

# Tracking the fate of digesta $^{13}\text{C}$ and $^{15}\text{N}$ compositions along the ruminant gastrointestinal tract: Does digestion influence the relationship between diet and faeces?

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**Abstract** Faecal stable isotope compositions reflect wildlife diets, if digestive processes along the gastrointestinal tract (GIT) do not alter diet–faeces isotopic relationships in an unpredictable way. We investigated  $^{13}\text{C}$  and  $^{15}\text{N}$  compositions of digesta along the ruminant GIT, using Saanen dairy goats kept on pure grass hay or browse for >20 days. Isotopic changes occurred in the ventral rumen, and in the small intestine, where digesta had significantly higher  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  (associated with lower C or higher N content, respectively) values relative to other GIT sites. However, effects on isotope fractionation were small ( $\sim 1.0\text{‰}$  for  $\delta^{13}\text{C}$  and  $\sim 2.0\text{‰}$  for  $\delta^{15}\text{N}$ ), and were reversed in the hindgut such that faecal isotope compositions did not differ from the foregut. No other substantial isotopic changes occurred across GIT sites, despite the morphophysiological complexity of the ruminant GIT. We found similarly small differences across

GIT components of rheem gazelles (*Gazella leptoceros*) fed a mixture of  $\text{C}_3$  lucerne and  $\text{C}_4$  grass, although in this case faeces were  $^{15}\text{N}$ -depleted relative to other GIT components. Along with differences in  $\delta^{15}\text{N}$  between goats fed browse or grass, this result implies a systematic difference in diet–faeces  $\delta^{15}\text{N}$  relationships, contingent on the botanical composition of ruminant diets. Thus, while our results support faecal  $\delta^{13}\text{C}$  as a reliable proxy for wildlife diets, further work on factors influencing faecal  $^{15}\text{N}$  abundance is needed. Finally, we note high levels of isotopic variability between individuals fed the same diets, even accounting for the relatively short duration of the experiments, suggesting an important influence of stochasticity on isotope fractionation.

**Keywords** Browse · Hindgut · Saanen goat · Fractionation · Grass · Rheem gazelle · Rumen

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## Introduction

Stable isotope analysis (SIA) offers a diversity of applications to wildlife ecology, and to ecology in general, at all levels of organization (Layman et al. 2007; Newsome et al. 2007; Crawford et al. 2008). One of the biggest advantages of SIA is that behaviors like diet and habitat use can be approached from a number of spatial and temporal scales. The various body tissues and excreta of animals differ in their respective metabolic, growth, and turnover rates, so that analysis of each type of material provides insights at a specific time scale (Tieszen et al. 1983; Hobson 1999; Sponheimer et al. 2009). Persistent tissues that are continuously remodeled through life, such as bone collagen, archive integrated lifetime diet averages; incremental inert tissues like hair, horns, and teeth can be used to reconstruct chronological histories of individuals; and analysis of materials such as faeces and breath CO<sub>2</sub> provide dietary information over daily or even hourly time scales (Ambrose and Norr 1993; Voigt et al. 2008; Cerling et al. 2009; Codron and Codron 2009; Sponheimer et al. 2009; Vander Zanden et al. 2010).

Of these materials, analysis of faeces is amongst the most promising for SIA in wildlife research, because collections do not necessitate interference with animals, and samples are easily obtained over heterogeneous spatiotemporal contexts (Treydte et al. 2006; Codron et al. 2007; Codron and Codron 2009). Laboratory and field experiments have demonstrated that faecal stable isotope compositions are generally consistent with diet isotope compositions, at least amongst large mammal herbivores (Sponheimer et al. 2003; Codron and Codron 2009; Norman et al. 2009; Wittmer et al. 2010; Codron et al. 2011). Yet since faeces are comprised of undigested food remains and various endogenous losses (e.g. gut microbes, sloughed epithelial tissues, and secreted enzymes), variations in their stable isotope composition may be derived from non-dietary effects. Indeed, faeces–diet isotope discriminations (numerical offsets) reported in the literature vary substantially between independent experiments (Steele and Daniel 1978; Jones et al. 1981; Sutoh et al. 1987; Sponheimer et al. 2003), and even within them (Codron et al. 2011). It is widely agreed that variations in animal–diet discriminations in any type of material are the biggest constraint for accurate and reliable diet reconstructions by SIA (Phillips et al. 2005; Caut et al. 2008; Newsome et al. 2010).

A prerequisite for interpreting patterns of faecal SIA is that faeces and diet isotope compositions should be strongly correlated, differing only systematically, without any significant changes to their relationship occurring along the gastrointestinal tract (GIT). Few studies have empirically compared the stable isotope composition of faeces with that of digesta in different GIT components (Sutoh et al.

1987; Hwang et al. 2007). The most recent study (Hwang et al. 2007) reported significant differences in <sup>13</sup>C/<sup>12</sup>C and <sup>15</sup>N/<sup>14</sup>N ratios of faeces (colon contents) compared with digesta of the stomach, small intestine, and caecum in various hindgut-fermenting small mammal species (voles, deer mice, and chipmunks). These differences did not appear to be systematic across species and, in some cases, even across individuals. If left unaccounted for in wildlife studies, such changes along the GIT would lead to inaccurate diet reconstructions (Hwang et al. 2007). This uncertainty could be exacerbated in large mammal herbivores, especially ruminants, given the increased complexity of their digestive systems (see Stevens and Hume 1995). For example, rumen contents of many species are stratified into layers of differing density, particle sizes, and possibly microbial activity (reviewed in Hummel et al. 2009), which may contribute to isotopic variability. There are also known differences in chemical composition of digesta across ruminant GIT organs, consistent with each organ's digestive function (Boyne et al. 1956; Ash 1968).

