Plant and Soil **162**: 31–37, 1994.

Labelling of animal manure nitrogen with ^{15}N

P. Sørensen[1], E.S. Jensen[1] and N.E. Nielsen[2]
[1] *Plant Biology Section, Environmental Science and Technology Department, Risø National Laboratory, DK-4000 Roskilde, Denmark and* [2] *Section of Soil, Water and Plant Nutrition, Department of Agricultural Sciences, The Royal Veterinary and Agricultural University, Thorvaldsensvej 40, DK-1871 Frederiksberg C, Denmark*

Received 23 June 1993. Accepted in revised form 18 January 1994

Key words: homogeneity of labelling, mineralization, mobile nylon bag, ryegrass hay, sheep manure, water-soluble N

Abstract

A sheep was fed on ^{15}N-labelled ryegrass hay during a period of 9 days in order to obtain ^{15}N-labelled manure. After 9 days of feeding, the total N in faeces contained 3.70 atom % ^{15}N excess, which was equivalent to 82% of the ^{15}N enrichment of the hay N. The easily-decomposable fraction of the faecal N was less labelled (2.89 atom % ^{15}N excess) than the slowly-decomposable fraction. The ^{15}N enrichment of mineralized faecal N did not change significantly during 32 weeks of incubation in sand. About 25% of the faecal N was water-soluble. This N had a higher ^{15}N enrichment than the total faecal N, indicating that a part of the water-soluble N was indigestible feed N. The faeces contained only small amounts of NH_4^+-N, which had a ^{15}N enrichment similar to the ^{15}N enrichment of N mineralized during incubation in sand. It is suggested that the labelled faecal N obtained after a few days of feeding on labelled feed could be divided in two N pools: A decomposable N fraction (about 60%) with a ^{15}N enrichment similar to the enrichment of N mineralized in sand (2.89 ± 0.09 atom % ^{15}N excess), and a very slowly-decomposable N fraction (about 40%) with a ^{15}N enrichment similar to that of the feed (4.52 atom % ^{15}N excess).

Introduction

Due to the many processes involved in the turnover and losses of manure N in soils (Castellanos and Pratt, 1981; Thompson et al., 1987; van Faassen and van Dijk, 1987), the plant-availability of nitrogen in animal manures is difficult to estimate. The fate of animal manure nitrogen applied to soil may be studied by using ^{15}N-labelled manure. Different methods have been used for labelling of animal manures with ^{15}N. Herbst et al. (1987) and Vilsmeyer and Gutzer (1990) labelled the NH_4^+-N pool of a cattle slurry by adding a small amount of highly enriched NH_4^+-N. Labelling the organic N fraction of manure homogeneously with ^{15}N is more difficult. Rauhe et al. (1973) and Peschke (1982) fed cows on ^{15}N-labelled plant material for a single day. Rauhe and Bornhak (1970) used the excrements of a cow, that had been given a feed containing ^{15}N labelled urea. Kirchmann (1985) produced ^{15}N labelled poultry manure by feeding a cock on ^{15}N enriched barley grain.

The aim of the present study was to evaluate the homogeneity of ^{15}N enrichment of sheep faecal N. The sheep was fed on ^{15}N-enriched ryegrass hay for 9 days.

Theoretical considerations

According to its origin, faecal N from ruminants may be partitioned into the following fractions:

a) indigestible feed N, of which the pool size is dependent upon the nature of the feed;
b) microbial N from the rumen, contributing about 17% of faecal N, as estimated from Danfær (1990);
c) nitrogen in digestive secretions, mucus and dead cells from tissues in the digestive tract;

d) nitrogen in living and dead microbes from the intestine and the hind gut, contributing about 33% of faecal N (Danfær, 1990).

During the initial days of feeding on ^{15}N-labelled feed, ^{15}N in the faeces is diluted by unlabelled indigestible feed N remaining in the digestive tract from the period preceding ^{15}N feeding and by endogenous N excreted to the digestive tract (Nolan, 1975). Subsequently, ^{15}N in faeces continues to be diluted by partly-labelled endogenous N. Thus homogeneous labelling of manure requires a long feeding period on ^{15}N-labelled feed.

