

Isotopic composition of cow tail switch hair as an information archive of the animal environment

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Abstract

Using isotopic signatures from animal tissue, it is possible to recover certain information about the environment of the animal – notably the diet – at the time the hair was laid down. In the case of tail switch hair of cattle, a single hair may often represent an archive of information spanning a year or more in time. Isotopic analysis by mass spectrometry is now becoming cheap enough to be considered accessible for routine diagnostic or scientific investigation. The ratios of ¹³carbon (C):¹²carbon and ¹⁵nitrogen (N):¹⁴nitrogen are ideal for such investigation, since C and N are constituents of all animal proteins. This paper explains the theory of isotopic analysis in layman's terms, and reports an experiment in which tail switch hair of 9 cattle from three Northland dairy farms was analysed in a 'proof of concept' study, to demonstrate the information-retrieval potential offered by isotopic analysis. Changes in isotopic abundance are measured in parts per thousand (‰). When matching signatures on replicate hairs, the average distance from the 'interpolation' line was $\pm 0.13\text{‰}$ for $\delta^{13}\text{C}$, and $\pm 0.11\text{‰}$ for $\delta^{15}\text{N}$. In contrast to this, differences in $\delta^{13}\text{C}$ between different hair segments analysed exceeded 11‰, while between farm differences in $\delta^{15}\text{N}$ exceeded 2.0‰. We suggest possible reasons for these differences in isotopic signature.

Keywords: ¹³C, ¹⁵N, isotopic archive, nutritional ecology, stable isotope

Introduction

Animal proteins are largely comprised of the elements carbon (C), hydrogen, nitrogen (N) and oxygen. All elements, including these four, have two kinds of particles in their nucleus, protons and neutrons. The number of protons is fixed for a particular element, but the number of neutrons can vary. For example, C has 6 protons in the nucleus, but may have 5 neutrons (¹¹C, radioactive), 6 neutrons (¹²C, stable), 7 neutrons (¹³C, stable), or 8 neutrons (¹⁴C, radioactive). These different forms of the same element, with different total numbers of

protons and neutrons (i.e. different atomic weight), are known as isotopes. For the stable isotopes of C, the natural abundance of the lighter ¹²C isotope is 98.89% and of the heavier ¹³C isotope is 1.11%. Similarly, N has 7 protons, and the stable isotopes of N, ¹⁴N with 7 neutrons and ¹⁵N with 8 neutrons, have a natural abundance of 99.63% and 0.37%, respectively.

While different isotopes of the same element have identical chemical properties, certain environmental and biological processes may operate selectively in favour of either the heavier or lighter isotope. However, such discrimination is typically in the range of the third or fourth decimal place. The abundance of the heavier isotope is thus expressed in parts per thousand (‰), as compared to an international standard, and is referred to as the δ -value, calculated as follows:

$\delta^{13}\text{C}$ (or $\delta^{15}\text{N}$) = $[(R_{\text{sample}}/R_{\text{standard}}) - 1] \times 10^3$, where R is the respective ¹³C/¹²C or ¹⁵N/¹⁴N ratio. The C isotopic composition ($\delta^{13}\text{C}$) is presented relative to 'PDB' (fossil carbonate) standard and the $\delta^{15}\text{N}$ relative to N in air. Thus, a negative sample δ -value indicates depletion, and a positive δ indicates enrichment, respectively, of the heavy isotope, compared with the standard.

Many grasses of tropical origin such as paspalum and kikuyu (C4 grasses) have a different photosynthetic system from temperate (C3) grasses such as perennial ryegrass (e.g. Campbell *et al.* 1996). During photosynthesis in C3 plants carbon dioxide containing the heavier ¹³C is less likely to be assimilated ($\delta^{13}\text{C}$ $-27 \pm 2\text{‰}$; O'Leary 1988). This discrimination against ¹³C is somewhat suppressed under water deficit, and C4 plants exhibit little or no preference for ¹²C ($\delta^{13}\text{C}$ $-13 \pm 1\text{‰}$; O'Leary 1988). Reduced discrimination of C3 plants for ¹³C when under water deficit has led to a test for water use efficiency (Farquhar *et al.* 1989) based on the ratio of ¹³C:¹²C in plant tissue, though the test must be used with caution (Virgona 1993). Similarly, various ecosystem processes can change the natural abundance of the common isotopes of N (Högberg 1997). While N from biological fixation has $\delta^{15}\text{N}$