The aim of this study was to determine whether the stable carbon and nitrogen isotope compositions of digesta vary significantly along the GIT of ruminants, leading to inconsistent relationships between diet and faecal isotope compositions. We analyzed digesta sampled along the GIT of Saanen dairy goats (*Capra hircus* (L., 1758)), using specimens obtained from individuals kept on fixed diet regimes of either grass hay or browse for c. 3 weeks, after which animals were euthanized and frozen with viscera in living position. These results are supplemented with a smaller dataset for rheem gazelles (*Gazella leptoceros* (F. Cuvier, 1842)), sampled from individuals fed a mixture of dicot and grass hays for c. 3 weeks before slaughter. We test the hypothesis that faecal stable isotope compositions are consistent with those of the diet. To evaluate the hypothesis, we assess whether digesta isotope compositions change significantly along the GIT, whether these changes are linked with changes in digesta chemical composition and hence digestive function of particular GIT sites, and whether differences in diet (and associated differences in chemical content and nutritive value) influence these patterns. In other words, our null hypothesis is that stable isotope compositions are static along the ruminant GIT, regardless of changes in extrinsic (diet composition) and intrinsic (digestive processes) conditions.

## Materials and methods

Animals for this study were from two independent experiments. The first experiment was part of a larger clinical trial at the Vetsuisse Faculty, University of Zürich, Switzerland (see e.g. Becker-Birck 2009; Imer 2010). Five female

Saanen goats were kept on a monospecific C<sub>3</sub> grass hay diet (mean  $\delta^{13}\text{C} = -27.8\% \pm 0.16 \text{ S.D.}$ ,  $\delta^{15}\text{N} = 1.8 \pm 0.14\%$ ; Table 1) for 20 days. All animals were clinically healthy. After the adaptation period, individuals were fasted for about 12 h, immobilized with xylazine/ketamine, and euthanized with potassium chloride. They were then frozen at  $-18^\circ\text{C}$  in sternal recumbency, to retain internal structures in living position. Additionally, another five females that were not included in the larger study were kept on a mixed browse diet (poplar, raspberry, and chestnut) for 20 days (mean  $\delta^{13}\text{C} = -28.0 \pm 0.18\%$ ,  $\delta^{15}\text{N} = 0.4 \pm 0.16\%$ ), and then euthanized and frozen in the same way. Only one or two individuals were euthanized per day; thus, some individuals continued on the experimental diet for up to 10 days longer than others. Abdomens of frozen animals were sliced transversely. Contents were sampled from transverse sections at multiple sites along the GIT, working anterior–posteriorly, and also dorso–ventrally within some organs (e.g. within the rumen) (see Fig. 1). From each sampling point, most or all of the material was removed, ground and homogenized, from which a small (~5 mg) subsample was taken for isotope analysis.

The second experiment used captive rheem gazelles, reared as part of a captive carnivore feeding regime at the Al-Wabra Wildlife Preservation, Qatar. Seven individuals were fed diets comprising a mixture of lucerne (*Medicago sativa*; mean  $\delta^{13}\text{C} = -28.9 \pm 0.15\%$ ,  $\delta^{15}\text{N} = 4.9 \pm 0.28\%$ ) and a C<sub>4</sub> grass hay (mean  $\delta^{13}\text{C} = -13.7 \pm 0.38\%$ ,  $\delta^{15}\text{N} = 1.1 \pm 0.27\%$ ), for 20 days. Individuals favoured lucerne over grass (weighing of forage components before and after feeding revealed that lucerne accounted for between 75 and 80% of bulk intake by all individuals, i.e., diet isotope compositions were roughly  $-25.4\%$  and  $4.1\%$  for  $\delta^{13}\text{C}$

and  $\delta^{15}\text{N}$ , respectively; see Table 1), but intake likely varied at different times and time scales. All rheem gazelles were slaughtered on the same day, and GIT contents were sampled from the dorsal rumen, ventral rumen, reticulum, omasum, and faeces.

GIT contents sampled at different sites were oven-dried to constant weight and mill-ground to a homogeneous powder. Powdered specimens were individually weighed into tin capsules and loaded into the autosampler of a Thermo Flash elemental analyzer (Finnigan, Bremen, Germany) for combustion. Resultant CO<sub>2</sub> and N<sub>2</sub> gases were introduced via a continuous flow inlet system to a Delta V Mass Spectrometer (Finnigan, Bremen, Germany), calibrated with the international standards NBS 22 and IAEA CH-6, for determination of stable isotope compositions.  $^{13}\text{C}/^{12}\text{C}$  and  $^{15}\text{N}/^{14}\text{N}$  ratios are presented in the conventional delta ( $\delta$ ) notation, in per mil (‰) relative to the Vienna PeeDee Belemnite (VPDB) and atmospheric N<sub>2</sub> standards, respectively. Standard deviations for repeated measurements of atropine and peptone standards were less than 0.2%. These analyses yielded elemental concentrations (%C and %N), by weight.

Forage fibre fractions (neutral detergent fibre (NDF) and acid detergent fibre (ADF)) were determined using an ANKOM Fiber Analyzer (Ankom Technology, Macedon, USA). Acid detergent lignin (ADL) of the forages was determined by reacting ADF fractions in a 72% sulphuric acid solution. NDF, ADF, and ADL are reported as percentages of dry matter. A subsample of each fraction was retained and subjected to stable isotope analysis. Only the goat forages were available for these analyses. Stable isotope compositions, %N, and fibre fractions of

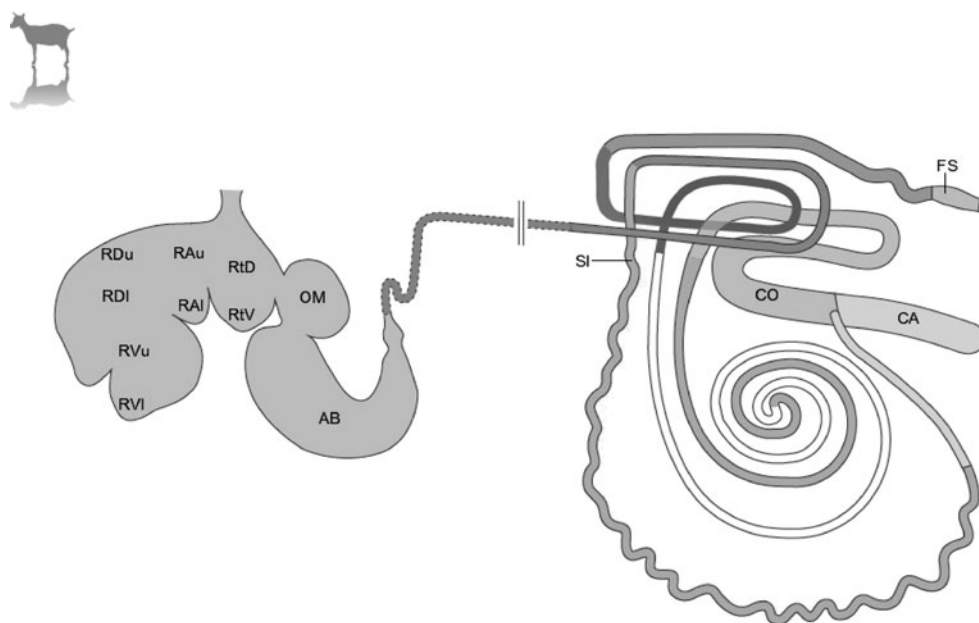
**Table 1** Stable isotope compositions, %N, and fibre fractions (with their respective isotopic compositions) of forages used in the experiments for this study. Numbers in parentheses are standard deviations for five independent measurements