It is suggested that the faecal N fractions a, b, c and d may be divided into two main N pools. Pool 1 consists of undigested feed N and similarly-enriched microbial N from the rumen, after a few days of feeding on labelled feed (even though there is some endogenous secretion of N to the rumen (Nolan, 1975)). Pool 1 is considered to be relatively slowly-decomposable in soil, because its components have passed the digestive tract having been exposed to microbes, acids and digestive enzymes in the animal without being assimilated.

Pool 2 of the faecal N consists of N compounds in living microbes, partly-decomposed microbial tissues, digestive secretions and dead cells from tissues in the digestive tract (Nolan, 1975). Consequently this N pool is relatively more-decomposable in soil than pool 1. Nitrogen in pool 2 is diluted by endogenous N originating from the animal, and is consequently less highly-labelled than the feed N.

Materials and methods

Production of ^{15}N-labelled sheep manure

Italian ryegrass (*Lolium multiflorum* L. cv. Ninak) was grown in 20 L pots containing quartz sand. The pots were irrigated automatically with a circulating nutrient solution containing basic nutrients, which included ^{15}N-labelled N fertilizer. Each pot was fertilized with 2.0 g KNO_3-N with 5.0 atom % ^{15}N excess. The grass was cut and air-dried after 54 days, and another 2.0 g ^{15}N-labelled KNO_3-N and basic nutrients were applied. The second cut was taken after 44 days. After air-drying the two cuts were chopped into 2 cm lengths, and mixed. Other pots were fertilized with KNO_3 of natural ^{15}N abundance for production of unlabelled hay. The ^{15}N-labelled grass hay contained 2.17% total N (in DM) and 4.523 atom % ^{15}N excess.

Table 1. Composition of a mixture of sheep faeces collected 8 and 9 days after commencement of feeding on ^{15}N-labelled hay

	Content (% of DM)
Total N	3.08%
Total C	46%
NH_4^+-N	0.036%
NO_3^--N	Not detectable
Water-soluble N	0.70%
Water-soluble C	9.1%

An adult castrated sheep was fed in sequence at a rate of 950 g DM per day on Italian ryegrass hay grown in the field, followed by unlabelled, pot-grown hay for 7 days, ^{15}N-labelled pot-grown hay for 9 days, and finally unlabelled pot-grown hay for a 3 further days. Faeces was quantitatively collected in plastic bags attached to the sheep (urine was collected separately). The faeces was pooled and mixed after 24 hours collection, and frozen (−18°C). The composition of faeces collected on days 8 and 9 after starting the ^{15}N feeding is shown in Table 1.

To estimate the content of indigestible feed N in the faeces, a mobile nylon bag procedure was used (Hvelplund, 1985). The feed was milled pass a 1 mm sieve. One gram hay samples were sealed in nylon bags (pore size 9 μm; dimensions 60 × 60 mm). Ten bags were placed in the rumen of a fistulated dairy cow for 16 h and thereafter incubated in a pepsin-HCl solution for 2 h at 37°C. Finally, all the bags were inserted into the duodenum of the cow and recovered in the faeces. After washing and drying, the nylon bag content was analysed for total N and ^{15}N enrichment.

Experimental procedures

Incubation experiments were carried out to evaluate the homogeneity of ^{15}N-labelling of the sheep faeces.

Experiment 1

Labelled faeces from different sampling days was incubated in quartz sand in order to evaluate the ^{15}N enrichment of mineralized N. Moist and homogenized faeces, sampled on days 2, 5, 8 and 10 after commencement

of feeding on ^{15}N-labelled hay, and containing 10.0 mg N, were mixed with 50 g dry quartz sand in 250 mL bottles. The water content was adjusted to 55% of the water-holding-capacity (WHC) of the sand, and the bottles were loosely covered by lids of aluminium foil in order to reduce losses of water and incubated at 20°C. The moisture content was readjusted weekly. After 21 and 84 days of incubation, three replicates of the sand-faeces mixtures were extracted with 2 *M* KCl (1:10) (Keeney and Nelson, 1982) and analysed for NO_3^--N, NH_4^+-N and ^{15}N enrichment of inorganic N.

