department of Agriculture (NDA), 2000). Variation among animals was reduced by randomly selecting ten carcasses according to age (class A, no permanent incisor teeth), fatness (class 2 or 3) and weight (less than 20 kg). In South Africa carcasses are classified according to age, fat, conformation and damage classes as described under the Product Standards Act No.119 of 1990 and its regulations (National Department of Agriculture (NDA), 1990, 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) and class 3 measures 4.1–7.0 mm (medium fatness). Following slaughtering, the carcasses were cooled at 4 °C for 24 h. The *Longissimus thoracis et lumborum* (LTL) muscles were excised from the left side of the carcasses. Subcutaneous fat and visible sinews were removed and the LTL divided so that the *Longissimus lumborum* (LL) was homogenised for stable isotope ratio analysis.

2.7. Sample preparation

The fat of a 5-g homogenised meat sample was extracted (Lee, Trevino, & Chaiyawat, 1996). The meat was defatted to correct for variation in isotopic ratios between proteins and lipids due to the effect of the biochemical isotopic fractionation (Camin et al., 2007). As the lipid fraction contains little nitrogen, stable isotope analysis of meat was only conducted on the protein fraction (Camin et al., 2007). The 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.

2.8. Isotope ratio analysis

Powdered meat samples were weighed into tin cups to an accuracy of 1 microgram and combusted individually in a Flash 2000 organic elemental analyser, and the resultant CO_2 and N_2 gas introduced to a isotopic mass spectrometer (Delta V Plus) using a Conflo IV gas control unit (Thermo Scientific, Bremen, Germany). Isotope ratios are expressed in the conventional delta (δ) notation in parts per mil (‰) and correspond to Vienna PeeDee Belemnite (PDB) and nitrogen air (N_2) (internal standards) according to the following general formula:

$$\delta\ ‰ = \frac{R_{\text{sample}} - R_{\text{standard}}}{R_{\text{standard}}} \times 1000 \quad (1)$$

where R represents the ratio between the abundant isotopes i.e. $^{13}C/^{12}C$ and $^{15}N/^{14}N$. Standard deviations of repeated measurements of in-house standards were less than 0.25‰. The in-house standards used were: choc, a commercial chocolate/egg mixture; sucrose, Australian National University (ANU) sucrose; Valine, DL-valine purchased from Sigma; MG (Merck Gel), a proteinaceous gel produced by Merck; and Seal, which is a seal bone crushed, demineralised and dissolved in acid, and then reconstituted in gel form. All in-house standards were calibrated against the International Atomic Energy Agency (IAEA) standards. PDB has relatively more ^{13}C than most of the terrestrial biosphere (Sandberg et al., 2012). Accordingly, the tissues of plants and animals have negative $\delta^{13}C$ values.

2.9. Statistical analysis

Statistical analysis of data was performed using GLM (General Linear Models) procedure of SAS™ statistical software (Statistical Analysis System, Version, 9.2, 2006, SAS Institute Inc., Cary, NC) for analysis of variance (ANOVA) and XLSTAT® statistical software (Version 2014.2.03; Addinsoft, New York, NY) for the multivariate statistical analysis. Pre-processing of the data involved using the Shapiro–Wilk test to test for deviation from normality (Shapiro & Wilk, 1965). When the deviation from normality was significant ($p \leqslant 0.05$) the outliers in the data were identified and removed until the data was normal or symmetrically distributed. Following the confirmation of normality of the data, one-way ANOVA was carried out for each stable isotope ratio with farm as factor. Fisher's Least Significant Differences (LSD) was calculated at a 5% significance level to compare farm means. A probability level of 5% was considered significant for all the significance tests. Multivariate statistical techniques were used to find significant patterns and associations in the collected data. Discriminant analysis (DA) was carried out to determine whether lamb meat from the different farms could be mathematically distinguished on the basis of its stable isotope ratios. Farm separation was described and elucidated by means of linear functions of the variables (discriminant functions) that best separated farms, using the first six observations from each farm as a training set. The model was then validated using leave-N-out (LNO) cross-validation by using the last four observations from each farm as a test set. The grouping of the farms in separate clusters, on the basis of their meat stable isotope ratios, was performed applying Ward's hierarchical clustering technique (Ward, 1963). This technique clusters animals into smaller numbers of mutually exclusive groups, each having members that are similar with respect to specified characteristics.

