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TECHNICAL REPORT

Short-term diet changes revealed using stable carbon isotopes in horse tail-hair

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Summary

1. We demonstrate the potential of extracting high-resolution dietary information from stable carbon isotopes ($\delta^{13}C$) in horse tail-hair, in response to short-term changes in diet in controlled feeding experiments.

2. Tail hairs were sampled from six horses that had been equilibrated to C3 forage and were then subjected to a series of short-term diet switches to the C4 Coastal Bermuda Grass (*Cynodon dactylon* L.). Four of these horses were equilibrated to Alfalfa (*Medicago sativa* L.) and were then subjected to 1-, 3- and 7-day diet spikes of the C4 grass. The remaining two horses were equilibrated to a C3 grass mix (*Dactylis glomerata* L. and *Festuca arundinacea* Schreb.) and then subjected to a 7-day diet spike of C4 grass.

3. The effects of the short-term diet switches were easily observable in the hair. The 1-, 3- and 7-day spikes showed an increasing deviation from the prespike equilibrium value of 1·0‰, 2·9‰ and 5·6‰ (7-day treatments averaged).

4. Isotopic chronologies of individual hairs were created and plotted against a three-pool, exponential-decay model. With small alterations to the original model parameters, our data are well explained by this model.

5. This study indicates that information about diet is recorded with high resolution in hair. This method could be applied to both modern and ancient samples, greatly enhancing the temporal resolution of diet reconstruction studies.

Key-words: C3–C4, diet reconstruction, isotope chronology

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Introduction

A common problem faced in animal ecology is the difficulty of observing or accurately reconstructing responses of animals to ecological events in the field. This is especially the case with rare and endangered species and animals with temporal habits or habitats that make observation difficult. Ideally, one would be able to collect a detailed record of a given animal's past diet, movements and nutritional status, in a non-destructive manner and without time-consuming and potentially biased observational studies. Stable isotope analyses of animal tissues have been regarded as a potential solution to this problem (Gannes, Martinez & Koch 1998). Isotopic techniques have been applied to animal ecology for decades to characterize diet (Ambrose & Deniro 1986; Deniro & Epstein 1978; Tieszen *et al*. 1983), trace large-scale movements (Hobson 1999) and assess general nutritional condition (Hobson, Alisauskas & Clark 1993; Schell & Saupe 1993). Typically, previous studies have examined large-scale or long-term patterns such as migration on a continental scale or diet over an entire season or lifetime. Recently there have been studies indicating that there is hope for extracting detailed chronological information from biological archives such as feathers, baleen, tooth enamel and hair (Ambrose & Deniro 1986; Best & Schell 1996; Cerling *et al*. 1997; Chamberlain *et al*. 1997; Hobson & Schell 1998; Macko *et al*. 1999a; Cherel, Hobson & Weimerskirch 2000; Hobson, McLellan & Woods 2000; Schoeninger & Bada 2001; Bol & Pflieger 2002; Passey & Cerling 2002; Schwertl, Auerswald & Schnyder 2003). However, the goal of finding a high-resolution (daily) archive has not yet been realised. In this paper we aim to demonstrate that hair may well be such an archive.

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Previous studies have shown that hair is a chronological, biological archive of ecological, physiological and geographical information that can be interpreted through isotopic analyses (Jones *et al*. 1981; Schoeninger, Iwaniec & Nash 1998; Macko *et al*. 1999a; O'Connell & Hedges 1999; Bol & Pflieger 2002; Schwertl *et al*. 2003). Hair is composed of a protein complex (keratin) formed from amino acids that are derived from both exogenous (diet, environmental water) and endogenous sources (metabolic turnover of endogenous tissues). The isotopic composition of hair thus potentially provides information on the animal's diet ($\delta^{13}C$, $\delta^{15}N$), nutritional status ($\delta^{15}N$) and location and movements ($\delta^{13}C$, $\delta^{18}O$, δD, $\delta^{34}S$, $\delta^{87}Sr$). Hair is particularly suited to isotopic studies. Hair growth is fast (van Scott, Ekel & Auerbach 1963) and generally continuous (in the anagen phase) and once formed is biologically inactive and resistant to degradation (Macko *et al*. 1999a). This allows isotopic chronologies to be established, limited only by the length of the hair. Such analyses can be performed for both modern and ancient samples (Macko *et al*. 1999b; O'Connell & Hedges 1999; White, Longstaffe & Law 1999; Bol & Pflieger 2002).

The isotopic composition of hair has been used to reconstruct diet in a variety of taxa (Schoeninger *et al*. 1998; Macko *et al*. 1999a; O'Connell & Hedges 1999; White *et al*. 1999; Hobson *et al*. 2000; Schoeninger & Bada 2001). These studies examined the isotopic values of hair samples with low temporal resolution, focusing on long-term changes in diet and seasonality. There have been no studies investigating the $\delta^{13}C$ patterns in hair relating to rapid changes (in the order of days) between food sources. Work at this scale is needed if we hope to be able to use hair analyses as a surrogate for observational studies.

Recently, progress has been made on understanding the isotopic physiology of hair formation, albeit not for short-term changes in diet. After an initial paper reporting the slow approach to equilibrium after a diet change (Jones *et al*. 1981), Ayliffe *et al*. (2004) conducted a long-term, controlled feeding trial with horses. They showed that the change in $\delta^{13}C$ of hair can be explained by a three-pool, exponential-decay model. The three pools represent contributions of amino acids from different sources to hair synthesis at the current time. These three pools can be described as (1) a fast pool, reflecting amino acids sourced directly from the diet, with a half-life of less than a day (~12 h), (2) a fast pool reflecting amino acids derived from biosynthesis or the breakdown products of metabolic proteins, with a half-life of several days (~4 days) and (3) a slow pool representing amino acids contributed from the breakdown of structural proteins, with a half-life of months (~136 days). The modelled proportions of these pools indicate that roughly half the amino acids incorporated into the hair are derived from the two fast pools and half from the slow pool (Ayliffe *et al*. 2004). This leads to an attenuation of the diet signal recorded in the hair that decreases as the pools approach equilibrium with the diet. The fast pools reach equilibrium rapidly but the slow pool only attains equilibrium after many months. Thus, although a change in the $\delta^{13}C$ of the hair can be detected rapidly after a diet change, the time to full equilibration is in the order of many months. This attenuation of the diet signal leads one to ask whether high-resolution dietary information (i.e. short-term changes in diet) can be obtained from hair.

In this paper we examine the sensitivity of hair $\delta^{13}C$ analyses in detecting short-term changes in diet. We aim to: (1) determine whether short-term diet changes (in the order of days) are detectable with high-resolution sampling along a single hair to create isotopic chronologies and (2) test the model proposed by Ayliffe *et al*. (2004) to see if it accurately predicts our data. Sampling on the scale of a day of hair growth provides us with the opportunity to examine the physiological processes involved in hair formation that have hitherto been unavailable. In addition, understanding how the $\delta^{13}C$ in the hair changes with rapid changes in diet would indicate the potential detail that can be captured from the isotopic analysis of hair and could vastly enhance the detail of isotopic analyses of animal feeding ecology and behaviour.

Methods

EXPERIMENTAL DESIGN

Four horses that had been on a constant diet of Alfalfa (*Medicago sativa* L.) for a period of approximately 6 months were subjected to short-term changes in diet. The horses were switched from Alfalfa (a C3 dicot; –26·8‰; hereafter Alf) to a C4 grass, Coastal Bermuda Grass (*Cynodon dactylon* L.; –13·5‰; hereafter CBG) for periods of 1 day, 3 days or 7 days and then returned to the original alfalfa diet. Two horses were given discontinuous C4 spikes of 1 day and 3 days, and two horses were given a single 7-day spike (Table 1). In this manner two replicates per spike duration were obtained.

In a second experiment two horses that had been on a constant diet of Afalfa were placed on a C3 Grass Hay (a *Dactylis glomerata* L. and *Festuca arundinacea* Schreb. mix; –26·4‰; hereafter GH) for a period of 4 weeks. They were then subjected to a 7-day spike of CBG before being returned to GH for 35 days (Table 1). Thus, only the C3 feed used varied between the two experiments.

The horses were moved between paddocks that contained either Alfalfa, GH or CBG in order to efficiently administer the diet changes. All changes between paddocks were made at exactly the same time of day. Food and water were provided *ad libitum*.

Prior to the commencement of the diet switches, a patch of tail-hair was shaved to the skin. Hair was sampled from this shaved patch and from adjacent unshaved areas at the completion of the diet switches. Previous studies have suggested that there is no change in growth rate associated with shaving (Lynfield &

Table 1. The type and duration of the diets for the horses in this study

	Horse					
	SQ	PR	TS	NS	BY	CL
Prior diet	Alf (6 months)	Alf (6 months)	Alf (6 months)	Alf (6 months)	GH (4 weeks)	GH (4 weeks)
Switch 1	1-day CBG 8 days Alf	3-days CBG 16 days Alf	7-days CBG 21 days Alf	7-days CBG 21 days Alf	7-days CBG 35 days GH	7-days CBG 35 days GH
Switch 2	3-days CBG 16 days Alf	1-day CBG 8 days Alf				
Experiment duration	28 days	28 days	28 days	28 days	42 days	42 days

Alf, Alfalfa (*Medicago sativa* L.): C3.
GH, Grass Hay (*Dactylis glomerata* L. and *Festuca arundinacea* Schreb.): C3.
CBG, Coastal Bermuda Grass (*Cynodon dactylon* L.): C4.