Experiment 2

In order to evaluate the enrichment of mineralized faecal N during a longer period of decomposition, a mixture of freeze-dried faeces sampled on days 8 and 9 was ground to pass a 2 mm sieve, mixed with quartz sand (5 mg faecal N in 25 g sand) and incubated as in Exp. 1. After 1, 2, 4, 8, 16 and 32 weeks of incubation three replicates were extracted with 2 *M* KCl (1:10) and extracts analysed as in Exp. 1.

Manure C mineralization rates were determined from CO_2 evolution. Separate triplicates of the sand-faeces mixture were placed in 2-L jars with a beaker of 1 *M* KOH for absorption of CO_2 and a beaker of water to maintain the water content in the sand-faeces mixture. Samples of sand without manure were used as blanks. The CO_2 absorbers were changed and analysed after 1, 2, 4, 8, 16 and 32 weeks.

Experiment 3

In order to evaluate whether the enrichment of mineralized faecal N changed during incubation in soil, the ^{15}N-labelled faeces used in experiment 2 was also incubated in a sandy soil at 25°C. The soil contained 0.12% total N, 2.2% organic matter, 4.5% clay (< 0.002 mm), 4.2% silt (0.002–0.02), 19% fine sand (0.02–0.2 mm) and 70% coarse sand (0.2–2 mm), soil pH (H_2O) was 5.8. Dry faeces (103 μg N g^{-1} soil) was mixed with 50 g air-dried soil and water was added to 55% of the WHC. The water content was adjusted weekly. Control soil without faeces was also incubated. After 2, 7, 14, 28, 56, 84 and 112 days of incubation, 3 replicates were extracted with 2 *M* KCl, and the extract was analysed for NO_3^--N, NH_4^+-N and ^{15}N enrichment of inorganic N.

Chemical analysis

Total N and C in faeces were determined by elemental analysis using a Carlo Erba NA1500 N/C analyzer according to Jensen (1991) after drying the material at 80°C for 24 h. The ^{15}N enrichment was determined on a mass spectrometer (Delta, Finnigan MAT) coupled on-line to the elemental analyzer according to Jensen (1991). Ammonium-N and NO_3^--N in centrifuged and filtered extracts were measured on a Technicon Auto-Analyzer II using the sodium salicylate-sodium nitroprusside-hypochlorite method for NH_4^+-N (Technicon, 1974), and the sulfanilamide-naphthylethylenediamine method for NO_3^--N after reducing nitrate to nitrite with hydrazine (Kamphake et al., 1967).

Inorganic N in extracts was concentrated before ^{15}N analysis using a diffusion procedure; N was diffused as NH_3 to an acidified glass filter enclosed in polytetrafluoroethylene (teflon) tape by adding MgO and Devarda's alloy to the solution (Sørensen and Jensen, 1991).

The CO_2-C absorbed in KOH was determined by titration with 0.2 *M* HCl, after addition of $BaCl_2$ to precipitate carbonate.

The water-soluble N in faeces was extracted by shaking one gram of freeze-dried sample in 20 mL water for 15 minutes. The extract was centrifuged (20000 *g*, 20 min, 4°C), and the supernatant was filtered through a 0.22 μm filter, freeze-dried and analysed for total N and ^{15}N.

All results are expressed on an oven-dry basis (soil 105°C, 24 h; faeces 80°C, 24 h).

Statistical analysis

Analysis of variance was carried out on data using the SAS procedure GLM (SAS, 1989).

Results and discussion

The ^{15}N enrichment of faecal N from a sheep fed on ^{15}N-labelled hay (4.52 atom % ^{15}N excess), increased with time reaching 3.70 atom % ^{15}N excess after 9 days of feeding (Fig. 1). By the end of the allocated experimental period for feeding on ^{15}N-labelled hay, the ^{15}N enrichment of faecal N continued to increase slightly. Thus, a longer period of feeding on ^{15}N hay would probably have resulted in a higher ^{15}N enrichment of the faecal N.