3. Results and discussion

On the basis of production, seven groups (farms) of different geographic origin can be identified. Farms can further be divided in three groups based on diet (Table 2). Lamb from the Northern Cape were mainly raised on Karoo bushes/shrubs and grasses (CK, NK, HK/LO, KV, HK/CAL), while lamb from the Western Cape and Free State were raised on lucerne/alfalfa (*M. sativa*) (RU) and grass (FS), respectively (Table 2). It is important to note that although one would expect the isotopic differences of the plants to be exactly replicated in the tissues of animals living on these plants; this is not the case as isotope fractionation takes place during metabolism and results in varying isotope ratios between different tissues of the animal (Vogel et al., 1978). Therefore, for the purpose of this study only the protein fraction was used (i.e. defatted meat).

3.1. Variability of meat stable isotope ratios

Mean values of the $^{13}C/^{12}C$ and $^{15}N/^{14}N$ isotope ratios of meat protein are shown in Table 2. The $\delta^{13}C$ values are affected by the

Table 2
The means (±*SD*) of $\delta^{13}C$ and $\delta^{15}N$ values (in ‰, relative to the PeeDee Belemnite and atmospheric N_2 standards, respectively) of lamb meat from different farms.

Code	Diet	*n*	$\delta^{13}C$ (‰)	$\delta^{15}N$ (‰)
CK	Shrubs/bushes and grass	10	$-19.6^{c} \pm 1.07$	$8.9^{c} \pm 0.41$
NK	Shrubs/bushes and grass	10	$-20.1^{c} \pm 0.82$	$12.1^{f} \pm 0.80$
HK/LO	Shrubs/bushes and grass	10	$-18.7^{d} \pm 0.47$	$10.3^{e} \pm 0.22$
KV	Shrubs/bushes and grass	10	$-24.3^{a} \pm 0.24$	$9.5^{d} \pm 0.26$
HK/CAL	Shrubs/bushes and grass	10	$-22.9^{b} \pm 0.41$	$12.9^{g} \pm 0.56$
RU	Lucerne/alfalfa	10	$-22.7^{b} \pm 0.20$	$6.7^{a} \pm 0.36$
FS	Grass	10	$-15.8^{e} \pm 1.16$	$8.2^{b} \pm 0.45$
LSD ($p = 0.05$)			0.65	0.43

(*SD*) Standard deviation; (*n*) number of samples (animals); (CK) Central Karoo; (NK) Northern Karoo; (HK/LO) Hantam Karoo/Loeriesfontein; (KV) Knersvlakte; (HK/CAL) Hantam Karoo/Calvinia;(RU) Rûens; (FS) Free-State; (LSD) Least Significant Difference; $^{a-g}$Values in the same column for $\delta^{13}C$ and $\delta^{15}N$ with different superscripts are significantly different ($p \leqslant 0.05$).

origin of the meat, mainly reflecting the diet. KV had the lowest and significant ($p \leqslant 0.05$) $\delta^{13}C$ value (-24.3 ± 0.24‰), followed by HK/CAL (-22.9 ± 0.41‰) and RU (-22.7 ± 0.20‰), which did not differ from each other (Table 2). The Free State lamb (FS) had the least negative $\delta^{13}C$ value (-15.8 ± 1.16‰) (Table 2). It is known that the carbon isotope ratio is largely influenced by the amount of C_3 and C_4 plant material incorporated into the animal's diet. The C_3-pathway (typical of temperate pasture plants) causes a large change in the carbon isotope proportions relative to atmospheric carbon dioxide (Piasentier et al., 2003). However, the C_4-pathway (typical of tropical plants) incorporates more ^{13}C and results in a smaller change i.e. less negative $\delta^{13}C$ values. Hence, C_4 plants have higher $^{13}C/^{12}C$ ratios in their tissues compared to C_3 plants (Gibson, 2009; Vogel et al., 1978). The same difference in $^{13}C/^{12}C$ ratios is recorded in the tissues of the animals raised on C_3 and C_4 plants, respectively (Codron, Codron, Sponheimer, 2005). Perini et al. (2009) found that lambs predominantly fed forage have the lowest (most negative) $\delta^{13}C$ values, while those which had concentrates included in their diets had less negative values. This is attributed to the use of grains and by-products of maize (a C_4 plant) in the concentrates. A similar ^{13}C-enrichment was reported for lambs fed milk (Camin et al., 2007).

The $\delta^{13}C$ values reported in Table 2 for the Western Cape (RU) and Free State farms (FS) are similar to the $\delta^{13}C$ values reported by Codron et al. (2005b) for lucerne/alfalfa (C_3 plant) diet (-26.8‰) and Bermuda grass (C_4 plant) diet (-13.6‰), respectively. Given that the RU lambs were raised on lucerne/alfalfa and the FS lamb on grass, the $\delta^{13}C$ values of the meat are similar to that of the diet. Hence, one can assume that the carbon isotope ratios expressed in the meat is representative of the diet of the animal. Although Codron et al. (2005b) determined the carbon isotope ratios of the faeces and not of the meat, they also established a direct link between diet and the faeces with some discrimination resulting in lower faecal $\delta^{13}C$ values. Typically, pure grazer animals consuming C_4 plants had the highest $\delta^{13}C$ values (-14.7 to -13.5‰), while browsers consuming C_3 plants had the lowest (-27.2 to -26.2‰) and mixed-feeders consuming both C_4 and C_3 plants had intermediate values (-23.0 to -19.0‰) (Codron, Codron, Sponheimer, 2005). Similarly, Vogel et al. (1978) found C_3 plants to have $\delta^{13}C$ values more negative than -20‰ and C_4 plants more positive than -16‰. Following these findings and the results presented in Table 2 in combination with the plants collected from the different areas (Table 1), one can interpret the results in a similar manner.

The CK and NK farms had intermediate $\delta^{13}C$ values (not significantly different) (Table 2), which is expected as the plants collected from the areas included both C_3 and C_4 plants (Table 1). The Dorper is known to be a more general or non-selective grazer, focussing on the woody plant types of the veld, such as Karoo bushes, trees and shrubs (Du Toit, 1998). However, they do graze sparingly on the latter if there are still soft forages available in the veld. Soft forage may consist of perennial grasses known to be very palatable grazing plants (Acocks, 1988). Hence, the main contributor towards the increase in $\delta^{13}C$ values of CK and NK is likely to be the C_4 grass species, such as *Fingerhuthia africana*, *Stipagrostis ciliata* and *Stipagrostis obtusa* (Table 1). Karoo bushes are mostly consumed during winter and times of drought when the perennial grasses are depleted (Acocks, 1988). Similarly, Radloff et al. (2013) reported that the dietary intake of cattle is largely dependent on the forage conditions to which they are exposed to. Cattle, typically considered grazers, would increase their browse intake to compensate for the decrease in grass quality during the late dry season.

HK/LO had slightly higher $\delta^{13}C$ values than that of CK and NK, differing significantly from the rest ($p \leqslant 0.05$) (Table 2). Although the plants collected from the HK/LO area did not include C_4 grass species, CAM plants (*Stoeberia beetzii* and *Tetragonia fruticosa*) were prevalent in the area and these are known to have intermediate $\delta^{13}C$ values (Table 1) (Capuano et al., 2013; Codron, Codron, Sponheimer, 2005). The plants collected from the HK/CAL farm mainly included C_3 Karoo bushes/shrubs (*Chrysocoma ciliata*, *Eriocephalus ericoides*, *Justica orchioides*, *Lycium* spp., *Pentzia incana* and *Pentzia sphaerocephala*) and some succulents (*Drosanthemum hispidum*, *Eberlanzia ferox* and *Mesembryanthemum vaginatum*) known to provide negative and intermediate $\delta^{13}C$ values, respectively (Table 1) (Capuano et al., 2013). As a result the HK/CAL lamb had low $\delta^{13}C$ values (Table 2). Vogel (1978) reported similar results with lower ^{13}C values for springbok living on Karoo bushes/shrubs from the Northern Cape compared to those consuming more grass from Namibia. In contrast, HK/CAL lamb had the highest $\delta^{15}N$ value (12.9 ± 0.56‰).

Environmental parameters such as climate and soil condition are known to influence the ^{15}N content of feed, which in turn affects the $\delta^{15}N$ values of the products produced from it. Furthermore, organic fertilisers increase the ^{15}N level of nitrogen compounds of soil and plants, whereas the presence of legumes has the opposite effect on grassland ^{15}N-content, because it uses the nitrogen in the air as a nitrogen source (DeNiro & Epstein, 1981; Perini et al., 2009; Piasentier et al., 2003). Perini et al. (2009) proposed that an increase in the $\delta^{15}N$ values of lamb meat could be due to the application of organic fertilisers or linked to arid conditions of the production site. Hence, the high $\delta^{15}N$ values for HK/CAL are likely due to the arid conditions of the farm. Another contributor towards ^{15}N-enrichment could also be the prevalence of CAM plants, as Codron et al. (2005a) observed the highest $\delta^{15}N$ values for succulents. Conversely Devincenzi et al. (2014) found that $\delta^{15}N$ decreased linearly with the proportion of legume in the diet of lambs. This is likely the reason for the decrease in $\delta^{15}N$ values for KV (9.5 ± 0.26‰) (Table 2). The plants collected from the KV farm included several of these leguminous

plant species, such as *Lebeckia leipoldtiana*; *Wiborgia monoptera* and *Wiborgia sericea* (Table 1).

Piasentier et al. (2003) found differences in the $\delta^{15}N$ values of lamb meat fed similar diets, but in different countries. Therefore, the different $\delta^{15}N$ values for the lamb meat of the various farms were expected (Table 2). This was specifically related to lamb from the Northern Cape, which is produced on typical Karoo plants, which vary depending on the origin within the region. In fact, the lamb from the Western Cape and Free State farms (RU and FS) had the lowest ($p \leqslant 0.05$) $\delta^{15}N$ values, followed by the Northern Cape farms (CK, KV, HK/LO, NK and HK/CAL), HK/CAL having the highest $\delta^{15}N$ value. Overall $\delta^{15}N$ values showed the clearest discrimination between farms in the stable isotope ratios examined, as a result of low within-group (animal to animal) variation and the greatest between-farm variability (Table 2). According to Piasentier et al. (2003), the reported $\delta^{15}N$ values were not indicative of a specific region as distant regions might be similar. This was also evident in the current study as farms largely varying in diet and location had similar values such as CK (8.9 ± 0.41) and FS (8.2 ± 0.45) (Table 2).

3.2. Discriminating geographical origin from meat stable isotope ratios

Discriminant analysis (DA) was performed to test if the stable isotope ratios in lamb meat could discriminate the different farms of origin. The results are reported in Table 3 as the number and percentage of correctly classified observations. The stable isotope ratios allowed 97.62% correct classification of the meat samples for the estimation model and 96.43% for the validation model (Table 3). With the exception of CK, all the meat samples were 100% correctly classified (Table 3). The 25% misclassification of the CK is a result of the variation in $\delta^{13}C$ and $\delta^{15}N$ values between animals sourced from the CK area. The results obtained for the classification of lamb meat according to farm of origin confirm the potential of using stable isotope ratio analysis as a tool for the characterisation of the origin of meat, i.e. the authentication of farm of origin lamb meat and its traceability linked to a diet typical of the defined region.

Ward's hierarchical clustering technique (Ward, 1963) was applied for grouping farms based on the dissimilarities in the stable isotope ratio values of the meat samples. During the clustering process samples, which minimise the agglomeration criterion, are clustered together. A binary clustering tree (dendrogram), whose root is the class that contains all the samples, is produced by all the succeeding clustering processes. The dendrogram of the clustering is provided as Supplementary Data (Fig. S1). Six classes were identified and the resulting classification sample codes used as observation labels in the DA plot.

