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Fractionation of nitrogen isotopes by animals: a further complication to the use of variations in the natural abundance of ^{15}N for tracer studies

By K. W. STEELE* and R. M. DANIEL
University of Waikato, Hamilton, New Zealand

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SUMMARY

A study of the fractionation of nitrogen isotopes in the diet by cattle is described and the results discussed.

Compared with the diet, urine had a lower ratio of ^{15}N to ^{14}N, but faeces, blood and milk all had a higher ratio.

It is argued that the use of natural ^{15}N as a tracer in grazed ecosystems is more complicated than was at first thought.

INTRODUCTION

It is well documented that the natural abundance of ^{15}N in total and mineralizable soil nitrogen varies between soils (Cheng, Bremner & Edwards, 1964; Bremner & Tabatabai, 1973; Feigin *et al.* 1974*a*, *b*), and is also often significantly different from that of fertilizer nitrogen (Kohl, Shearer & Commoner, 1971; Freyer & Aly, 1974; Edwards, 1973; Shearer, Kohl & Commoner, 1974; Rennie & Paul, 1975). This latter difference led Kohl *et al.* (1971) to postulate that the contribution of fertilizer nitrogen to nitrogen in surface water could be estimated by measurement of its isotopic composition. This approach has been severely criticized (Hauck *et al.* 1972), and it is generally considered that, because of the small difference in ^{15}N abundance between soil and fertilizer nitrogen, and its high variability, the use of natural variations in the abundance of ^{15}N can at best be semi-quantitative (Bremner & Tabatabai, 1973; Feigin *et al.* 1974*a*, *b*). In field studies, even qualitative estimates may be of doubtful validity (Black & Waring, 1977). Many nitrogen isotopic fractionations which occur during chemical and biological reactions in soils have been investigated (Delwiche & Steyn, 1970). We report here a previously unmeasured fractionation that occurs in cattle.

* Present address: Ruakura Agricultural Research Centre, Private Bag, Hamilton, New Zealand.

MATERIALS AND METHODS

In order to determine the effect of animals on the isotopic composition of nitrogen, a selection of animals housed in indoor stalls were fed on a fixed diet for 21 days with complete collection of urine, faeces and milk over the final 7 days. Blood samples were collected from the jugular veins of two animals at the conclusion of the 7 day period. Four types of diet were compared. Ryegrass (*Lolium perenne*)/white clover (*Trifolium repens*) pasture stored frozen for 6 months; hay and silage made from this pasture; and cake made from macerated pasture from which the juice had been removed from the fibrous residue by means of a belt press. This latter process removed approximately 35% of the protein.

Fresh milk, urine and blood samples, and freeze dried feed and faeces samples were analysed. Total nitrogen was converted to ammonium nitrogen by Kjeldahl digestion after pretreatment of the sample with reduced iron and H_2SO_4 to convert any nitrate and nitrite nitrogen to ammonium nitrogen (Goh, 1972). Ammonium nitrogen was converted to molecular nitrogen using the method of Ross & Martin (1970) and isotopic composition determined on a Micromass 602C mass spectrometer equipped with a twin inlet system and dual collector plates. Six successive comparisons of sample and reference gas were made for each determination. Results are expressed as the

difference ($\delta^{15}N$) from the ratio of the number of atoms of ^{15}N to the number of atoms of ^{14}N in atmospheric nitrogen, where

$$\delta^{15}N = \frac{(^{15}N/^{14}N)_x - (^{15}N/^{14}N)_{\text{Atmospheric N}}}{(^{15}N/^{14}N)_{\text{Atmospheric N}}} \times 1000.$$

Table 1. *Determination of $\delta^{15}N$ of six replicates of a pasture, urine and faeces sample*

	δ^{15}N relative to atmospheric N		
	Pasture	Urine	Faeces
	+0·63	−1·49	+2·74
	+0·77	−1·37	+3·32
	+0·89	−1·72	+3·09
	+0·69	−1·18	+3·38
	+0·83	−1·49	+3·13
	+0·77	−1·67	+3·01
Mean	+0·76	−1·49	+3·11
S.D.	0·09	0·20	0·23

Table 2. *Variations in $\delta^{15}N$ values in feed, urine, faeces, blood and milk of animals fed on different diets*

		δ^{15}N				
Animal	Diet	Feed	Urine	Faeces	Milk	Blood
Jersey cow						
1	Pasture	0·6	−1·7	2·6	4·3	—
2	Pasture	0·6	−1·5	2·6	4·2	—
3	Cake	0·7	−1·4	2·3	4·3	—
4	Cake	0·7	−2·4	3·1	4·3	—
Angus steer						
1	Hay	0·6	−0·8	2·5	—	—
2	Hay	0·6	−0·6	2·7	—	—
3	Silage	0·6	−2·1	2·5	—	4·7
4	Silage	0·6	−2·8	2·1	—	4·9

A working standard of reagent grade ammonium sulphate with a $\delta^{15}N$ value of 0·22 compared with atmospheric nitrogen was used. The precision of the method used for determination of $\delta^{15}N$ is shown in Table 1, where results for the determination of $\delta^{15}N$ of six replicate samples of pasture, urine and faeces are presented.

RESULTS AND DISCUSSION

Results presented in Table 2 show significant differences in the nitrogen isotope-ratio of feed, urine, and faeces examined. Food was enriched in $\delta^{15}N$ by about 2 during passage through the gut, while urine was depleted by about 2 with respect to feed and by up to 7 with respect to blood. This nitrogen isotope fractionation is of the same order of magnitude as the natural variation (Hauck, 1973). The isotopic composition of urine was found to vary throughout the day (Table 3), the depletion of ^{15}N being greatest during the middle of the day, presumably when the animals were feeding. It is also of interest to note that the urine of steers fed on hay was less depleted in ^{15}N than the urine of

Table 3. *Diurnal variation in $\delta^{15}N$ values in the urine of steers receiving silage ($\delta^{15}N$ = 0·6)*

	δ^{15}N	
Collection period (h)	Angus steer 5	Angus steer 6
12.00–15.00	−3·2	−3·2
15.00–18.00	−2·0	−1·6
18.00–09.00	−1·1	−1·0
09.00–12.00	−3·2	−3·5

Table 4. *Seven-day nitrogen balance estimates for four Jersey cows*

Animal	Diet	Total nitrogen intake (kg/7 days)	Total nitrogen losses* (kg/7 days)	^{15}N intake (g/7 days)	^{15}N losses* (g/7 days)
Jersey cow					
1	Pasture	2·72	2·67	10·72	10·52
2	Pasture	2·61	2·37	10·28	9·34
3	Cake	2·26	2·25	8·91	8·86
4	Cake	2·22	1·99	8·75	7·84

* Calculated from values for faeces, urine and milk.

animals fed on cake, grass or silage. Despite the variation in $\delta^{15}N$ of feed, faeces and urine, nitrogen inputs match nitrogen outputs reasonably closely, confirming that the animals were in approximate nitrogen balance during the 7 day period under study (Table 4), and supporting the validity of the technique used. One further feature of the reported data is the relative consistency of the faeces enrichment in ^{15}N over that of the feed. If faeces enrichment is constant for a given feed, it may be possible to use the nitrogen isotopic composition of faeces to determine the proportional intake of two feeds which differ in nitrogen isotopic composition.

A necessary condition for the valid use of methods based on differences in the natural isotopic composition of soil, fertilizer and atmospheric nitrogen to determine the contribution of fertilizer and soil nitrogen to nitrogen in rivers, lakes and plants, is that each source of nitrogen must maintain its isotopic identity throughout all transformations.

The present results suggest that this condition is unlikely to be met where grazing animals are present, since more nitrogen is excreted in urine than in faeces, and loss of nitrogen is normally greater from urine than from dung (Whitehead, 1970). This further complicates the use of small natural variations in ^{15}N to trace nitrogen flow in grazed ecosystems.

Rumen microflora are unlikely to be the sole source of the observed fractionation since we have preliminary evidence showing a similar fractionation in humans and pigs.

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