values close to 0‰, values of $\delta^{15}\text{N}$ generally increase with increasing trophic level of the N (Handley & Raven 1992). Herbivores show higher $\delta^{15}\text{N}$ -values than their diet, carnivores show higher $\delta^{15}\text{N}$ -values than their prey. In a number of studies the $\delta^{15}\text{N}$ of animals was about 3‰ higher (enriched in the heavier isotope) than in their diet (e.g. De Niro & Epstein 1981; Hobson *et al.* 1996).

Signatures of animal forages are both incorporated into animal tissues, and modified by metabolic processes of the animals themselves (De Niro & Epstein 1978; De Niro & Epstein 1981; Tieszen *et al.* 1983; Rundel *et al.* 1988). Animal hair, for example, represents an archive of the isotope signatures of food eaten by that animal at the time the hair was laid down. Nowadays, analysis of isotope ratios in animal hair by mass spectrometry, to recover information on isotope signatures is reasonably cheap, and recently some researchers have explored the possibility of using isotopic analysis of animal hair to recover information about the feeding habits and environment of that animal (Hobson *et al.* 1996; Schoeninger *et al.* 1997; McIlwee & Johnson 1998; Schwertl *et al.* 2003). Changes in δ -value along the length of a hair or between hairs therefore allow inference about seasonal ecosystem changes, or may identify differences between production systems. This paper reports the results of isotopic analysis of cow tail switch hair from three dairy farms located near Dargaville, where both seasonal and between-farm difference in pasture botanical composition (i.e. C4 grass proportion in the diet) would be expected to occur. While it was expected from previous overseas work cited above that animal hair from the respective farms would reflect dietary differences in isotopic abundance, and that mass spectroscopy would detect such differences, we wished to confirm this for data from New Zealand.

Methods

Hair samples were collected from an organic dairy farm (3 cows sampled as replicates for statistical analysis), a conventional dairy farm with ryegrass dominant pastures (4 cows), and a conventional dairy farm with a significant kikuyu grass component in pastures (2 cows). A tuft of hairs was cut in early December 2002, as close to the skin as possible, from the tail switch of each of these nine cows. To remove faeces and other contaminants, hair was washed by ultra-sound agitation in deionised water, shaken in a 2:1 mixture of methanol/chloroform for 2 hours, and rinsed with deionised water. After drying (40°C, 48 h), duplicate hairs from each animal were selected in order to

verify repeatability of analytical procedures. Hairs were stretched on a frame, cut to 10 mm lengths and each alternate length analysed for $\delta^{13}\text{C}$ and for $\delta^{15}\text{N}$ value. Each hair segment was enclosed in a tin cup (4 x 6 mm) and combusted in an elemental analyzer (NA 1108; Carlo Erba, Milan) interfaced (ConFlo II; Finnigan MAT, Bremen, Germany) to an isotope ratio mass spectrometer (Delta Plus; Finnigan MAT) located at the Institute of Grassland Science, Technical University of Munich. The standard deviation was $\pm 0.2\text{‰}$ for $\delta^{13}\text{C}$ and $\pm 0.3\text{‰}$ for $\delta^{15}\text{N}$. For further details of the isotopic measurements see Schwertl *et al.* (2003).

The isotope patterns of two hairs from the same animal can be displaced relative to each other. Two different mechanisms may contribute to such a mismatch. A 'cutting error' may arise from a difference in stubble length. A 'growth cycle error' can originate from sampling hair in different growth phases. A single hair grows for a certain time (anagen phase), remains in the follicle channel for some time afterwards and finally drops. After a phase of follicle quiescence (telogen phase) a new hair is produced. When cut, an anagen hair cannot be distinguished from a telogen hair, but the latter does not contain recently-formed hair tissue and, hence, misses recent isotopic information. To correct for the mismatch, the isotope signature of one hair had to be shifted relative to the other in 1 mm steps, until a best fit to the second hair was obtained, as described by Schwertl *et al.* (2003). In total, analysis of 18 hairs (2 hairs per animal, 9 animals from three farms) required mass spectrometry measurement of 81 hair samples.

Results

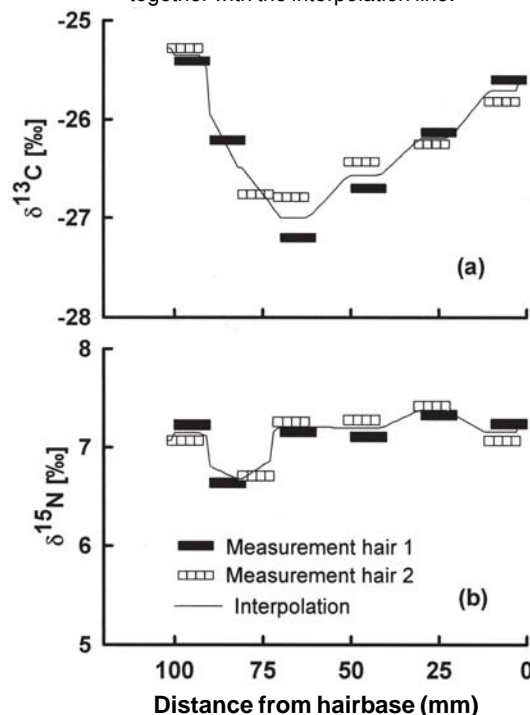
Repeatability of analysis for different hairs of the same animal

The isotopic patterns of two replicate hairs from the same animal typically showed remarkable similarity after optimum shift (Figure 1). Close agreement between duplicate hairs was indicated by high correlation coefficients for C and N signatures (mean $r = 0.82$). Similar mean correlation coefficients were found for the two hairs of each of the other animals analysed (0.80 ± 0.15). Over the 9 pairs of hairs the mean distance of individual measurement points from the interpolation line was $\pm 0.13\text{‰}$ for $\delta^{13}\text{C}$, and $\pm 0.11\text{‰}$ for $\delta^{15}\text{N}$.

Differences between farms

For $\delta^{15}\text{N}$, all three farms differed significantly in their isotopic signature, with the organic farm having the lowest $\delta^{15}\text{N}$ value, indicating comparative

Figure 1 Isotopic signature for (a) $\delta^{13}\text{C}$ and (b) $\delta^{15}\text{N}$ of alternate 10-mm sections along two hairs of cow number 13 (Ryegrass farm) shown together with the interpolation line.



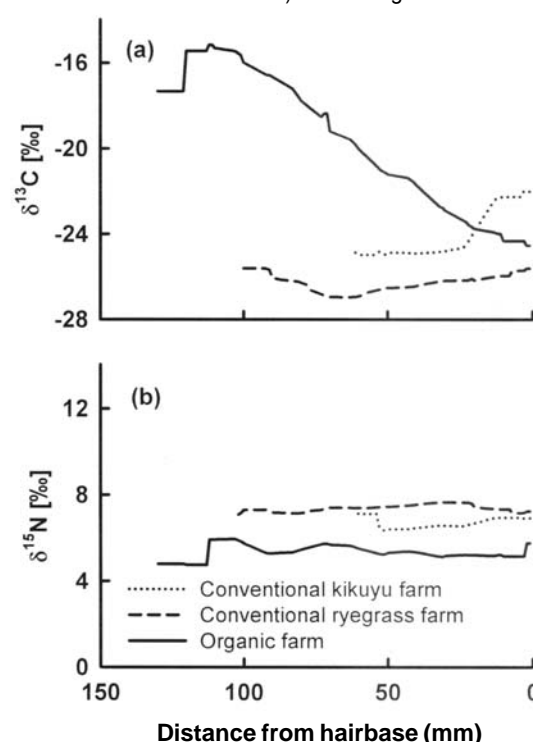
enrichment of the heavier ^{15}N isotope in hair samples from the two conventional farms (Table 1). For $\delta^{13}\text{C}$, each of the farms again exhibited a highly significant difference in mean value (Table 1). However, plotting $\delta^{13}\text{C}$ versus the distance of the particular hair segment from the hair base (Figure 2a), indicated unique temporal patterns in the isotopic signature of each farm, in addition to the difference in farm average $\delta^{13}\text{C}$ value (Table 1). In contrast to the variability over time of $\delta^{13}\text{C}$ for isotopic signature, $\delta^{15}\text{N}$ signatures for all farms did not change significantly over time (Figure 2b).

Discussion

Repeatability

Given that differences in $\delta^{13}\text{C}$ between different hair

Figure 2 Average isotopic signatures for (a) $\delta^{13}\text{C}$, and (b) $\delta^{15}\text{N}$ of three farms, calculated from individual 2-hair averages. Mean deviations of individual lines from average farm line were 0.53 and 0.55‰ (Organic farm, 3 animals), 0.15 and 0.27‰ (Conventional kikuyu farm, 2 animals), and 0.09 and 0.21‰ (Conventional ryegrass farm, 4 animals) for $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ respectively. The X-axis reflects a time progression from old hair (left, hair tip) to recently formed hair (right, hair base). The data cover a period of approximately 4 months (early August to 2 December 2002) for the longest hairs.



segments analysed exceeded 11‰ on the organic farm (Figure 2a), and that between farm differences in $\delta^{15}\text{N}$ exceeded 2‰ (Figure 2b), while mean distance from the interpolation line (Figure 1) was less than 0.15‰ for both $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$, the present results underline the sensitivity of mass spectrometry, as a forensic tool to recover historical

Table 1 Mean isotopic signature for $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ on the three farms and 95% and 99.9% confidence intervals (CI) for the means. Means different at the 99.9% level are indicated by different letters.

Isotope	Farm	Mean	95% CI	99.9% CI	SD	n
$\delta^{13}\text{C}$	Kikuyu	-24.13 A	0.81	1.61	1.35	13
	Ryegrass	-26.29 B	0.16	0.28	0.48	39
	Organic	-20.42 C	1.18	2.12	3.10	29
$\delta^{15}\text{N}$	Kikuyu	6.67 a	0.29	0.57	0.48	13
	Ryegrass	7.39 b	0.12	0.21	0.37	39
	Organic	5.28 c	0.28	0.50	0.74	29

information on certain aspects of animal environment, as 'sampled' through dietary intake.

Differences between farms

The notable features of the data are time-trends in $\delta^{13}\text{C}$ signature, unique to each of the farms sampled (Figure 2a), and between farm differences in $\delta^{15}\text{N}$ (Figure 2b). Hence, the experimental hypothesis that such differences in isotopic signature would be detected is clearly confirmed. However, since detailed records such as hair growth rate, and animal intake and diet composition on each farm were not collected, interpretation of these results was necessarily intuitive. Each of the farmers was interviewed, and relevant data on cow husbandry and diet obtained.

At the organic farm, the three cows from which hair was collected in early December 2002 had calved in July 2001, December 2001 (both milked over), and July 2002, respectively. Tail switch hairs for cows milked over were trimmed in July 2002, and for the cow calving in July 2002, tail switch hairs were trimmed on completion of colostrum milking (late August 2002), untrimmed tail hair being a sign for the person milking, that milk must be discarded. This husbandry practice on the organic farm allows the start of the record for that farm (120 – 140 mm from the hair base, Figure 2a) to be placed between late July and late August, depending on the length of stubble left when tail hair was cut. At this time, the $\delta^{13}\text{C}$ -value of around -16‰ indicates a high presence of C4 material in the diet. Assuming that: (i) C4 plant material typically has $\delta^{13}\text{C}$ -values of about -13‰ , (ii) pastures grazed in early spring comprised only C3-plants with $\delta^{13}\text{C}$ -values about -27‰ (O'Leary 1988), and (iii) that $\delta^{13}\text{C}$ of hair is about 1–2‰ higher than diet (De Niro & Epstein 1978; Jones *et al.* 1981), C4 material made up about 70% of the animal diet at that time. On our enquiry, the farmer confirmed that in August and part of September, cows were receiving feed supplements of molasses, maize silage, and sorghum, all products derived from C4 plants. However, the quantity of C4-derived supplementary feed was estimated at 5 kg DM/cow/day, rather less than 70% of the diet for a lactating cow. Since experience to date is that the assumptions above are fairly accurate for estimating proportion of C4 material in the diet, this apparent discrepancy, and the variability in signal between cows on this farm (Figure 2a), are two points for follow up study. We can speculate that between cow differences in $\delta^{13}\text{C}$ -value at the organic farm (Figure 2a) could arise from differences in the amount of supplement consumed, more negative values being