Food	$\delta^{13}\text{C}_{\text{VPDB}}$ (‰)				$\delta^{15}\text{N}_{\text{Air}}$ (‰)				Percent nitrogen	Percentage of DM		
	Bulk	NDF	ADF	ADL	Bulk	NDF	ADF	ADL		NDF	ADF	ADL
Goat forages												
Grass hay	<b>-27.8 (0.16)</b>	-26.6	-26.8	-25.6	<b>1.8 (0.14)</b>	1.1	0.9	0.9	<b>1.2 (0.14)</b>	57.3	36.9	3.3
Poplar	-27.8 (0.26)	-27.5	-26.9	-28.9	-0.2 (0.24)	-0.2	-0.3	0.0	2.0 (0.06)	35.4	25.4	7.2
Raspberry	-26.8 (0.13)	-25.8	-25.7	-28.8	3.0 (0.14)	1.0	0.8	2.3	2.9 (0.06)	34.3	20.8	5.1
Chestnut	-29.5 (0.15)	-29.5	-29.1	-31.4	-1.6 (0.09)	-1.1	-1.4	-0.6	1.8 (0.07)	41.3	23.0	4.1
Browse average	<b>-28.0 (0.18)</b>	-27.6	-27.2	-29.7	<b>0.4 (0.16)</b>	-0.1	-0.3	0.6	<b>2.2 (0.43)</b>	37.0	23.1	5.5
Rheem gazelle forages												
Grass hay	-13.7 (0.38)				1.1 (0.27)				1.1 (0.39)			
Lucerne	-28.9 (0.15)				4.9 (0.28)				4.6 (0.53)			
Average <sup>a</sup>	<b>-25.4 (0.20)</b>				<b>4.1 (0.20)</b>				<b>3.8 (0.50)</b>			

<sup>a</sup> Average for rheem gazelle weighted for mean lucerne intake of 77.4% and mean grass intake of 22.6%. Values in bold typeface were used in calculations of digesta diet discrimination (i.e.,  $\epsilon^*_{\text{DD}}$ )

DM dry matter, NDF neutral detergent fibre, ADF acid detergent fibre, ADL acid detergent lignin

**Fig. 1** Diagram of ruminant GIT showing sites from where digesta were sampled for isotope analysis: *R* rumen, *Rt* reticulum, *D* dorsal, *V* ventral, *A* atrium, *u* upper, *l* lower, *OM* omasum, *AB* abomasum, *SI* small intestine, *CA* caecum, *CO* colon, *FS* faeces



the forages used in these experiments are presented in Table 1.

#### Data analysis

Our main objective was to test whether  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  values (and %C and %N) of digesta differ significantly across components of the GIT, which would lead to non-systematic differences between diet and faecal isotope composition. To test these hypotheses, we used mixed effects models (GLM module of STATISTICA Version 8.0; Statsoft, Inc. 2007), with “GIT component” entered as a (fixed) categorical effect. “Individual” was included as a random effect to account for variations across animals, and in the case of goats “Diet” (grass hay or browse) was another main effect. Subsequently, we nested “Individual” within “Diet”. We used Tukey's post hoc test for multiple mean comparisons, and we report specifically on comparisons between faecal isotope composition with that of digesta in other GIT components. We then replaced the effect “GIT component” with measured C:N ratios (continuous variable) to test whether isotopic differences across GIT components are linked to changes in digesta biochemical composition.

For comparability with common practice in the experimental stable isotope literature, we calculated isotopic discriminations (offsets) between digesta and diet ( $\varepsilon^*_{\text{DD}}$ ) for each GIT component of every animal, using the formula

$$\varepsilon^*_{\text{DD}} = \left( \frac{10^3 + \delta_{\text{digesta}}}{10^3 + \delta_{\text{diet}}} - 1 \right) 10^3.$$

The asterisk depicts that isotopic equilibrium between digesta and diets is not assumed in the calculations (Passey

et al. 2005). Diet values are from analysis of bulk forage material (Table 1). Estimated  $\varepsilon^*_{13\text{C}}$  and  $\varepsilon^*_{15\text{N}}$  were used as additional dependent variables, alongside raw  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  values, in the mixed models described above.

## Results

### Goats

Although  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  values of digesta did differ significantly across GIT components within individuals ( $p < 0.0001$ ; Table 2), the effects were relatively weak (eta-squared=0.13 and 0.18, respectively), and changes were numerically small and infrequent. For  $\delta^{13}\text{C}$ , small intestine contents were about 1.0‰ higher (mean =  $-27.6 \pm 0.7\%$ ;  $p < 0.001$ ), and lower ventral rumen contents about 0.5‰ higher (mean =  $-28.1 \pm 1.1\%$ ;  $p < 0.001$  to 0.03), than other GIT components (combined mean =  $-28.5 \pm 0.2\%$ ; Fig. 2a and b). All other post hoc comparisons indicate that  $\delta^{13}\text{C}$  values of digesta were static within individuals (overall range was  $< 0.05\%$ ;  $p = 0.09$  to 0.99). Faeces, too, had  $\delta^{13}\text{C}$  values (mean  $-28.7 \pm 1.1\%$ ) similar to those of digesta from all GIT components ( $p = 0.52$  to 0.99), except for the small intestine ( $p < 0.0001$ ) and the lower ventral rumen ( $\delta^{13}\text{C}$  only;  $p < 0.01$ ). Digesta  $\delta^{15}\text{N}$  values also showed a slight ( $\sim 0.6\%$ ) enrichment in the lower ventral rumen (mean =  $2.8 \pm 1.8\%$ ) compared to other foregut components (combined mean =  $2.2 \pm 0.2\%$ ;  $p = 0.02$  to 0.04; Fig. 2c and d). Larger changes ( $\sim 1.0$  to  $2.0\%$ ) in  $\delta^{15}\text{N}$  values of digesta occurred between the foregut and posterior GIT components, with the small intestine ( $4.1 \pm 1.1\%$ ), caecum ( $3.4 \pm 1.7\%$ ) and colon ( $3.2 \pm 1.7\%$ ) having higher values compared with all foregut