Table 2. Atom % ^{15}N excess in N fractions of sheep faeces after 2 to 10 days of feeding on ^{15}N-labelled Italian ryegrass hay and atom % ^{15}N excess of inorganic N after incubation of the faeces in quartz sand (Experiment 1)

Labelling period (days)	Atom % ^{15}N excess at sampling			Atom % ^{15}N excess of inorganic N after incubation	
	Total N	NH_4^+-N	Water-soluble N	21 days	84 days
2	1.14	1.07	1.77 (0.013)[a]	1.03 (0.09)	1.09 (0.002)
5	3.09	2.41	3.38 (0.046)	2.31 (0.09)	2.36 (0.02)
8	3.57	2.83	ND[b]	2.69 (0.05)	2.74 (0.03)
10	3.70	2.96	3.83 (0.001)	2.77 (0.08)	2.83 (0.12)
8+9	3.55 (0.005)	2.93 (0.007)	3.94 (n = 1)	ND	ND

[a] standard deviation; standard deviation is not shown where n = 2.

[b] ND = not determined.

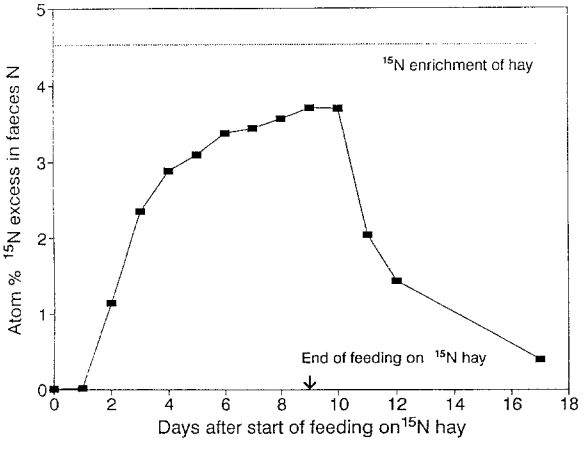

Fig. 1. ^{15}N enrichment of sheep faeces sampled during a period of 9 days of feeding on ^{15}N-labelled ryegrass hay.

The NH_4^+-N in faeces constituted 1.2% of total N (Table 1), and the ^{15}N enrichment of NH_4^+-N in the faeces was lower than the enrichment of total N (Table 2). This indicates that a higher proportion of the NH_4^+-N was derived from endogenous unlabelled N, than was that of the total N. The water-soluble N constituted 25% of the total N (Table 1), and the water-soluble fraction had a higher ^{15}N enrichment than the total N (Table 2), even though NH_4^+-N and other soluble endogenous compounds with a low enrichment were probably included in the water-soluble N fraction. This indicates that a high proportion of the water-soluble N was derived from unassimilated feed N and rumen-derived microbial N, both with a high ^{15}N enrichment. The water-soluble N compounds with a high enrichment were probably mainly included in colloids and macromolecules, which could not be absorbed in the digestive tract of the animal. Aoyama (1985) also found membranous and filamentous material in the water-soluble fraction of composts.

Experiment 1

After 21 days of incubation in quartz sand, 7–9% of the organic N in the manure was mineralized (data not shown). The ^{15}N enrichment of mineralized N was significantly lower than that of faecal total N and close to the enrichment of the faecal NH_4^+-N (Table 2). The lower enrichment of mineralized N indicates that it mainly originated from the partly-labelled N pool (pool 2), consisting of secretions, tissue cells and microbes from the intestine and the hind gut. The ^{15}N enrichment of mineralized N after 84 days was not significantly different from the mineralized N after 21 days. There seemed to be no major mineralization from the water-soluble N fraction, since the ^{15}N enrichment of the mineralized N was much lower than the enrichment of the water-soluble N.

The ^{15}N enrichment of N mineralized from samples of faeces excreted after 2 days feeding on labelled hay was close to the enrichment of the total N of the faeces (Table 2). The explanation may be that the main part of the slowly-decomposable fraction of the faecal N (pool 1) at the day 2 sampling was still unlabelled N, originating from the preceding feeding period on unlabelled hay. The ^{15}N enrichment of the water-soluble N fraction was also higher than the ^{15}N enrichment of the total N at this stage of the feeding period (Table 2).

Experiment 2

Labelled faeces from sampling days day 8 and 9 was incubated in quartz sand for 32 weeks. The ^{15}N enrichment of released inorganic N did not change significantly between week 1 and week 32, averaging 2.89 ± 0.09 atom % excess, compared to 3.52 atom % ^{15}N excess in the faecal total N. After a few weeks incubation inorganic N concentration decreased due to net immobilization, since no losses of total N were detected (data not shown). The observed nearly-constant enrichment of the inorganic N therefore represents that of the manure N actively turning over during the period. After 16 weeks of incubation 20% of the faeces C was recovered as CO_2. The CO_2 evolution rate from the manure in sand was about the half of that observed from a similar manure in soil with a natural microbial population (unpublished results).