Macwilliams 1970). The hair was plucked from the tail using forceps so that the root was obtained. Only hairs in the anagen growth phase, as determined from the condition of the root (Harding & Rogers 1999), were used for analysis.

SAMPLE PREPARATION AND ANALYSIS

Individual hairs were repeatedly wiped with 95% ethanol and allowed to dry. The density of the hair was determined and the length required to obtain sufficient mass for isotopic analysis was calculated. The hair was then sectioned using a razor blade and the length of each section was measured with digital callipers. Sections were then weighed on a microbalance and loaded into tin capsules for continuous flow isotopic analysis. At least two hairs from each animal were analysed (Table 3).

Samples were analysed for $\delta^{13}C$ in a Carlo Erba elemental analyser (EA 1108, Milan, Italy) coupled with a Delta-S continuous-flow isotope ratio-mass spectrometer (Finnigan Mat, Bremen, Germany). By turning off the dilution of CO_2 in the elemental analyser, samples as small as 25 μg could be analyzed for $\delta^{13}C$.

Isotope ratios are expressed in ‰ as:

$$\delta^{13}C = (R_{sample}/R_{standard} - 1) \times 1000,$$

where R is the ratio of the heavy to light isotope ($^{13}C/^{12}C$). The δ-values are reported relative to the international standard of PDB.

DATA ANALYSIS

Scaling

For the purposes of our study it was desirous to scale the isotope measurements from length (mm along hair) to time (days since diet switch). The isotope measurement of a particular hair segment represents an integrated signal of the variation expressed in that segment. Thus initially the $\delta^{13}C$ data were plotted against the midpoint of the segment for which the isotope measurement was made, added to the total length of the hair prior to that segment. Scaling from millimetres to days was done using a linear calibration taken from the known times of (1) the start of the C4 spike and (2) the end of the experiment.

Using a method for detecting peaks in gas chromatography, the beginning of the C4 spike was determined to have begun when the difference between the $\delta^{13}C$ of the current sample and the previous three-point mean was greater than two standard deviations of that mean. That sample was assumed to mark the commencement of the spike. We assigned the centre point of that segment the value of zero days plus a lag factor of 12 h. The lag factor accounts for the time taken for ingested food to be digested and laid down as keratin in the hair follicle. The lag factor was estimated based from the response of breath data to diet change (Ayliffe *et al*. 2004) and radioisotope work (Ryder 1958), indicating that it takes on the order of 12 h for a new diet to be incorporated into hair keratin. The actual commencement of the spike may have occurred at any time during the formation of that hair segment and not necessarily at the mid-point. Thus, there is a small error associated with our chronology calculated in this manner. We minimized this error by examining how well our predicted chronology measures up to what we would expect to observe. By comparing the calculated date for the end of the spike from our chronology, with the known date of the end of the spike, the chronology could be adjusted to obtain maximum accuracy. This was done by varying the assigned zero point within the segment that had been identified as the start of the peak and therefore did not violate any of the previous assumptions.

Root analysis

We tested whether the $\delta^{13}C$ of hair segments containing the root were significantly different from segments containing keratin only. The hairs selected were from the animals that had experienced a 7-day diet switch. Only the last five segments of the hair were used (the final segment containing the root) as by then the $\delta^{13}C$ of the hair had started to approach an equilibrium value with the diet.

Table 2. Model parameters for the three-pool exponential-decay model showing half-life and best-fit fractional contributions for three diet change scenarios. The first set of parameters are taken from Ayliffe *et al*. (2004). The second and third sets represent diet changes from Alf-CBG and from GH-CBG, respectively. The numbers and letters in the far right column refer to the panes in Figs 3 and 4 for which these parameters were used

	Pool 3	Pool 2	Pool 1	Figure
Half-life	0·5 days	4·3 days	136 days	
Fractional contribution (from Ayliffe *et al*. 2004)	0·41	0·15	0·44	3a,b; 4a,b
Fractional contribution (Alf–CBG)	0·33	0·17	0·50	3c; 4c,d
Fractional contribution (GH–CBG)	0·31	0·17	0·52	3d

Alf – Alfalfa (*Medicago sativa* L.): C3.
GH – Grass Hay (*Dactylis glomerata* L. and *Festuca arundinacea* Schreb.): C3.
CBG – Coastal Bermuda Grass (*Cynodon dactylon* L.): C4.

Model

The three-pool exponential-decay model proposed by Ayliffe *et al*. (2004) was fitted to our data. The model prediction was scaled to the sampling resolution by integrating the model output over the length of each segment in the same manner as occurs, *de facto*, for the isotope sampling. Initially we used Ayliffe *et al*.'s original parameters (Table 2) to examine how well these described our data. These parameters were then changed to achieve the best fit with our data (Table 2). The degree of model fit to the data was determined by least squares. In fitting the model, we limited ourselves to only modifying the proportions of the three pools. The half-lives of all three pools were interpreted as representing biochemical pathways that would not change substantially over the experiment and were thus held constant. We reasoned that the factor most likely to change between the experiments would be the amount and assortment of amino acids derived directly from the diet, a feature thought to be represented by Pool 3 (Ayliffe *et al*. 2004). We assumed that the ratio of amino acids resulting from the turnover of metabolically active tissues (Pool 2) and structural tissues (Pool 1) would remain constant. Although this is probably a vastly oversimplified approach, our present level of understanding does not permit greater sophistication. Further experimentation may well provide greater insight.

Results

There was exceptional congruence shown between the $\delta^{13}C$ of hair samples from the same animal as well as between animals on the same treatment (Figs 1 and 2). In all cases, the standard error of the $\delta^{13}C$ of hairs within a particular treatment was less than that associated with the isotopic measurement (± 0·1‰).

The $\delta^{13}C$ data are shown plotted against the derived chronologies (Figs 1 and 2). Growth rates associated with these chronologies were ~0·7 mm day^{-1} (Table 3). In all hairs sampled, a comparison between the calculated chronology and the expected chronology (based on the feeding schedule) indicated that there was no evidence of a substantial deviation from a linear growth rate throughout the duration of the study. There was also no detectable effect of shaving on hair growth rates. The C4 spikes observed in the hair chronologies vary with the duration of the change in diet (Figs 1 and 2). The longer the time spent on the new diet, the greater the magnitude and breadth of change. The 1-, 3- and 7-day spikes (from animals on the Alf–CBG–Alf diet) show an increasing deviation from the prespike equilibrium value by 1·0‰, 2·9‰ and 5·8‰, respectively (Fig. 1). A similar deviation of 5·3‰ was observed for the 7-day spike in the GH–CBG–GH experiment (Fig. 1).

Table 3. Mean growth rate (MGR) of tail-hair for all horses sampled in this study

Horse	MGR (mm day^{-1}) ± 1 SE	*n*
SQ	0·64 ± 0·02	5
PR	0·63 ± 0·03	5
TS	0·77 ± 0·16	2
NS	0·76 ± 0·01	2
BY	0·88 ± 0·00	2
CL	0·66 ± 0·00	2
Mean	0·72 ± 0·03	6

The three-pool, exponential-decay model (Ayliffe *et al*. 2004) was plotted against the data from the 7-day spikes (Fig. 3) and the double spikes (Fig. 4). This model with the original parameters (Table 2) seems to fit our observations fairly well. However, the model predicts a larger magnitude of change than seen in our observations (Figs 3a,b and 4a,b). By altering the proportions of the three pools (Table 2) a better fit is obtained (Figs 3c,d and 4c,d). With these changes in parameters, the model accurately predicts our observed values with the exception of the 3, 1-day double spike treatment (Fig. 4d). In this case, the model predicts spikes of a larger magnitude than measured.

The $\delta^{13}C$ of segments containing the root of the hair were significantly different from the $\delta^{13}C$ of segments containing only keratin (Table 4). These root segments were on average ~0·6‰ more depleted in $\delta^{13}C$ than keratin segments. The effect of including the root

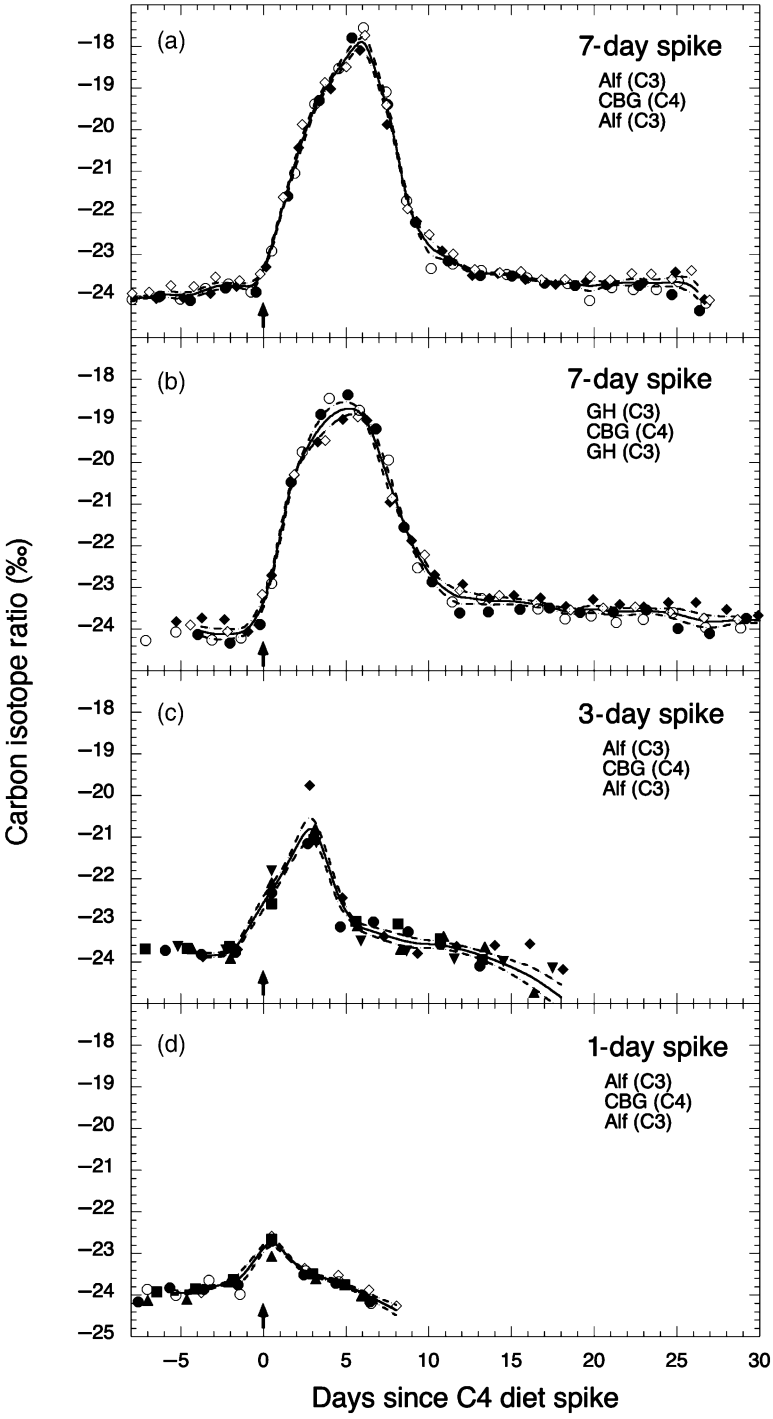

Fig. 1. Isotope chronologies for horses fed a C3 diet that was then changed to C4 for 7-day, 3-day and 1-day periods and then returned to C3. Two different C3 feeds, Grass Hay (GH) and Alfalfa (Alf), were used in the 7-day experiments. The C4 feed, Coastal Bermuda Grass (CBG), was common for all experiments. In each panel, data represent two animals with at least two replicate hairs per animal. The interpolated mean, with standard error is plotted through these data. Arrows on the *x*-axis indicate the start of the C4 spike.

segments in a chronology can be seen in the $\delta^{13}C$ chronology plots, with the interpolated mean showing a decline below the previously obtained equilibrium value due to the isotopically lighter root material (Figs 1 and 2).

Discussion

RESOLUTION

The effects of short-term changes between C3 and C4 diets can be easily observed in horse tail-hair. In fact changes in diet as short as 1-day can be detected (Figs 1 and 2). The $\delta^{13}C$ of the hair records an attenuated diet signal owing to the contribution of endogenously produced amino acids (Ayliffe *et al*. 2004). Despite this, short-term diet changes are clearly discernable. This indicates that diet reconstruction studies using hair have the potential to be extremely sensitive and of high resolution.

The factor limiting the maximum resolution obtainable from hair analyses appears to be analytical rather than biological. As a certain mass is needed for isotopic analyses, one is limited to the increment of hair that can be sampled. Current analytical restraints restricted us to the use of $\delta^{13}C$ only as it was not possible to obtain sufficient sample mass for $\delta^{15}N$ analyses at the desired sampling resolution (~1 mm increments). Combining multiple hairs would potentially increase the resolution, however, this is undesirable as differing growth rates of individual hairs, even within an animal (Table 3), makes it nearly impossible to be confident of sampling identical time periods on both hairs. Thus the resolution obtainable hinges on the growth rate and density of the hair in question, with thick, fast growing hair providing the greatest temporal resolution.

Segments containing the root are consistently depleted relative to their expected value (Table 4). This is consistent with an interpretation of root material containing more lipids (in blood, sebaceous secretions, actively dividing cells) than found in keratin as lipids are isotopically depleted relative to proteins (Deniro & Epstein 1977). Failing to take this into account when examining hair chronologies could lead to erroneous interpretations.

The double spikes (Figs 2 and 4) show the potential utility of extracting dietary information from a more complex chronology. It seems reasonable to assume that any field-based studies would encounter chronologies with a multitude of spikes rather than the simple example of an equilibrium situation. Using the three-pool exponential decay model of Ayliffe *et al*. (2004) and only changing the isotopic values of the feeds (information which would be readily available from the field), we were able to predict the $\delta^{13}C$ values measured in the hair with reasonable accuracy. The only major deviation from the model prediction is in the first 3-day spike (Fig. 4d). In this case the measured values are much lower than the predicted values. Slight errors in scaling the chronology, variation in food intake and physiology of the animal in question are all potential reasons for this departure and are likely to represent the minimum level of noise in field-based studies of this nature.

Our data support the use of this application in examining changes in diet where there is a large disparity

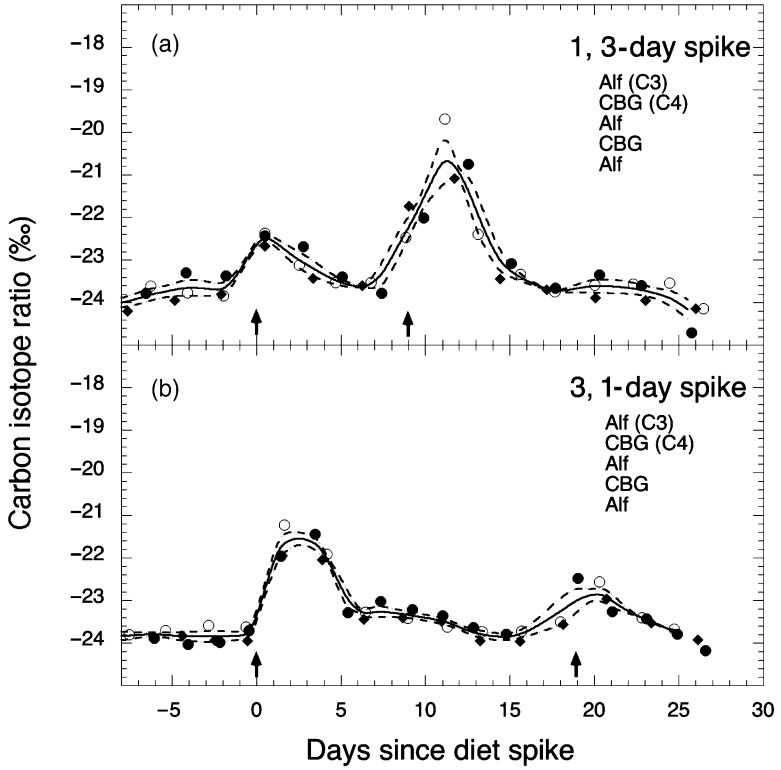

Fig. 2. Isotope chronologies for horses fed a C3 (Alfalfa) diet and then given two discontinuous C4 (Coastal Bermuda Grass, CBG) diet spikes 1 and 3 days in duration (although in different sequence). In each panel, data represent three replicate hairs from one animal. The interpolated mean, with standard error is plotted through these data. Arrows on the *x*-axis indicate the start of the C4 spikes.

in $\delta^{13}C$ between the diets (i.e. between C3 and C4 plants). The ability to detect changes in diet diminishes as the difference between the $\delta^{13}C$ of the two diets approaches zero. However, it is likely that additional isotopes (δD, $\delta^{18}O$, $\delta^{15}N$) could soon be used to resolve difference between other forages or water sources, which would vastly expand the applicability of this method.

MODELLING THE SPIKE

The three-pool, exponential-decay model (Ayliffe *et al.* 2004) describes our data well. The model, with the original parameters, accurately predicts the timing of our spikes but fails to predict the magnitude of change as accurately (Figs 3 and 4). The data from the two 7-day spike experiments indicate that there is a range in the magnitude of response but not in the duration or timing of the spike (Fig. 3). Ayliffe *et al.*'s model was developed from a similar experiment (a C3–C4 diet switch on horses) using the same C4 feed as in this study (CBG) but a different C3 feed (Orchard Meadow Brome, *Bromus inermis* Leyss.). Thus perhaps it is unsurprising that the exact parameters from this study do not completely describe our data. Altering the proportions of the three pools slightly (Table 2), but retaining the half-lives, results in a much closer prediction of our data. We feel this is additional evidence for the utility of this model.

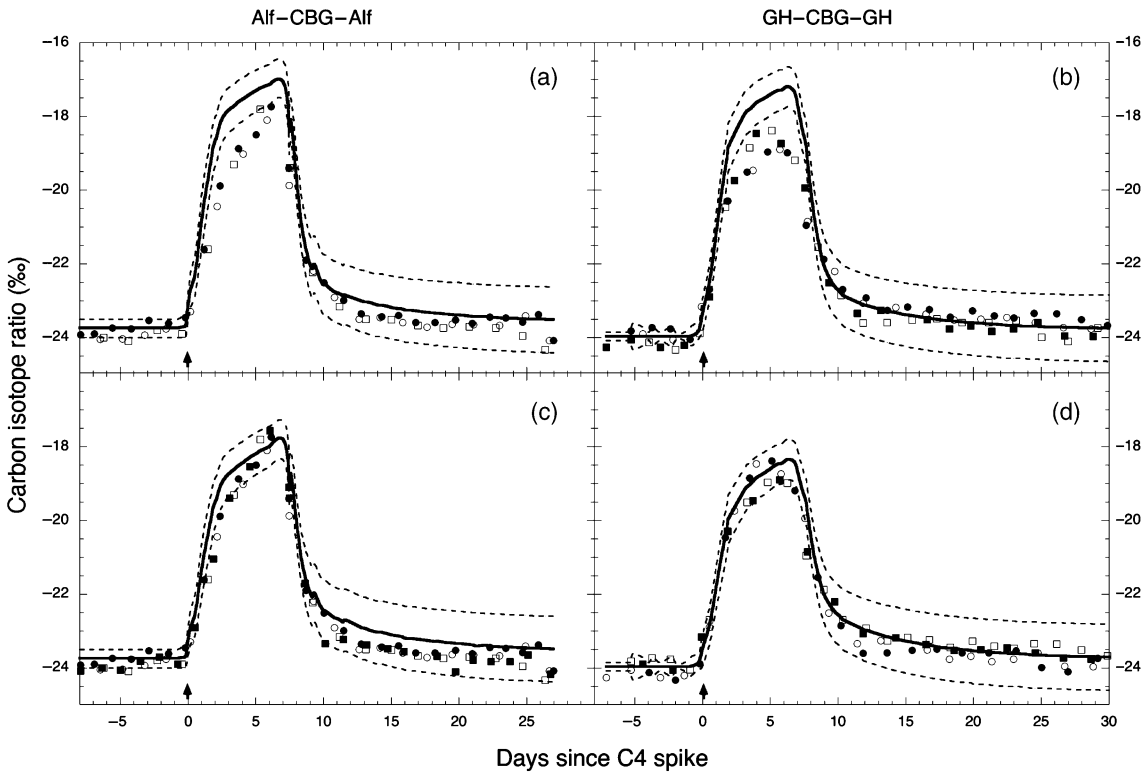

Fig. 3. Model predictions (solid lines, with error in broken lines) plotted with measured data for the two 7-day experiments. The original parameters from (Ayliffe *et al.* 2004) were used in the model for panels (a) and (b). Panels (c) and (d) represent the best fit achieved by modifying the fraction of the fastest turnover pool (pool 3). Model parameters are reported in Table 2.

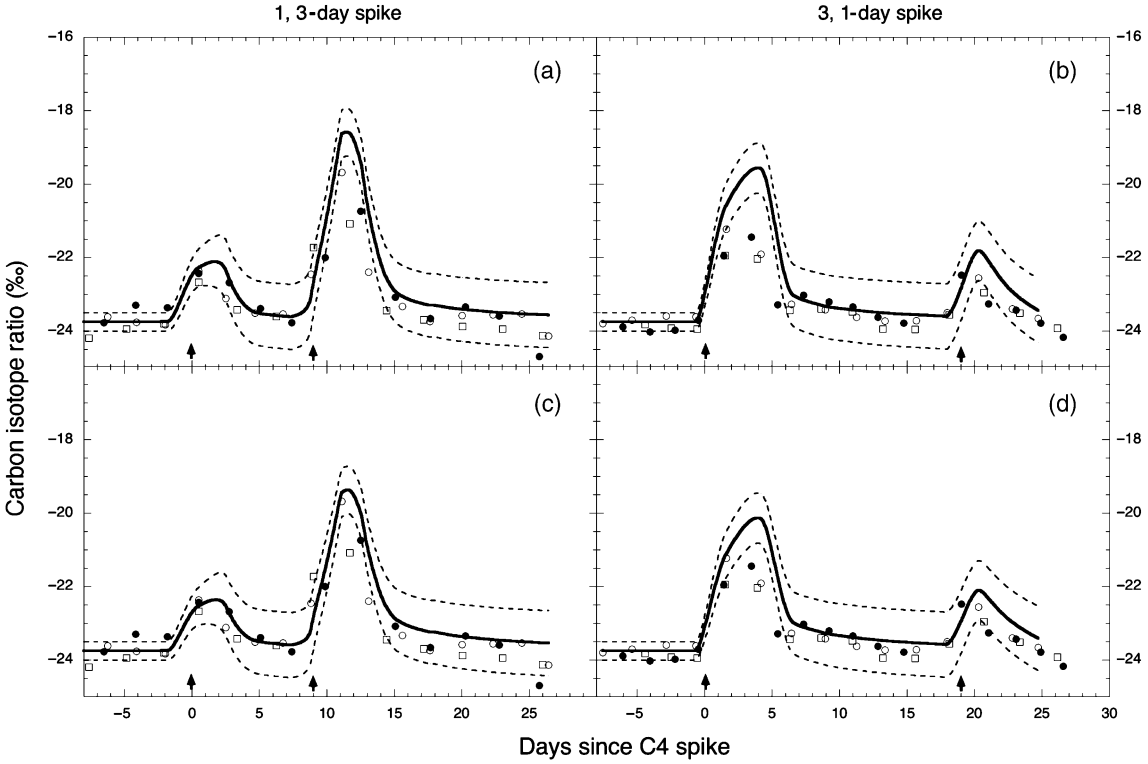

Fig. 4. Model predictions (solid lines, with error in broken lines) plotted with measured data for the two double spike experiments. The original parameters from (Ayliffe *et al*. 2004) were used in the model for panels (a) and (b). Panels (c) and (d) represent the best fit achieved by modifying the fraction of the fastest turnover pool (pool 3). Model parameters are reported in Table 2.

Table 4. $\delta^{13}C$ values and results of paired *t*-tests for the root segment (Root) and the four preceding segments (R-4 to R-1) on all hairs analysed from the 7-day spike animals. The difference from the root segment and the preceding segment is highlighted in the last column

Horse	R-4	R-3	R-2	R-1	Root	(R-1) – Root
TS 1	–23·64	–23·65	–23·67	–23·41	–24·08	0·67
TS 2	–23·45	–23·47	–23·58	–23·38	–24·09	0·71
NS 1	–23·74	–23·71	–23·74	–23·96	–24·34	0·38
NS 2	–23·79	–23·83	–23·83	–23·65	–24·17	0·52
BY 1	–23·93	–24·03	–24·06	–24·22	–24·69	0·47
BY 2	–23·81	–23·79	–24·00	–23·98	–24·45	0·48
CL 1	–23·93	–23·89	–23·78	–23·91	–24·53	0·62
CL 2	–23·77	–23·87	–23·88	–23·85	–24·52	0·67
Mean root difference						0·57
Paired *t*-test[a]	R-4 *vs* R-3	R-3 *vs* R-2	R-2 *vs* R-1	R-1 *vs* Root		
P <	0·2828	0·2801	0·7010	0·0000		
T =	1·163	1·171	–0·400	13·48		
d.f.	7	7	7	7		

[a]The only significant difference was found between segments R-1 and Root.

FACTORS AFFECTING THE SPIKE MAGNITUDE

The observation that the magnitude of the spike changes in different experiments warrants further discussion. In this study we observed different spike magnitudes in the two 7-day experiments (Figs 1 and 3). Ayliffe *et al*. (2004) also observed different magnitudes of response between spikes in their study. All of these experiments had a common C4 diet but different C3 diets prior to the C4 spike. Thus it seems as if the previous diet may be affecting the degree of response to the new feed.

A variety of factors could be responsible for this observed variation. Factors such as the quality of the feed, the amount of intake of the new diet and the nutritional status of the animal might all affect the response to the new diet. Normal hair growth rates appear to be near maximal (Chase 1958; van Scott

et al. 1963) and do not appear to change, consistently, with variations in nutrition (Galbraith 2000) or semi-starvation (Loewenthal 1956). Observable changes only seem to occur under extreme conditions of starvation (Bradfield 1979). Thus it seems that the absolute amount of amino acids supplied to the follicle remains constant regardless of diet. However, as these amino acids are derived from both endogenous tissue breakdown and ingested exogenous proteins, it is probable that the proportions of these sources could change with diet. One could predict that a higher-quality feed (higher in the appropriate amino acids) would be incorporated to a greater extent into the newly forming hair than a low-quality feed, where endogenous tissue breakdown products would comprise a relatively greater proportion of the amino acids available for hair synthesis. A similar effect may be seen in animals that do not have sufficient food intake, or food of sufficient quality, to meet their nutritional requirements. One would expect to see a greater proportion of endogenous tissue turnover in the hair of these animals, attenuating the spike. This would also be the case for an animal with a lower absolute intake relative to a neighbour. This may account for the variation seen between animals within the same experiment (Fig. 1) but unfortunately this was not controlled for in this study. At present we cannot discern between the effects of the above factors. This is the subject of ongoing research.

Conclusions

Changes in diet as short as 1 day can be observed using fine-scale sampling (~1 mm increments) of horse tail-hair. This result indicates that diet reconstruction studies using hair have the potential to be extremely sensitive and of high resolution. Currently this methodology has only been tested with $\delta^{13}C$ due to the analytical constraints of the fine-scale sampling resolution. The data we present here support the use of this application in examining changes in diet between C3 and C4 plants and in controlled-feeding studies. Future applications could include field studies in areas with an abundance of tropical grasses, studies of animals with access to a wide range of food types (migrants, humans) as well as controlled physiological studies. Additional isotopes (δD, $\delta^{18}O$, $\delta^{15}N$) could soon be used to resolve difference between other forages or water sources, which would vastly expand the scope of this method. The short-term changes were accurately described by a three-pool exponential-decay model (Ayliffe *et al*. 2004). Variation observed in the magnitude of the spikes potentially contains information relating to diet quality, relative food intake and nutritional status of the animal and should be the subject of further investigation.

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