**Table 2** Mixed effects models testing within- (GIT component) and between-individual sources of variation in  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  values of digesta, digesta diet discriminations ( $\epsilon^*$ ), and elemental composition of digesta (%C, %N, and C:N ratios)

Variable	Effects	Goats			Rheem gazelles	
		Diet	Individual (diet)	GIT component	Individual	GIT component
$\delta^{13}\text{C}$	<i>df</i> =	1, 114	8, 114	13, 114	6, 24	4, 24
	<i>F</i> ( <i>p</i> )	380.987***	46.835***	8.902***	30.084***	1.424 (0.256)
$\epsilon^*_{13\text{C}}$	Eta-squared	0.44	0.43	0.13	0.97	0.03
	<i>F</i> ( <i>p</i> )	264.479***	46.831***	8.902***	35.547***	1.425 (0.256)
$\delta^{15}\text{N}$	Eta-squared	0.35	0.50	0.15	0.97	0.03
	<i>F</i> ( <i>p</i> )	749.462***	28.180***	16.167***	88.636***	20.566***
$\epsilon^*_{15\text{N}}$	Eta-squared	0.63	0.19	0.18	0.87	0.13
	<i>F</i> ( <i>p</i> )	108.193***	28.193***	16.161***	75.702***	20.567***
%C	Eta-squared	0.08	0.47	0.44	0.85	0.15
	<i>F</i> ( <i>p</i> )	62.574***	0.850 (0.561)	5.547***	0.936 (0.487)	2.993*
%N	Eta-squared	0.44	0.05	0.51	0.32	0.68
	<i>F</i> ( <i>p</i> )	247.657***	4.445**	4.808***	2.57*	11.370***
C:N	Eta-squared	0.72	0.10	0.18	0.25	0.75
	<i>F</i> ( <i>p</i> )	155.582***	5.292***	5.337***	1.746 (0.153)	11.903***
	Eta-squared	0.58	0.16	0.26	0.18	0.82

“Individual” is a random effect; in the case of goats, this parameter is nested within the fixed effect “Diet” (grass hay or browse)

\* $p < 0.05$

\*\* $p < 0.001$

\*\*\* $p < 0.0001$

components ( $p < 0.001$  to 0.03). Faecal  $\delta^{15}\text{N}$  values were slightly lower ( $< 1.0\text{‰}$ ) than those of digesta in the hindgut (mean =  $2.8 \pm 1.8\text{‰}$ ), but only differed significantly from the small intestine ( $p < 0.0001$ ).

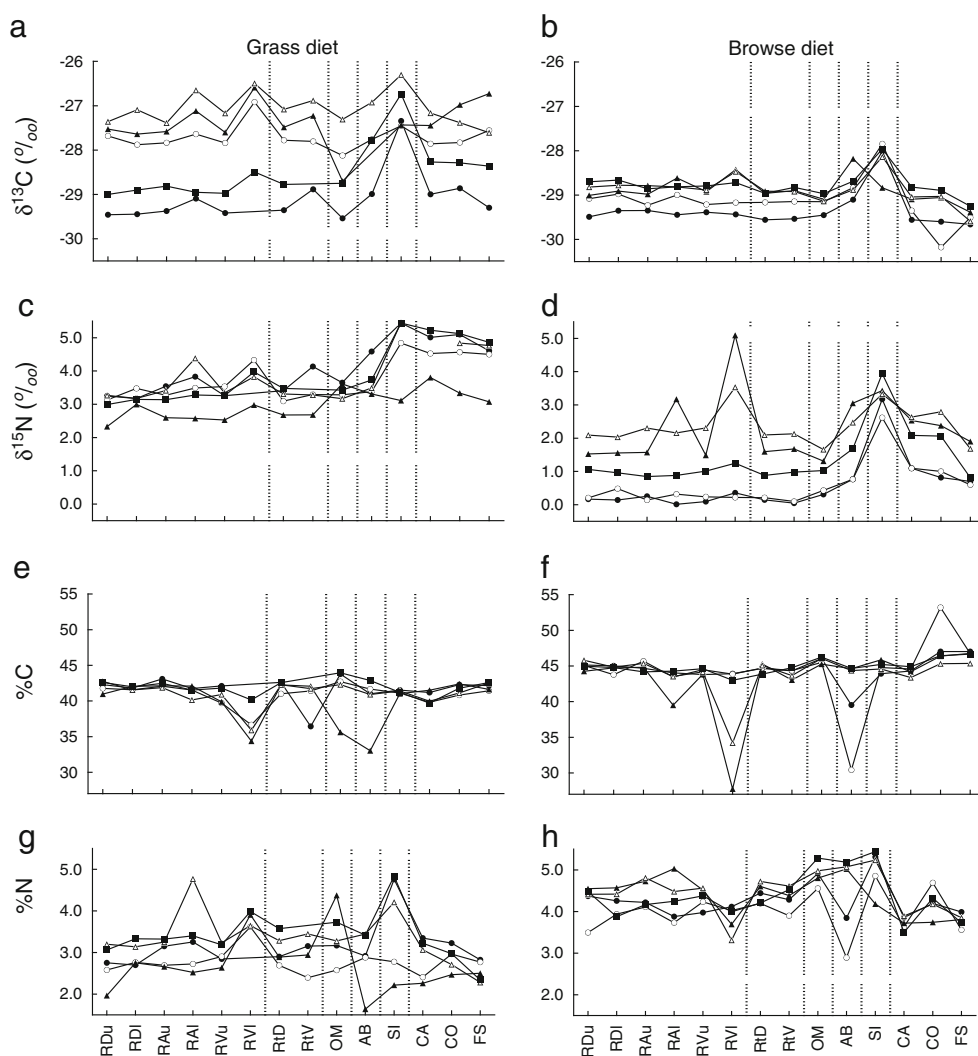
There were differences in  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  values across individuals, both between and within diet treatments ( $p < 0.0001$  for the “Diet” and “Individual nested in diet” effects; Table 2). These between-individual effects were stronger (eta-squared 0.19 to 0.63) than those ascribed to changes across GIT components within individuals, and were of a larger magnitude (up to 1.6‰ and 1.8‰ for  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$ , respectively) than isotopic variation within any one diet (for which the largest minimum–maximum ranges were 0.7‰ and 0.6‰ for  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$ , respectively), ruling out selective foraging as a contributing factor. The diet effect emerges because individuals on grass hay had higher mean  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  values ( $-27.9 \pm 0.9\text{‰}$  and  $3.7 \pm 0.8\text{‰}$ , respectively) compared with animals fed browse ( $-29.0 \pm 0.4$  and  $1.5 \pm 1.1\text{‰}$ , respectively). This difference in goat  $\delta^{13}\text{C}$  values occurred despite both forages being  $\text{C}_3$  and had similar bulk  $\delta^{13}\text{C}$  values ( $-27.6$  and  $-27.8\text{‰}$  for grass and browse, respectively). Accordingly,  $\epsilon^*_{13\text{C}}$  for the grass diet was higher (less negative) than for the browse diet (mean for all GIT components =  $-0.1 \pm 0.9\text{‰}$  and  $-1.0 \pm 0.4\text{‰}$ , respectively). The diet effect on goat  $\delta^{15}\text{N}$  values is consistent with the higher  $\delta^{15}\text{N}$  of the grass hay compared

with browse (1.8 and 0.4‰, respectively), but  $\epsilon^*_{15\text{N}}$  was still slightly higher for grass compared with browse diets ( $1.9 \pm 0.8\text{‰}$  and  $1.1 \pm 1.1\text{‰}$ , respectively). However, the diet effect on  $\epsilon^*_{15\text{N}}$  was relatively weak (eta-squared = 0.08).

Within the diet groups, differences in  $\delta^{13}\text{C}$  values across individuals were most common amongst animals fed grass hay: all but one individual differed significantly from the others in the group ( $p < 0.001$ ; Fig. 2a). Amongst individuals fed browse, all but one had similar mean  $\delta^{13}\text{C}$  values ( $p = 0.11$  to 0.99; Fig. 2b). By contrast, differences in  $\delta^{15}\text{N}$  values across individuals were less prevalent for the grass diet ( $p = 0.81$  to 0.99, except for one individual with mean approximately 1.0‰ lower than the others,  $p < 0.01$ ; Fig. 2c) than for the browse diet ( $p < 0.01$ , except for one pair of animals  $p = 0.99$ ; Fig. 2d). Another potential effect of diet regime on  $\delta^{15}\text{N}$  data is that the higher values for posterior GIT components (see above) were only visible in individuals on the grass diet (Fig. 2c), whereas values seemed to decline in the caecum, colon, and faeces of individuals fed browse (Fig. 2d). However, the sample size is too small to test for an interaction between diet and GIT component.

Elemental composition of digesta differed significantly across GIT components ( $p < 0.0001$  for %C and %N; Table 2), although again the variation was limited to only a few sites (Fig. 2e to h). Digesta %C only varied within

**Fig. 2** Variation in  $\delta^{13}\text{C}$  (a, b),  $\delta^{15}\text{N}$  (c, d), %C (e, f), and %N (g, h) values of digesta along the GIT of five goats fed  $\text{C}_3$  grass hay (a, c, e, g) and five goats fed  $\text{C}_3$  browse (b, d, f, h) for 20 days. Each symbol represents a different individual. GIT components are ordered approximately from anterior to posterior along the x-axis, i.e., from rumen to faeces (see legend of Fig. 1). Dotted vertical lines separate GIT organs (from left to right): rumen, reticulum, omasum, abomasum, small intestine, and hindgut



individuals because lower ventral rumen contents had ~5.0 to 7.0% lower values than other GIT components ( $p < 0.01$ ;  $p$ -values ranged from 0.39 to 0.99 for all other post hoc comparisons), and variation in %N of digesta was only significant because the small intestine had values ~0.5 to 1.0% higher compared with most other GIT sites ( $p < 0.05$ ). Since  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  values were also both higher in the lower ventral rumen and small intestine than in the rest of the GIT, a significant negative relationship emerged between the C:N ratio and isotope composition of digesta ( $p < 0.0001$  for  $\delta^{13}\text{C}$  and  $p < 0.01$  for  $\delta^{15}\text{N}$ ). However, when both diets were evaluated separately, the effect did not persist for the grass diet (Fig. 3).

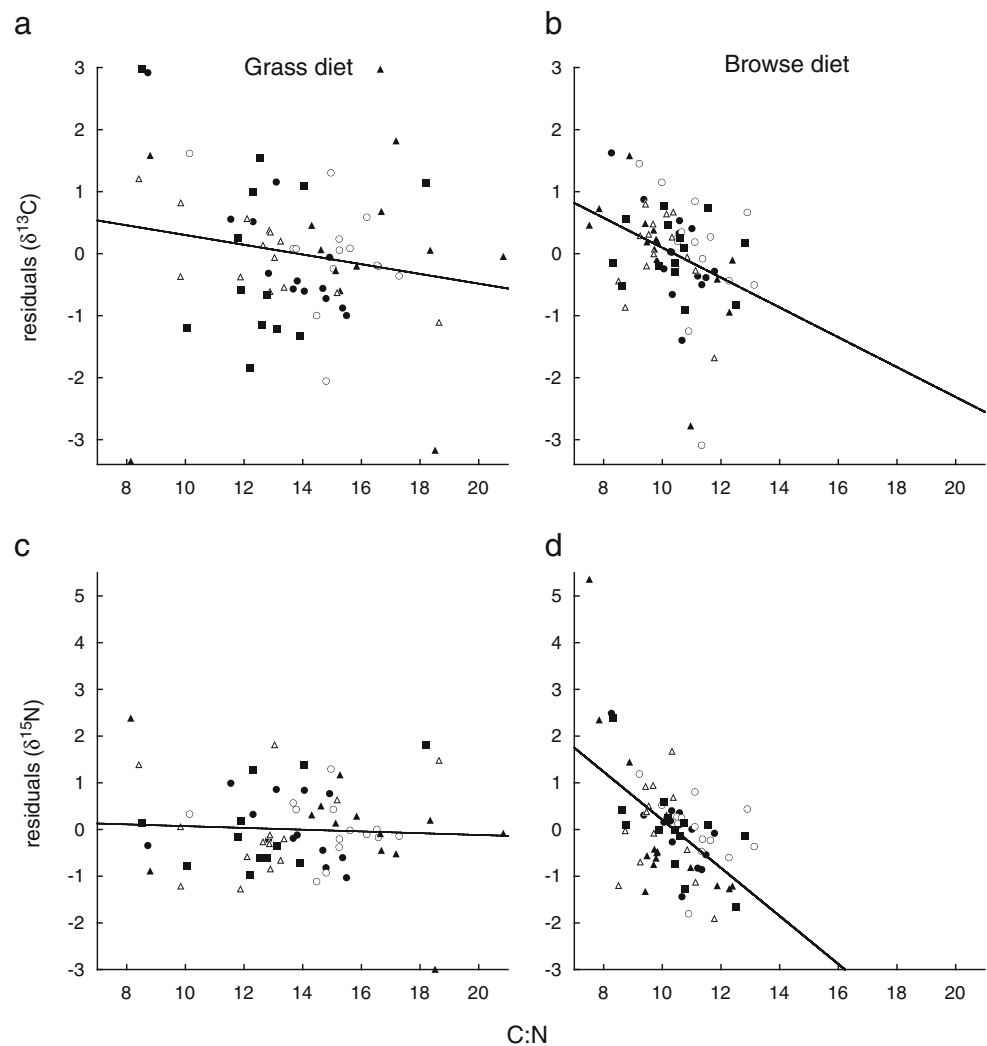
#### Rheem gazelles

There were no significant changes in  $\delta^{13}\text{C}$  values of digesta along the GIT in this species ( $p = 0.26$ ; Table 2). Visually, there appeared to be a difference between faeces and other components in some individuals (Fig. 4a); thus, the lack of

significant effect may be due to low power of the small sample size (seven individuals). Nonetheless, the effect of GIT component was weak compared with the differences across individuals ( $p < 0.0001$ ; eta-squared = 0.97). A similar result was obtained for  $\delta^{15}\text{N}$  data ( $p < 0.0001$ ; eta-squared = 0.87 for the individual effect), although in this case the effect of GIT component is significant, with faeces being slightly (~0.7‰)  $^{15}\text{N}$ -depleted compared with other GIT components (mean =  $5.8 \pm 0.6\text{‰}$  and  $6.5 \pm 0.1\text{‰}$ , respectively;  $p < 0.001$ ; Fig. 4b).

The rheem gazelle data showed no evidence for significant changes in %C of digesta through the GIT ( $p = 0.68$ ; Table 2 and Fig. 4c), and though changes in %N were significant ( $p < 0.0001$ ), this was only because faeces had ~0.5% lower N content than other digesta ( $p < 0.001$ ;  $p$ -values ranged from 0.82 to 0.99 for all other post hoc contrasts). Thus, the pattern of %N changing across GIT components in this species paralleled the trend for  $\delta^{15}\text{N}$  (Fig. 3b and d), which resulted in a significant negative relationship between this isotope and C:N ratio of the digesta ( $p < 0.0001$ ). By contrast,

**Fig. 3** Relationships between  $\delta^{13}\text{C}$  (a, b) and  $\delta^{15}\text{N}$  (c, d) values with C:N composition of digesta of goats fed grass hay (a, c) and browse (b, d). Each symbol represents a different individual and each observation is a separate GIT component. Residuals on the y-axes are from mixed effects models with “Diet” and “Individual” (nested within “Diet”) as factors. *Fit lines* represent simple linear correlations: **a**  $r=-0.176$ ,  $p=0.153$ ; **b**  $r=-0.360$ ,  $p<0.01$ ; **c**  $r=-0.056$ ,  $p=0.647$ ; **d**  $r=-0.560$ ,  $p<0.0001$



replacement of “GIT component” with C:N ratio in the mixed-effects models revealed no significant effect of C:N on digesta  $\delta^{13}\text{C}$  values ( $p=0.87$ ).