Experiment 3

The microbial population in soils may be able to decompose organic N compounds more efficiently than the microbial population developed in quartz sand. Therefore the labelled manure was also incubated in a sandy soil, in which the immobilization of N was expected to be small. The ^{15}N enrichment of mineralized N was assumed to be the same as in the quartz sand incubation (viz. 2.89 atom % ^{15}N excess). After 7 days of incubation the soil contained 11 μg N g^{-1} soil as inorganic labelled N (Fig. 2) corresponding to 10% mineralization of the labelled organic N. The mineralization of labelled N continued through the incubation period. After 112 days the soil contained 33 μg N g^{-1} soil as inorganic labelled N (Fig. 2) corresponding to 32% mineralization of the labelled organic manure N. There was a highly significant linear correlation, between the net mineralization from manure N, calculated by difference, and the recovery in the soil of labelled inorganic N from the manure (Fig. 3). This indicates that the manure N mineralized during incubation was homogeneously labelled.

Nitrogen pools in sheep faeces

Approximately 9.1% of the total N and 5.7% of the labelled N from the hay was recovered in the nylon bags after passage through the digestive system of a cow. The apparent digestibility of the feed N (N in feed minus N in sheep faeces during 7 days) was determined to be 64.7%. From the ^{15}N recovery in the mobile

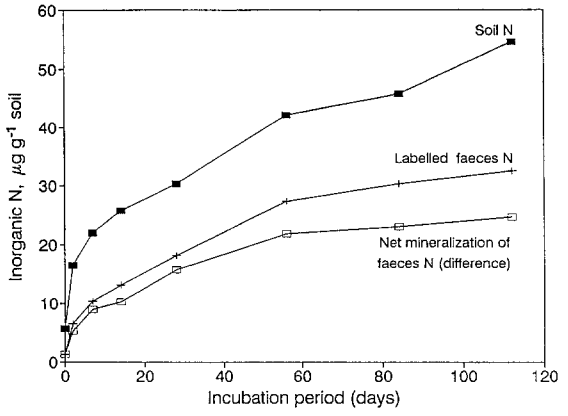

Fig. 2. Net mineralization of soil N (control soil), labelled faeces N (103 μg N g^{-1} soil) and faeces N (difference between experimental and control soil) in an incubation experiment. The mean standard deviations of inorganic N determinations were 0.9 in control soils, 1.1 in soils with manure and 0.5 in labelled N.

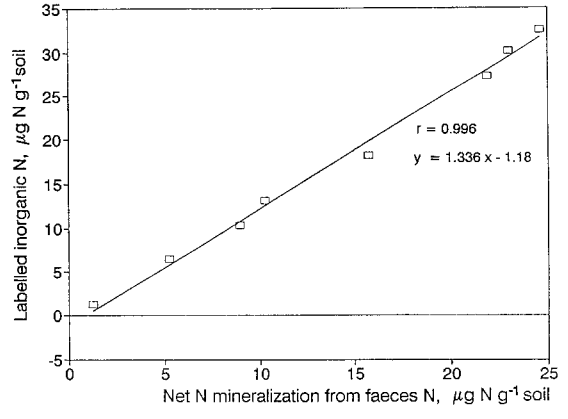

Fig. 3. Linear relationship between net mineralization of faeces N and accumulation of labelled inorganic N from sheep faeces from day 8 and 9 (see Fig. 1) incubated in soil.

nylon bags and the apparent N digestibility, it can be calculated that 16% of the faecal N was indigestible feed N (% indigestible feed N in faecal N = fraction of feed N recovered in nylon bags × 100/(total N in faeces/total N in feed) = 0.0566 × 100/(1–0.647)). This estimate is probably too low, because indigestible water-soluble N is lost from the nylon bags.

Estimates of the true digestibility are often based on the total N recovery in nylon bags (Hvelplund, 1985). Using total N recovery in nylon bags it can be estimated that 26% of the faecal N was indigestible feed N (0.091 × 100/(1–0.647)). