An experimental study of nitrogen flux in llamas: is ^{14}N preferentially excreted?

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Abstract

Nitrogen isotope analysis is now commonly used to investigate the diets, and to a lesser extent, the environments of ancient populations. These studies assume that mammals are predictably enriched in ^{15}N over their food, and concomitantly, that ^{15}N becomes increasingly concentrated as one moves up the food chain. The literature commonly states that this ^{15}N-enrichment of mammalian tissues is due to preferential excretion of light nitrogen (^{14}N), but there are few data to support this assertion. To address the gap, we conducted two nitrogen flux trials in which four llamas (*Lama glama*) were fed high- and low-protein diets. The ratios of fecal nitrogen loss to urinary nitrogen loss were 0.30 and 0.88 on the high- and low-protein diets respectively. Feces were enriched in ^{15}N by approximately +3‰ on both diets, whereas urinary nitrogen was depleted in ^{15}N (-2.1‰) on the low-protein diet, but not significantly different from intake δ^{15}N on the high-protein diet. Most importantly, there was no statistically significant difference between dietary and total excreta δ^{15}N on either diet. Given these data and theoretical considerations, we argue that the nitrogen influx and efflux of adult mammals at steady state should be isotopically commensurate. However, during growth, diet change, thermal or nutritional stress, animals may not be at steady state and fractionation between intake and excreta δ^{15}N may occur.
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1. Introduction

Nitrogen isotope analysis has become a common tool for investigating the diets of ancient populations (e.g. [2,4,6,7,16,19,34,38–40,42,53]). Previous applications include determining the degree of carnivory by neanderthals [4,34], a population's relative dependence on marine and terrestrial resources [33,38,43,49], and even ancient weaning practices [6,7,16,39]. The success of such studies is predicated on knowing the fractionation that occurs between dietary and tissue δ^{15}N, which is widely believed to be about +3‰ [13,15,24,35,37].

There is evidence, however, that mammalian diet–tissue fractionation can be quite variable. For instance, a recent study showed that diet–hair spacing can range between +2.7‰ and +6.1‰ for mammals on identical diets [44], and several researchers have hypothesized that diet–collagen fractionation can be increased by thermal [1,2] or nutritional stress [14,42]. Unfortunately, the mechanisms that account for this variability are poorly known, thus complicating interpretation of archaeological δ^{15}N data. Despite this, there has been little experimental research into the factors that influence diet–tissue fractionation of ^{15}N, with a few significant exceptions [3,9,11,14,20].

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Table 1
Composition (dry matter) of the alfalfa (*M. sativa*) and
bermudagrass (*C. dactylon*) used in this study

	Alfalfa	Bermudagrass
Crude protein (%)	20.6	10.3
Neutral detergent fiber (%)	45.5	62.3
Acid detergent fiber (%)	36.6	28.6
Lignin (%)	6.5	5.4
Non-structural carbohydrate (%)	27.2	16.4
Acid detergent insoluble CP (%)	1.0	0.9
Crude fat (%)	3.7	1.6
Ash (%)	10.8	10.3
Digestibility (%)	69	60
$\delta^{15}N$ (‰)	0.4	5.8
C/N ratio	15.8	28.2

It is often stated that preferential excretion of ^{14}N is responsible for, or a consequence of, the ^{15}N-enrichment of mammalian tissues (e.g. [1,2,10,17,18,31,47]). There are a number of mechanisms that could lead to preferential excretion of ^{14}N: faster removal of ^{14}N-containing amine groups during deamination and transamination of amino acids is a leading candidate [9,20], however the fractionation might also occur during synthesis of carbamoyl phosphate and in the urea cycle [2,47]. Despite the near consensus in the literature that ^{14}N is preferentially excreted, there exist few, if any data to support this claim. Although mammalian urinary $\delta^{15}N$ is usually depleted compared to diet, fecal $\delta^{15}N$ is consistently enriched in ^{15}N [46–48]. The literature has tended to discount the ^{15}N-enrichment of feces as insignificant, as fecal nitrogen losses have been considered to be small [1,2,12,46]. Yet, fecal nitrogen loss can constitute 50% of total nitrogen efflux, particularly for herbivores on low-protein diets (e.g. [8,23,27,50]). Thus, we cannot be certain that when both fecal and urinary $\delta^{15}N$ are considered, excreta $\delta^{15}N$ is depleted in ^{15}N compared to diet.

As a result of this uncertainty, we decided to test the hypothesis that nitrogen influx and efflux have different $\delta^{15}N$ values. To this end, we conducted nitrogen flux trials with llamas (*Lama glama*) on high- and low-protein feeds. To our knowledge, this is the first study to present data on mammalian urinary and fecal nitrogen losses as well as the $\delta^{15}N$ of total excreta. Admittedly, one study with pseudoruminant llamas may not settle the question for mammals in general, particularly those with other digestive physiologies, but it should nevertheless prove a reasonable start towards that end.

2. Materials and methods

Four adult male llamas were used for two trials. In the first trial they were fed high-protein alfalfa hay (*Medicago sativa*, 20.6% crude protein; Table 1), and in the second they were fed low-protein coastal bermudagrass (*Cynodon dactylon*, 10.3% crude protein; Table 1). The animals were owned, housed, and fed under the auspices of the Stable Isotope Biology (SIB) Project, which is jointly run by Brigham Young University and University of Utah. We followed standard procedures for dietary studies with large animals (e.g. [25,36]). Both feeds were shredded to limit selectivity, and provided ad libitum at 12 h intervals. In order to ensure proper acclimation, the llamas were put on the experimental diets at least 21 days prior to the studies, and placed in individual metabolic crates with fecal collection bags and urine collectors five days before the trials commenced. After the acclimation periods, feces and urine were collected for five days. Urine was collected in a container with a small amount of dilute sulfuric acid to prevent ammonia loss. Urine volume and feces weight were recorded daily. Feces were dried in a forced convection oven at 60 °C for 48 h, weighed again, and homogenized. Urine was freeze-dried at −45 °C for 48 h. The nitrogen content of urine, feces and feeds was determined by Kjeldahl analysis. Although nitrogen is lost in hair and sloughed skin, these losses are extremely small compared to urinary and fecal nitrogen efflux, and were therefore not used in nitrogen balance calculations.

Homogenized urine and fecal samples for each individual were then combusted in an elemental analyzer (Carlo Erba Instruments, Milan, Italy) and nitrogen isotopes were measured using a flow-through inlet system coupled to a continuous flow isotope ratio mass spectrometer (Finnigan, Bremen, Germany). The standard deviation of replicate measurements of an internal standard was <0.3‰ for all runs. We used analysis of variance to test for differences between intake $\delta^{15}N$, urine $\delta^{15}N$, fecal $\delta^{15}N$, and total excreta $\delta^{15}N$ on both feeds. We also tested for differences in the ratios of fecal N loss to urinary N loss ratios (FN/UN), nitrogen balance (NB), diet–feces fractionation (ε_{d-f}), diet–urine fractionation (ε_{d-u}), and diet to total excreta fractionation (ε_{d-e}) (see Table 2 for definition of ε).

3. Results

Data from both trials are presented in Table 2. The llamas were in positive nitrogen balance on both diets excepting one individual on the bermudagrass. Even with this anomaly, however, nitrogen balance was not significantly different on the two feeds (P=0.23). In contrast, total nitrogen influx and total nitrogen efflux were over twice as high on the alfalfa diet (P<0.01), demonstrating that alfalfa crude protein levels far exceeded llama protein requirements. Hence, from the perspective of nitrogen balance, these two diets were nutritionally similar, or at least not as distinct as their crude protein levels would suggest.

Table 2
Daily nitrogen balance and δ^{15}N data for llamas in this study. Fractionation is expressed here as ε values $(((1000+\delta^{15}\text{N}_{\text{urine/feces/excreta}})/(1000+\delta^{15}\text{N}_{\text{diet}})-1)*1000)$, which are sometimes, but not always equivalent to Δ values (see [5]). Mean data are also provided for a pilot study of horses [45] and sheep [48]

	Intake δ^{15}N (‰)	Fecal δ^{15}N (‰)	Urinary δ^{15}N (‰)	Nitrogen intake (g/day)	Nitrogen feces (g/day)	Nitrogen urine (g/day)	Fecal N/urine N	Nitrogen balance (g/day)	Excreta δ^{15}N (‰)	ε diet-feces (‰)	ε diet-urine (‰)	ε diet-excreta (‰)
Medicago diet												
Jaxson	0.4	3.4	−0.2	51.9	8.9	34.4	0.26	8.6	0.5	3.0	−0.6	0.1
Patch	0.4	3.5	−0.2	53.4	10.1	36.0	0.28	7.4	0.6	3.1	−0.6	0.2
Slingshot	0.4	2.9	0.6	40.3	8.2	23.2	0.36	8.9	1.2	2.5	0.2	0.8
Theo	0.4	3.3	0.1	52.9	10.8	33.8	0.32	8.3	0.9	2.9	−0.3	0.5
Mean	**0.4**	**3.3**	**0.1**	**49.6**	**9.5**	**31.8**	**0.30**	**8.3**	**0.8**	**2.9**	**−0.3**	**0.4**
SD		0.3	0.4	6.2	1.2	5.8	0.04	0.7	0.3	0.3	0.4	0.3
Cynodon diet												
Jaxson	5.8	9.0	3.5	18.4	7.9	11.4	0.69	−0.9	5.7	3.2	−2.3	−0.1
Patch	5.8	8.3	3.7	25.4	9.1	11.1	0.82	5.3	5.7	2.4	−2.1	−0.1
Slingshot	5.8	9.2	3.9	25.2	8.7	7.3	1.19	9.3	6.8	3.4	−1.9	1.0
Theo	5.8	8.9	3.9	25.7	8.2	9.9	0.83	7.5	6.1	3.0	−1.9	0.3
Mean	**5.8**	**8.8**	**3.7**	**23.7**	**8.5**	**9.9**	**0.88**	**5.3**	**6.1**	**3.0**	**−2.1**	**0.3**
SD		0.4	0.2	3.5	0.5	1.9	0.22	4.5	0.5	0.4	0.2	0.5
E. caballus												
Medicago diet	0.4	3.0	0.3	309.4	78.0	200.4	0.39	31.0	1.1	2.6	−0.1	0.7
Cynodon diet	5.8	9.1	3.8	155.3	57.9	51.5	1.12	45.9	6.6	3.3	−2.0	0.8
Sutoh et al. [48]												
O. aries												
Medicago diet	0.8	3.8	0.6	36.7	9.0	21.8	0.4	5.8	1.6	3.0	−0.1	0.8

3.1. Fecal nitrogen/urinary nitrogen

Fecal nitrogen efflux was not significantly different on the two diets (8.5–9.5 g/d) (P=0.15). Urinary nitrogen loss was very similar to fecal loss on the bermudagrass (9.9 g/d), but increased by over three times on the alfalfa diet (31.8 g/d) (P<0.01). Hence, almost all of the alfalfa's excess nitrogen was lost in urine. As a result, the mean FN/UN ratios for the alfalfa and bermudagrass diets were significantly different (P<0.01; Table 2), with feces accounting for 46% of total nitrogen efflux on the low-protein coastal bermudagrass, but only 23% on the high-protein alfalfa. This suggests that dietary protein levels exert a strong influence on the relative proportions of nitrogen lost in feces and urine.

3.2. Excreted $\delta^{15}N$

Feces were enriched in ^{15}N over diet by about +3‰ on both feeds (P=0.87). Diet to urine fractionation (ε_{d-u}), in contrast, differed on the two feeds (P<0.01). There was no significant fractionation between diet and urine $\delta^{15}N$ for the alfalfa diet (P=0.58), although the urinary mean was slightly depleted (-0.3‰). On the bermudagrass, however, urine was highly depleted in ^{15}N (-2.1‰) relative to diet (P<0.01). Most importantly, total excreta $\delta^{15}N$ ($\delta^{15}N$ of urine and feces adjusted for their percentages of nitrogen efflux) was not significantly different from the $\delta^{15}N$ values of the alfalfa (P=0.43) or bermudagrass (P=0.30) hays. The reason for this was that fecal and urinary $\delta^{15}N$ and flux were counterbalanced. For instance, although diet–urine fractionation was more negative on the bermudagrass, it was offset by a relatively greater amount ^{15}N-enriched of fecal nitrogen efflux.

4. Discussion

These data challenge several assumptions found in the nitrogen isotope literature. For instance, they show that herbivore fecal nitrogen loss can make up nearly 50% of total nitrogen efflux on low-quality diets. This result is consistent with other studies of varied taxa [8,23,27,51], including a recent pilot study of horses (*Equus caballus*) in which fecal nitrogen loss made up 53% of total nitrogen efflux on a low-protein bermuda-grass hay diet [45] (Table 2). Most herbivore fecal nitrogen is not of dietary origin, but rather derived from sloughed endogenous tissues and microbial cell walls [50]. Endogenous tissues and gut microfauna are enriched in ^{15}N compared to diet [46], which likely explains the +3.0‰ fecal enrichment observed in this study. This is supported by preliminary data showing that feces treated with acid detergent to remove sloughed tissues

and microbes become depleted by -3‰ to -5‰ [45]. Therefore, it is unlikely that fecal ^{15}N-enrichment is due to selective removal of lighter nitrogen during digestion and assimilation (see [32]). Studies of cattle, sheep, goats, pigs, and locusts have also found that fecal $\delta^{15}N$ is enriched compared to dietary values [46–48,52], which suggests it is a nearly universal phenomenon (but see [11]). Exceptions are most likely to be found when animals are fed synthesized diets that have components with highly different $\delta^{15}N$ values and digestibilities.

Unlike the fecal results, diet–urine fractionation was different on the two diets. Diet–urine fractionation is also known to be variable in cattle, ranging from -1‰ to -5‰ [2,46]. The diet–urine fractionation observed here for llamas on bermudagrass (-2.2‰) was consistent with these findings, but the small, statistically insignificant fractionation on alfalfa (-0.3‰) was not. Although this latter result is unusual, it is not unique. Sutoh et al. (1993) showed that sheep (*Ovis aries*) fed alfalfa diets had nearly identical urinary and dietary $\delta^{15}N$ (Table 2). In tandem, these results strongly suggest that some intrinsic property of alfalfa hay was responsible for the lack of diet–urine fractionation. Alfalfa is a legume with more nitrogen and less fiber than grass hays [25,28,29]. Its high nitrogen content considerably exceeds the nitrogen requirements of most large herbivores [28,29], and as a result, much of its nitrogen must be purged to prevent ammonia toxicity. Grass hays such as the bermudagrass used here, however, barely exceed minimum nitrogen requirements for most herbivores, necessitating more complete utilization of dietary nitrogen (Table 1) [28,29]. It is probable that this differential utilization of dietary nitrogen by mammals on alfalfa and grass hays is responsible for the observed differences in diet–urine fractionation—perhaps because excreted dietary nitrogen is so much more common on the former that it "swamps" nitrogen derived from endogenous sources. Future tests of herbivores on homogenous, non-synthesized diets with differing protein contents will be necessary to test this hypothesis.

This study's most notable finding was that there were no significant differences between dietary and excretory $\delta^{15}N$ values on either diet, despite a large literature arguing that preferential excretion of ^{14}N leads to a relative enrichment of ^{15}N in tissues (e.g. [1,2,10,17,18,31,47]). It might be argued that this un-anticipated finding is an artifact of the study design. Animals in this study (and in most nitrogen balance studies) were placed on each test diet only 21 days before data collection. As a result, not all of their tissues were fully equilibrated with the test diets [49], and since body tissues (particularly those of the digestive tract) are an important component of nitrogen efflux, this could have impacted our calculated excreta $\delta^{15}N$. However, the fact that we obtained virtually identical results for two different diets with such highly distinct $\delta^{15}N$

values militates against this being a significant problem. Furthermore, there are other data that support our observation. Although Sutoh et al. [48] never calculated total excreta $\delta^{15}N$, they provided sufficient data for us to do so, and as in our study, the excreta of their sheep were not depleted in ^{15}N, but rather slightly enriched (+0.8‰) compared to diet (Table 1). Likewise, a recent pilot study of horses consuming alfalfa and bermuda-grass hay found that total excreta were not depleted in ^{15}N compared to either diet (Table 2). This is what we would expect for mammals at steady state. Adult mammals at steady state have tissues that are already enriched in ^{15}N relative to their diets [3,11,13,47]. To maintain the status quo, $\delta^{15}N$ of nitrogen influx and efflux must be the same according to the principles of mass balance [12,32]. Thus, if a mammal's excretions are ^{15}N-depleted its tissues must become correspondingly enriched. If this state of disequilibrium were to continue, the animal would become more and more enriched in ^{15}N throughout its life. Several studies have demonstrated that $\delta^{15}N$ does not usually increase in this manner [3,24,26,47,52], although there are possible exceptions [3,30,41]. Consequently, our finding that the $\delta^{15}N$ values of intake and excreta are not significantly different conforms to theoretical expectations of animals at steady state. Ironically, though, because our llamas were in positive nitrogen balance and thus not at steady state, their tissue $\delta^{15}N$ was probably changing despite similar influx and efflux $\delta^{15}N$ [32]. Regardless, these data demonstrate that we can no longer assume that ^{14}N is preferentially excreted by adult mammals. However, future studies of animals that have been on the same diet their entire lives are required to confirm this finding.

This does not mean that ^{15}N-depleted excreta play no role in determining mammalian tissue $\delta^{15}N$. It does suggest, however, that such depletion will be of import only when an animal is not at steady state, as would be the case during growth, pregnancy, dietary change, negative nitrogen balance, or thermal stress. Indeed, we have experimental evidence of one such instance. We recently conducted a nitrogen balance study of alpacas (*L. pacos*) eating bermudagrass. Alpacas, unlike their llama brethren, performed poorly on this grass hay and were in negative nitrogen flux during the study. Their urine was more depleted in ^{15}N (−2.6‰) and their feces less enriched (+1.5‰) relative to diet than the llamas in this study, resulting in a total excreta depletion of −1.2‰. Thus, it is clear that influx and efflux $\delta^{15}N$ differences can and do occur, but only when an animal is not at steady state.

This all begs the question, What role does excreta $\delta^{15}N$ play in determining the nitrogen isotope compositions of modern and archaeological mammals? Ambrose [2] has advanced the hypothesis that interspecific differences in mammalian collagen $\delta^{15}N$ might be explained by thermophysiological adaptations. Animals with adaptations to prevent heat stress and dehydration tend to lose more urinary nitrogen (primarily as urea) than their water-dependent counterparts [21,22]. Because urine tends to be depleted in ^{15}N [46,47], animals that lose more urinary nitrogen should lose relatively more ^{14}N, and concomitantly, have higher tissue $\delta^{15}N$. This "urinary nitrogen excretion" hypothesis fits nicely with the observation that water-independent taxa (which tend to lose more urinary N than water-dependent species) have higher $\delta^{15}N$ than those that drink regularly [2].

Moreover, there is evidence that the relationship holds for domestic animals. We recently fed llamas and goats pure alfalfa diets for a period of one year. The llamas lost relatively more urinary nitrogen than goats (77% and 70% of total nitrogen efflux respectively), and as predicted by Ambrose's hypothesis, had higher diet–hair fractionations (+6.3‰ ± 0.2) than the latter (+5.0‰ ± 0.3). Furthermore, the urinary nitrogen excretion hypothesis may also explain why diet–hair fractionation of ^{15}N increases in step with dietary protein levels [44], because as shown in this study and elsewhere, dietary protein and urinary N loss increase in tandem. Thus, there is considerable evidence that varying amounts of urinary nitrogen loss are at least partially responsible for the observed inter- and intra-specific differences in diet–tissue fractionation of ^{15}N. This influence, however, must be wholly exerted while the animal is in a state of isotopic disequilibrium, after which intake and excreta $\delta^{15}N$ will be equivalent. Nevertheless, it is likely that other factors (e.g., urea recycling) also play a significant role.

All told, these results suggest that most current models explicating mammalian $\delta^{15}N$ values need re-thinking. Understanding the basis of diet–tissue fractionation is of vital importance if we are to fully utilize the potential of nitrogen isotopes as paleoenvironmental and paleodietary proxies, yet it is clear that we still know little of the nutritional, physiological, and biochemical factors that determine mammalian nitrogen isotope compositions. Controlled-feeding studies of physiologically diverse taxa with varied diets and under a variety of conditions are necessary to improve our ability to interpret archaeological $\delta^{15}N$ data.

Acknowledgements

References

[1] S.H. Ambrose, M.J. DeNiro, The isotopic ecology of East African mammals, Oecologia 69 (1986) 395–406.

[2] S.H. Ambrose, Effects of diet, climate and physiology on nitrogen isotope abundances in terrestrial foodwebs, Journal Archaeological Science 18 (1991) 293–317.

[3] S.H. Ambrose, Controlled diet and climate experiments on nitrogen isotope ratios of rats, in: S.H. Ambrose, M.A. Katzenberg (Eds.), Biogeochemical Approaches to Paleodietary Analysis, Kluwer Academic, New York, 2000, pp. 243–259.

[4] H. Bocherens, D. Billiou, A. Mariotti, M. Patou-Mathis, M. Otte, D. Bonjean, M. Toussaint, Palaeoenvironmental and palaeodietary implications of isotopic biogeochemistry of last interglacial neandertal and mammal bones in Scladina Cave (Belgium), Journal of Archaeological Science 26 (1999) 599–607.

[5] T.E. Cerling, J.M. Harris, Carbon isotope fractionation between diet and bioapatite in ungulate mammals and implications for ecological and paleoecological studies, Oecologia 120 (1999) 347–363.

[6] T.L. Dupras, H.P. Schwarcz, S.I. Fairgrieve, Infant feeding and weaning practices in Roman Egypt, American Journal of Physical Anthropology 115 (2001) 204–212.

[7] M.L. Fogel, N. Tuross, D.W. Owsley, Nitrogen isotope tracers of human lactation in modern and archaeological populations. Annual Report to the Director, Geophysical Laboratory, Carnegie Institution, Washington, D.C. 1988–1989 (1989) 111–117.

[8] D.O. Freudenberger, I.D. Hume, Effects of water restriction on digestive function in two macropodid marsupials from divergent habitats and the feral goat, Journal of Comparative Physiology B 163 (1993) 247–257.

[9] O.H. Gaebler, T.G. Vitti, R. Vukmirovich, Isotope effects in metabolism of ^{14}N and ^{15}N from unlabeled dietary proteins, Canadian Journal of Biochemistry 44 (1966) 1249–1257.

[10] L.Z. Gannes, D.M. O'Brien, C.M. del Rio, Stable isotopes in animal ecology: assumptions, caveats, and a call for more laboratory experiments, Ecology 78 (1997) 1271–1276.

[11] P.E. Hare, M.L. Fogel, T.W. Stafford, A.D. Mitchell, T.C. Hoering, The isotopic composition of carbon and nitrogen in individual amino acids isolated from modern and fossil proteins, Journal of Archaeological Science 18 (1991) 277–292.

[12] R.E.M. Hedges, G.J. van Klinken, "Consider a spherical cow …" —on modeling and diet, in: S.H. Ambrose, M.A. Katzenberg (Eds.), Biogeochemical Approaches to Paleodietary Analysis, Kluwer Academic, New York, 2000, pp. 211–241.

[13] K.A. Hobson, D.M. Schell, D. Renouf, E. Noseworthy, Stable carbon and nitrogen isotopic fractionation between diet and tissues of captive seals: implications for dietary reconstructions involving marine mammals, Canadian Journal of Fishery and Aquatic Science 53 (1996) 525–533.

[14] K.A. Hobson, R.T. Alisauskas, R.G. Clark, Stable nitrogen isotope enrichment in avian tissues due to fasting and nutritional stress: implications for isotopic analysis of diet, Condor 95 (1993) 388–394.