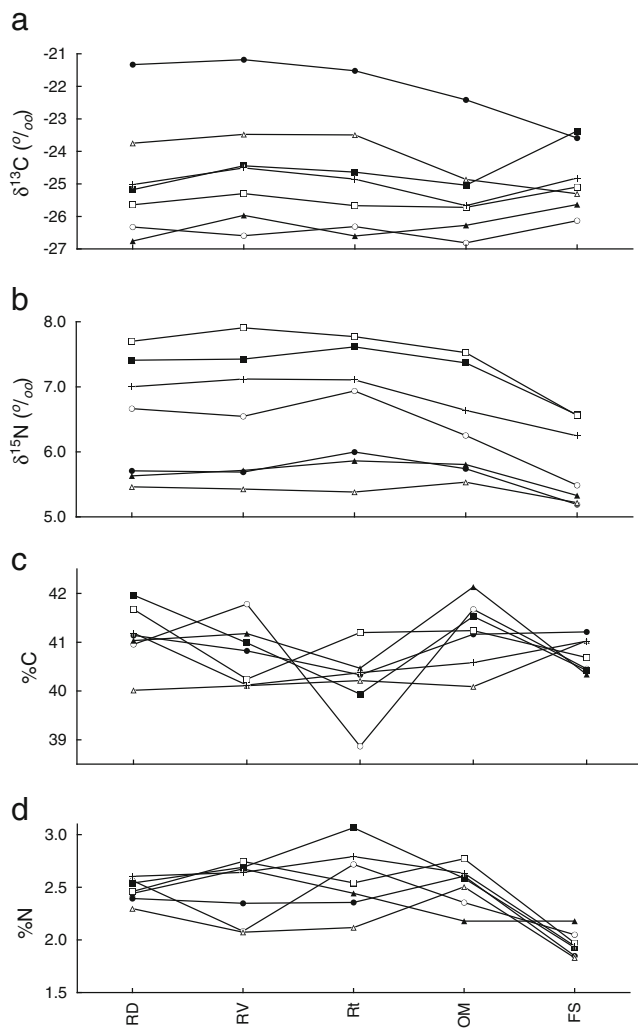
## Discussion

These results reveal few substantial changes in the stable isotope compositions, especially in  $\delta^{13}\text{C}$  values, of digesta from foregut, through the ruminant GIT, to production of faeces. Although we could not statistically accept the hypotheses that  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  values are static through the GIT, the changes that did occur were of small magnitude and occurred in only a few GIT components. Consequently, these differences seem unlikely to alter systematic relationships between diet and faecal isotope compositions.

Changes to digesta  $\delta^{13}\text{C}$  values within individuals only occurred in the lower (most ventral) ventral rumen, and in the small intestine, entailing  $^{13}\text{C}$  enrichment of no more

than 1.0‰; faecal  $\delta^{13}\text{C}$  values were not different from all other GIT components. Changes to digesta  $\delta^{15}\text{N}$  values along the GIT were in some cases larger (up to ~2.0‰), with relative  $^{15}\text{N}$  enrichment occurring in the lower ventral rumen, small intestine, and in the hindgut. Nonetheless, faecal  $\delta^{15}\text{N}$  values were lower than in hindgut components and thus did not reflect these change. But, two caveats are worth noting. First, amongst goats, the decline in  $\delta^{15}\text{N}$  values from small intestine to faeces did not appear to occur in individuals on the grass diet, resulting in higher (more positive)  $\epsilon^{*15}\text{N}_{\text{faeces-diet}}$  compared with animals fed browse. Second, amongst rheem gazelles, which were fed a mixed  $\text{C}_3$  (lucerne)/ $\text{C}_4$  (grass) diet, faecal  $\delta^{15}\text{N}$  values were significantly lower than in other GIT components sampled.

The quantitative description for goats benefitted from the fact that animals were frozen in living position immediately after euthanasia. This allowed us to sample digesta not only from multiple organs, but from multiple sites within certain organs, mainly the rumen. Such a sampling design is relevant for ruminant ecophysiology because the rumen



**Fig. 4** Variation in  $\delta^{13}\text{C}$  (a),  $\delta^{15}\text{N}$  (b), %C (c), and %N (d) values of digesta along the GIT of seven Rheem gazelle fed a mixture of  $\text{C}_3$  lucerne and  $\text{C}_4$  grass hays for 20 days. Each symbol represents a different individual. GIT components are ordered approximately from anterior to posterior along the x-axis, i.e., from rumen to faeces (see legend of Fig. 1)

content of many species is not homogeneous, but is stratified dorso–ventrally, especially in taxa that habitually consume significant amounts of grass (Hofmann 1973; Clauss et al. 2009a, b; Codron and Clauss 2010). In these taxa, rumen contents are layered, with a gas dome overlaying a fibrous raft in the dorsal region, and a fluid layer overlaying “sludge” of very dense particles in the ventral region. Such an effect was not seen in the ventral rumen of the rheem gazelles. This is most likely due to the fact that the goats had undergone a period of fasting before sampling, whereas the rheem gazelles had not: usually, the rumen content stratification becomes more pronounced after a prolonged fast (Hummel et al. 2009). It may also have been absent in the gazelles because they had not been frozen postmortem, but dissections did take place immediately after euthanizing, and postmortem mixing

in the rumen does not dampen stratification signals even in free-ranging ruminants (Clauss et al. 2009a, b; Codron and Clauss 2010).

Other factors in the experimental design were more limiting. In particular, the ~3-week feeding trials were likely not long enough for animals to reach isotopic equilibrium between their digesta and their diets. A recent longer-term study of goats found that isotopic equilibrium between diet and faeces may require as many as 120 days, or more, of feeding on a constant diet, given inputs from body stores and other non-dietary sources (Codron et al. 2011). The short duration of feeding trials employed for the present study probably accounts for some of the isotopic variation we observed between individuals, as individuals did not feed on experimental diets for long enough for all animals to converge on similar  $\delta$  values (see Martínez del Rio et al. 2009). Convergence in goat  $\delta^{13}\text{C}$  values seemed to occur amongst individuals on the browse diet, but not amongst animals fed grass, a finding consistent with previous results that showed slower  $^{13}\text{C}$  incorporation rates for animals on grass compared with dicot diets (Codron et al. 2011). We do not know the dietary or digesta isotope compositions of individuals at the start of the experiment, which would be needed to test hypotheses about differential turnover rates. Similarly, our data do not allow investigation of whether convergence in  $\delta^{15}\text{N}$  values amongst goats fed grass, but not amongst those fed browse, occurred because of slower  $^{15}\text{N}$  incorporation rates on the browse diet (different turnover patterns of  $^{13}\text{C}$  and  $^{15}\text{N}$  have been documented previously in birds; Bearhop et al. 2002). We observed even larger between-individual variation amongst rheem gazelles, probably related not only to the short duration of these experiments, but also to differences in digestibility of the  $\text{C}_3$  lucerne and  $\text{C}_4$  grass components of the diets fed to these animals (Wittmer et al. 2010; Codron et al. 2011).

Although we found only a few changes across GIT components, sites where differences did occur (ventral rumen and small intestine contents) are of interest for understanding processes that influence isotope discrimination patterns. Enrichment of  $^{13}\text{C}$  and  $^{15}\text{N}$  in the ventral rumen and small intestine was associated with lower C, and higher N, content, of digesta at these sites. The emergent trend is that there is a negative relationship between C:N ratios with  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  values (and with  $\epsilon^*_{\text{DD}}$ ) of digesta. One possibility is that isotope compositions of digesta at these sites are relatively enriched. For example, in a stratified rumen contents, the ventral rumen contains sedimented, digested plant material (Clauss et al. 2009a, b); consequently, the concentration of refractory materials, such as lignin, is likely to be highest here. However, lignin (ADL) components of the experimental diets were not consistently enriched in  $^{13}\text{C}$  and  $^{15}\text{N}$  relative to bulk diet (Table 1); thus, a difference in isotopic composition of diet components does not provide an



adequate explanation for the pattern. In the small intestine, composition of digesta changes mainly because of high fluid concentrations that aid nutrient absorption in this organ (Boyne et al. 1956; Ash 1968). The high fluid content (~95%) leads to dispersion of digesta particles, and consequently a relatively larger proportion of C and N in the small intestine is derived from body secretions. Since animal body tissues are usually enriched in both  $^{13}\text{C}$  and  $^{15}\text{N}$  relative to diet, digesta, and faeces (Tieszen et al. 1983; Sutoh et al. 1987; Ambrose and Norr 1993; Cerling and Harris 1999), it is reasonable to expect that secretions into the small intestine should be enriched relative to digesta. Fluid content is 10 to 15% lower (i.e., ~85%) in the caecum and colon (Ash 1968), which would have resulted in higher particle concentrations there and, consequently, a decrease in digesta  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  values to resemble values of foregut components. This hypothesis could be tested by a study of the quantities of C and N present in the small intestine that are derived from the body pool.

An alternative explanation, that would account for isotopic enrichment in both the ventral rumen and the small intestine, is that higher rates of microbial activity associated with lower C:N ratios lead to larger isotopic enrichment. It has been shown from in vitro experiments that cultured ruminal microbes have a lower (less positive)  $^{15}\text{N}$  discrimination effect when grown on substrates containing carbohydrates than on non-carbohydrate substrates, especially if the former entails substantial ammonification (Wattiaux and Reed 1995). In other words, more digestible substrates (lower C:N), entailing higher digestion/fermentation rates could be driving isotopic enrichment; this may have applied to the ventral rumen of the goats of this study.

The  $^{15}\text{N}$  depletion of digesta in hindgut compartments posterior to the small intestine did not seem to occur in goats fed grass hay. It has been suggested that ruminants with a browser-like GIT anatomy rely more heavily on hindgut (caecal) fermentation than species with a grazer-like anatomy—this should prevent lignified browse remaining in the forestomach too long and blocking passage of digesta (Hofmann 1973, 1989). Although the magnitude of this difference is debatable, such a mechanism could have evolved to enable browsers to maximize fibre digestion that may, in the rumen, be limited by a relatively higher passage rate of digesta (Hofmann 1989; Hummel et al. 2006). Within a species, a grass-based diet may actually result in longer digesta retention times in the rumen than a browse-based diet (Lechner et al. 2010; Clauss et al. 2011). If hindgut fermentation rates were significantly higher in browsed than grass-fed goats used in this study, the arising less positive  $^{15}\text{N}$  discriminations (see Wattiaux and Reed 1995) could explain the decrease in  $\delta^{15}\text{N}$  values in the caecum, colon, and faeces of browse-fed goats. Similar patterns of  $^{15}\text{N}$  enrichment from the stomach to the small

intestine, and depletion in the colon (faeces), have been documented in hindgut-fermenting rodents (Hwang et al. 2007).

Overall, our results demonstrate consistency in diet–faecal stable isotope compositions, with only limited variability arising from digestive processes through the GIT. Further, despite the short duration of feeding trials, estimated  $\epsilon^*_{\text{faeces-diet}}$  values for goats ( $-1.0 \pm 1.0\text{‰}$  and  $1.7 \pm 1.0\text{‰}$  for  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$ , respectively) are within the ranges reported from previous experimental studies of ruminants (Jones et al. 1981; Sutoh et al. 1987; Sponheimer et al. 2003; Codron et al. 2011). The study of isotopic changes in the GIT of rodents mentioned above (Hwang et al. 2007) found less consistency between faecal and diet isotope compositions than observed here. We had expected the opposite result, given that our animals were not kept on laboratory diets until equilibrium, and because we sampled a wider diversity of GIT components in species with even more complex digestive anatomies (ruminants). Some of the variation reported by Hwang et al. (2007) likely arose from their use of pelleted diets comprising numerous ingredients (as we observed in rheem gazelles fed mixed diets). However, disparity between their results and ours could also be due to inherent isotopic differences between individual animals. For example, we ascribed the somewhat high level of variation observed across individuals (relative to low variability within diets) to the fact that our experiments were too short for diets and digesta to reach isotopic equilibrium. Yet using a single pool turnover model (see e.g. Martínez del Río and Anderson-Sprecher 2008) with the slowest half-life reported for  $^{13}\text{C}$  in faeces (2.6 days; Codron et al. 2011), we predicted that after 20 days, individuals should have converged on more similar faecal  $\delta^{13}\text{C}$  values than is apparent in Fig. 2a. Thus, unless  $^{13}\text{C}$  incorporation rates of goats in this study were particularly slow, non-equilibrium only partially explains the substantial between-individual variations we encountered.

We conclude that faecal stable isotope compositions are not unpredictably influenced by digestive processes along the ruminant digestive tract, and thus are reliable archives of diet isotope composition. Two caveats are noted: first,  $^{15}\text{N}$  abundances (but not  $^{13}\text{C}$  abundances) in faeces may be influenced by digestive processes depending on diet type and nutritional value and second, while isotopic consistency persists along the GIT of individuals, the same levels of diet–consumer isotope fractionation do not necessarily occur across individuals of the same species. In our study, especially goats, digesta isotope compositions varied more widely across than within individuals fed the same diets—by magnitudes greater than the isotopic variability within any one diet. Variation between individuals also seems to have been a factor in the rodent study (see Figs. 1 and 2 in Hwang et al. 2007), and interindividual variations in tissue–diet discrimination have already been described in a study of

free-ranging sea otters (Newsome et al. 2010). Actually, stochasticity in animal isotope compositions at individual levels appear in virtually all experimental datasets; this source of variation has obvious consequences for studying wildlife diets, and should form an important component of future experiments, theoretical models, and metaanalyses.

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