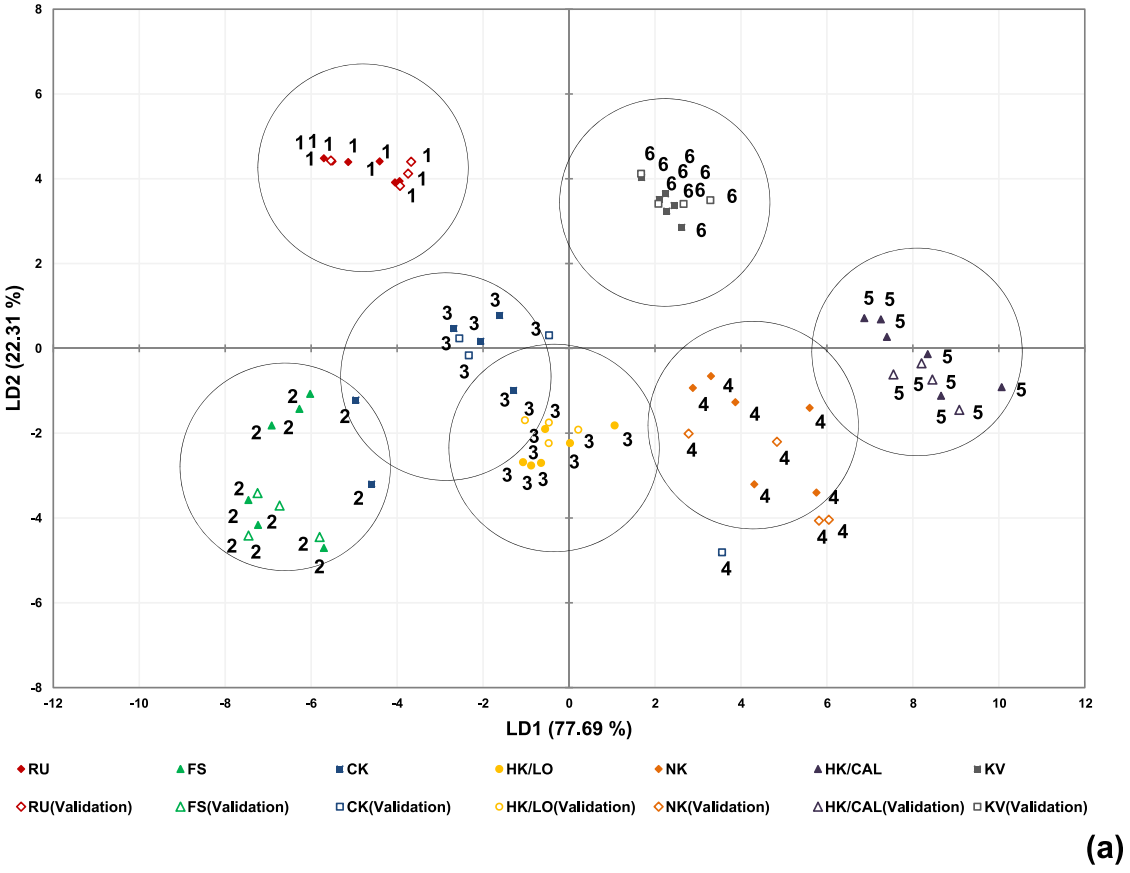
The DA plots of the observations (labelled according to Ward's clusters) and loadings on the linear discriminant function scores are illustrated in Fig. 2. The DA plots were used to visualise the grouping of observations within each farm of origin and the separation between farms of origin. The DA plots confirm that the farms of origin are very well discriminated on the axes extracted from the stable isotope ratios. In fact, Fig. 2b shows that both $\delta^{13}C$ and $\delta^{15}N$ values influence the location of samples along the axes. NK, HK/CAL and KV associate with higher $\delta^{15}N$ values, while RU, FS, CK and HK/LO associate with less negative $\delta^{13}C$ values. Fig. 2a also indicates that the seven farms of origin could be classified in six clusters. Each farm was assigned to its own cluster, except for CK and HK/LO which were clustered together (cluster 3) (Fig. 2a). CK and FS had the most variable $\delta^{13}C$ values, where the isotope ratios of some of the individual animals were far from the mean of the group (Fig. 2a and b). Such variation is likely, as the amount and type of plant species consumed by individual animals is a complex behavioural act influenced by various factors (Holechek, Vavra, & Pieper, 1982). Three of the ten CK samples were also assigned to different clusters, two to cluster 2 (FS) and one to cluster 4 (NK) (Fig. 2a). The other Northern Cape farms (CK, NK and HK/LO) grouped more to the middle of the graph (Fig. 2); hence, having lower $\delta^{15}N$ values combined with less negative $\delta^{13}C$ values (Fig. 2b). Some of the plants collected from the farms, apart from that of HK/LO, included C_4 grasses, a likely cause of increase in $\delta^{13}C$ values (Table 1). C_4 grasses have carbon isotope ratios ranging from −9‰ to −18‰ (Gibson, 2009). Although the HK/LO production area did not feature any of such grasses, it did have various succulent CAM plant species (Table 1). As CAM plants result in intermediate $\delta^{13}C$ values, a high inclusion in the diet could increase the $\delta^{13}C$ values (Capuano et al., 2013).