[15] K.A. Hobson, J.F. Piatt, J. Pitocchelli, Using stable isotopes to determine seabird trophic relationships, Journal of Animal Ecology 63 (1994) 786–798.

[16] M.A. Katzenberg, S.R. Saunders, W.R. Fitzgerald, Age differences in stable carbon and nitrogen isotope ratios in a population of maize horticulturalists, American Journal of Physical Anthropology 90 (1993) 267–281.

[17] C.I. Keeling, D.E. Nelson, Changes in the intramolecular stable carbon isotope ratios with age of the European cave bear (Ursus spelaeus), Oecologia 127 (2001) 495–500.

[18] J.F. Kelly, Stable carbon and nitrogen in the study of avian and mammalian trophic ecology, Canadian Journal of Zoology 78 (2000) 1–27.

[19] S.A. Macko, M.H. Engel, V. Andrusevich, C. Lubec, T.C. O'Connell, R.E.M. Hedges, Documenting the diet of ancient human populations through stable isotope analysis of hair, Philosophical Transactions of the Royal Society of London B 354 (1999) 65–76.

[20] S.A. Macko, M.L. Fogel, M.H. Engel, P.E. Hare, Kinetic fractionation of stable nitrogen isotopes during amino acid transamination, Geochimica et Cosmochimica Acta 50 (1986) 2143–2146.

[21] G.M.O. Maloiy, Water metabolism of a small East African antelope: the dik-dik, Proc R Soc Lond 184B (1973) 167–178.

[22] G.M.O. Maloiy, W.V. Macfarlane, A. Skolnik, Mammalian herbivores, in: G.M.O. Maloiy (Ed.), Comparative Physiology of Osmoregulation in Animals II, Academic Press, London, 1979, pp. 185–209.

[23] K. Milton, P.J. Van Soest, J.B. Robertson, Digestive efficiencies of wild howler monkeys, Physiological Zoology 53 (1980) 402–409.

[24] M. Minagawa, E. Wada, Step-wise enrichment of ^{15}N along food chains: further evidence and the relationship between δ^{15}N and animal age, Geochimica et Cosmochimica Acta 48 (1984) 1135–1140.

[25] D.J. Minson, Forage in Ruminant Nutrition, Academic Press, San Diego, 1990.

[26] H. Mizutani, M. Fukuda, Y. Kabaya, ^{13}C and ^{15}N enrichment factors of feathers of 11 species of adult birds, Ecology 73 (1992) 1391–1395.

[27] H.M. Mousa, K.E. Ali, I.D. Hume, Effects of water deprivation on urea metabolism in camels, desert sheep, and desert goats fed dry desert grass, Comparative Biochemistry and Physiology 74A (1983) 715–720.

[28] NRC, Nutrient Requirements of Goats, National Academy Press, Washington, DC, 1981.

[29] NRC, Nutrient Requirements of Beef Cattle, National Academy Press, Washington, DC, 1996.

[30] N.C. Overman, D.L. Parrish, Stable isotope composition of walleye: ^{15}N accumulation with age and area-specific differences in δ^{13}C, Canadian Journal of Aquatic Science 58 (2001) 1253–1260.

[31] B.J. Peterson, B. Fry, Stable isotopes in ecosystem studies, Annual Review of Ecol Syst 18 (1987) 293–320.

[32] S. Ponsard, P. Averbuch, Should growing and adult animals fed on the same diet show different δ^{15}N values? Rapid Communication in Mass Spectrometry 13 (1999) 1305–1310.

[33] M.P. Richards, P.B. Pettett, M.C. Stiner, E. Trinkaus, Stable isotope evidence for increasing dietary breadth in the European mid-Upper Paleolithic, Proceedings of the National Academy of Sciences 98 (2001) 6528–6532.

[34] M.P. Richards, P.B. Pettitt, E. Trinkaus, F.H. Smith, M. Paunovic, I. Karavanic, Neanderthal diet at Vindija and Neanderthal predation: the evidence from stable isotopes, Proceedings of the National Academy of Sciences 97 (2000) 7663–7666.

[35] J.D. Roth, K.A. Hobson, Stable carbon and nitrogen isotopic fractionation between diet and tissue of captive red fox: implications for dietary reconstruction, Canadian Journal of Zoology 78 (2000) 848–852.

[36] C. Rymer, The measurement of forage digestibility in vivo, in: D.I. Givens, E. Owen, R.F.E Axford, H.M. Omed (Eds.), Forage Evaluation in Ruminant Nutrition, CABI Publishing, New York, NY, 2000, pp. 113–134.

[37] M.J. Schoeninger, M.J. DeNiro, Nitrogen and carbon isotopic composition of bone collagen from marine and terrestrial animals, Geochimica et Cosmochimica Acta 48 (1984) 625–639.

[38] M.J. Schoeninger, M.J. DeNiro, H. Tauber, Stable nitrogen isotope ratios of bone collagen reflect marine and terrestrial components of prehistoric human diet, Science 220 (1983) 1381–1383.

[39] M.R. Schurr, Using stable nitrogen-isotopes to study weaning behavior in past populations, World Archaeology 30 (1998) 327–342.

[40] H.P. Schwarcz, T.L. Dupras, S.I Fairgrieve, ^{15}N-enrichment in the Sahara: in search of a global relationship, Journal of Archaeological Science 26 (1999) 629–636.

[41] C.M. Scrimgeour, S.C. Gordon, L.L. Handley, J.A.T. Woodford, Trophic levels and anomalous δ^{15}N of insects on raspberry (Rubus idaeus L.), Isotopes Environ Health Stud 31 (1995) 107–115.

[42] J.C. Sealy, N.J. van der Merwe, J.A. Lee-Thorp, J.L. Lanham, Nitrogen isotopic ecology in southern Africa: implications for environmental and dietary tracing, Geochimica et Cosmochimica Acta 51 (1987) 2707–2717.

[43] J.C. Sealy, N.J. van der Merwe, Social, spatial, and chronological patterning in marine food use as determined by δ^{13}C measurements of Holocene human skeletons from the South-Western Cape, South Africa, World Archaeology 20 (1988) 87–102.

[44] M. Sponheimer, T. Robinson, L. Ayliffe, B. Roeder, J. Hammer, A. West, B. Passey, T. Cerling, D. Dearing, J. Ehleringer, Nitrogen isotopes mammalian herbivores: hair δ^{15}N values from a controlled-feeding study, International Journal of Osteoarchaeology 13 (2003) 80–87.

[45] Sponheimer, unpublished data.

[46] K.W. Steele, R.M.J. Daniel, Fractionation of nitrogen isotopes by animals: a further complication to the use of variations in the natural abundance of ^{15}N for tracer studies, Journal of Agricultural Science 90 (1978) 7–9.

[47] M. Sutoh, T. Koyama, T. Yoneyama, Variations in natural ^{15}N abundances in the tissues and digesta of domestic animals, Radioisotopes 36 (1987) 74–77.

[48] M. Sutoh, Y. Obara, T. Yoneyama, The effects of feeding regimen on dietary sucrose supplementation on the natural abundance of ^{15}N in some components of ruminal fluid and plasma in sheep, Journal of Animal Science 71 (1993) 226–231.

[49] L.D. Tegland, T.F. Robinson, M. Sponheimer, B.L. Roeder, L. Ayliffe, Turnover of liver and muscle tissue determined by dietary stable isotope incorporation. Experimental Biology Meeting, San Diego, 2003.

[50] P.J. Van Soest, Nutritional Ecology of the Ruminant, second ed., Comstock, Ithaca, New York, 1994.

[51] B.G. Warmington, G.F. Wilson, T.N. Barry, Voluntary intake and digestion of ryegrass straw by llama × guanaco crossbreeds and sheep, Journal of Agricultural Science 113 (1989) 87–91.

[52] S.C. Webb, R.E.M. Hedges, S.J. Simpson, Diet quality influences the δ^{13}C and δ^{15}N of locusts and their biochemical componenents, Journal of Experimental Biology 201 (1998) 2903–2911.

[53] C.D. White, Isotopic determination of seasonality in diet and death from Nubian mummy hair, Journal of Archaeological Science 20 (1993) 657–666.