associated with reduced access to the C4-derived supplement. However, further measurements with supporting information on cow behaviour are needed to confirm this hypothesis. On the organic farm, the $\delta^{13}\text{C}$ -value took until late November to reach a value close to that normally found in C3 plant material (Figure 2a), even though C4-derived supplements were not fed after mid-September. This reflects the gradual elimination of C4-derived carbon from body tissues by dilution, after feeding of this material stopped. Jones *et al.* (1981) noted that a period of a little over 70 days was required for isotopic composition of animal tissue to fully adjust after a sudden change of diet. Finally, the proportion of C4 species in pastures of the organic farm must have been low, since the isotopic signature at the hair base (Figure 2a), based on our model assumptions (see above), indicated not more than 10% C4 material in the diet by late-November 2002.

In contrast with the organic farm, no C4-derived supplements were fed at the conventional farms in early lactation. For the ryegrass farm the $\delta^{13}\text{C}$ -values are as expected, consistent with there being little or no C4-derived plant material in the diet. Values for $\delta^{13}\text{C}$ at this farm did vary a little with time, being lowest about 60 mm from the hair base, but the reason for this is unknown. For the kikuyu farm, we were at first surprised by the $\delta^{13}\text{C}$ values, indicating an average of only 10% of C4 species in the diet, but with the C4 component increasing to approximately 30% for hair laid down just prior to sampling, that is in November (Figure 2a). However, on visiting the farm and inspecting the pastures in May 2003, it was learned that the farmer's practice is to 'mulch' kikuyu pastures in autumn with a heavy mower to break up the stem mat formed over summer and allow C3 species to provide winter forage. During this visit, C3-dominant pastures were observed, with annual dicotyledonous species such as chickweed (*Cerastium glomeratum*) also contributing significantly to the animal diet. The farmer estimated C3 grass content of spring pastures was at least 75%, and November is the month when kikuyu plants in pastures in this region begin to show strong summer growth. Hence, the isotopic record, although unexpected, proved to be explained very well, once the relevant facts were collected.

Although $\delta^{15}\text{N}$ differences between diet and animal are reported to deviate from the mean of about 3‰ in some physiological stress situations like fasting (Hobson *et al.* 1993) and water and/or N stress (Ambrose & De Niro 1986; Sealy *et al.* 1987; Ambrose 1991; Cormie & Schwarcz 1996), we would not expect that these factors influenced the

present results. The cows were of the same breed and were kept in the same region, in similar physiological condition (lactation), and nutrient and water supply was sufficient. Thus, differences in $\delta^{15}\text{N}$ between animals and between farms appear to arise from differences of $\delta^{15}\text{N}$ in the diet. We can only speculate on the reason for between-farm (Figure 2b) differences in $\delta^{15}\text{N}$ of herbage, but one possibility that could be investigated is that such differences reflect differences between farm systems in N loss by volatilisation as this is a process that strongly favours ^{14}N (Gormly & Spalding 1979). The lower $\delta^{15}\text{N}$ -value for the organic farm is consistent with lower gaseous N losses on this farm.

Potential applications of isotopic analysis

Based on these results, isotopic analysis is a sensitive tool for detection of certain types of variation in dietary composition of animals. In the present study, between-farm differences in the level of C4-plant-derived material in the animal diet were readily detected, and also a between farm difference in $\delta^{15}\text{N}$, of uncertain significance. The methodology could be utilised in animal behaviour studies where percentage of C4 species in the diet is of interest, potentially can detect practices such as feeding of animal protein to ruminants, or could be used to verify product origin, for example.

ACKNOWLEDGEMENTS

The NZ Grassland Trust, and the German Academic Exchange Foundation (DAAD) are thanked for assistance with travel of one of the authors (CM) from New Zealand to Germany in connection with this work. We also thank C & J Sanford, R & G Gillatt, and P & S Ball for making cow hair samples and farm data available for this study.

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