Given the assumed similarity in diets between HK/CAL and HK/LO one expected the $\delta^{13}C$ values to group closely. However, the results showed a different grouping, with HK/LO closer to the combined carbon and nitrogen isotope ratios of CK and NK (Fig. 2a), suggesting that the HK/CAL lambs consumed more

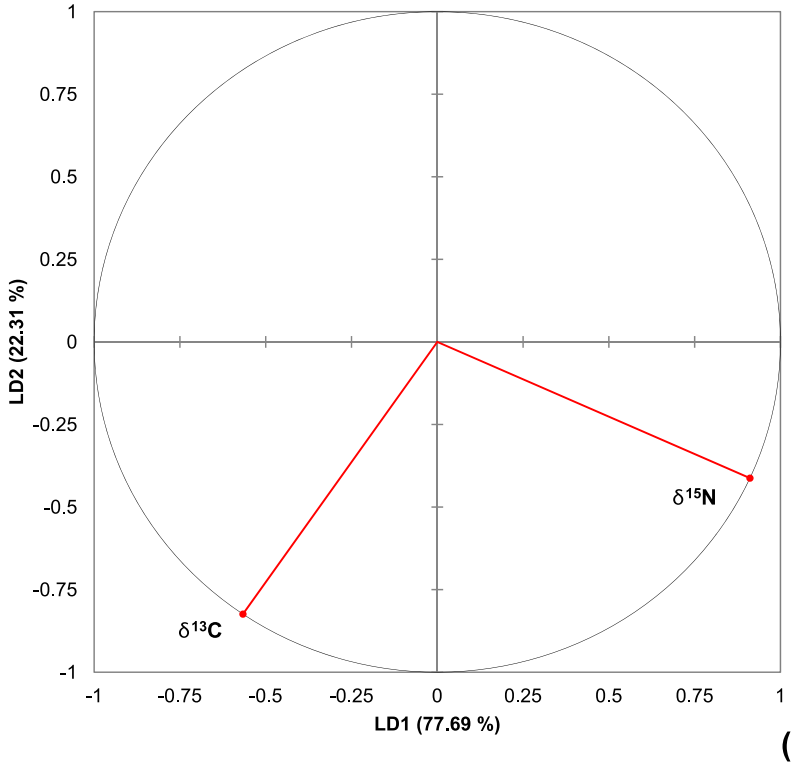
Table 3
Classification of individual lamb meat samples, on the basis of stable isotope ratios ($\delta^{13}C$ and $\delta^{15}N$) and percentage of observations correctly classified.

Model	Actual origin	Classified origin[a]							
		CK	NK	HK/LO	kV	HK/CAL	RU	FS	Total
Estimation	CK	5	0	0	0	0	0	1	6
	NK	0	6	0	0	0	0	0	6
	HK/LO	0	0	6	0	0	0	0	6
	KV	0	0	0	6	0	0	0	6
	HK/CAL	0	0	0	0	6	0	0	6
	RU	0	0	0	0	0	6	0	6
	FS	0	0	0	0	0	0	6	6
% Correctly classified		83.33	100	100	100	100	100	100	97.62[b]
Validation	CK	3	1	0	0	0	0	0	4
	NK	0	4	0	0	0	0	0	4
	HK/LO	0	0	4	0	0	0	0	4
	KV	0	0	0	4	0	0	0	4
	HK/CAL	0	0	0	0	4	0	0	4
	RU	0	0	0	0	0	4	0	4
	FS	0	0	0	0	0	0	4	4
% Correctly classified		75.00	100	100	100	100	100	100	96.43[c]

[a] The number of correctly classified observations are tabulated diagonally.
[b] 97.6% of the observations for the estimation model correctly classified.
[c] 96.4% of the observations for the validation correctly classified; (CK) Central Karoo; (NK) Northern Karoo; (HK/LO) Hantam Karoo/Loeriesfontein; (KV) Knersvlakte; (HK/CAL) Hantam Karoo/Calvinia;(RU) Rûens; (FS) Free-State.



(a)



(b)

Fig. 2. Discriminant analysis (DA) plot (a) and DA variable loadings plot (b) of the linear discriminant function scores on stable isotope ratios ($\delta^{13}C$ and $\delta^{15}N$) in lamb meat for the grouping of observations from different farms, using Ward cluster numbers (1–6) as observation labels. (LD) Linear Discriminant Score; (RU) Rûens; (FS) Free-State; (CK) Central Karoo; (HK/LO) Hantam Karoo/Loeriesfontein; (NK) Northern Karoo; (HK/CAL) Hantam Karoo/Calvinia; (KV) Knersvlakte.

Karoo bushes/shrubs compared to that of HK/LO and in effect also to that of the other areas. A more accurate approach to confirm the composition of the diet would be to identify the exact plant species consumed, by using techniques such as micro-histological analysis of the faeces (Holechek et al., 1982). Unfortunately such an approach was out of the scope of the current study, but for future work it would provide valuable information regarding the effect of diet on the isotope ratios of the meat. Although carbon and nitrogen isotopes allowed a good discrimination between farms, the use of other isotopes (i.e. ^{2}H, ^{18}O and ^{34}S) has the potential to provide an additional level of geographical resolution (Crawford et al., 2008; Kelly et al., 2005). For instance, Perini et al. (2009) explored the use of several isotopes (^{2}H, ^{13}C, ^{15}N, ^{18}O and ^{34}S) as an effective tool to trace the geographical origin and diet of lambs.

4. Conclusions

The study proved that stable isotope ratio analysis of meat is a promising tool to evaluate extensive meat production associated with type of diet of which the ^{13}C isotope are particularly useful for indicating dietary differences. The results were reasonably representative of the observed vegetation of the different farms. Hence, one can conclude that the collection of plants from the animal production site provides an indication of the likely diet of the animals. Stable isotope ratio analysis is also useful when a limited number of sample replicates is available. It provided evidence for the authenticity of extensively produced South African Dorper lamb, based on origin, as well as diet. By applying discriminant analysis 97.62% and 96.43% of the observations for the estimation and validation were correctly classified, respectively. The results demonstrate the usefulness of multi-element fingerprints as indicators for authenticating the geographical origin of lamb in South Africa. Through the analysis of additional isotopes, one can even further improve the discriminative power of the technique. Further research would entail determining baseline data of stable isotope ratios for regionally unique South African lamb so as to development robust classification models, using a larger sample set, which is representative of the different regions. The latter would provide the means of moving from the current farm of origin to region of origin authentication of sheep meat.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.foodchem.2015.07.121.

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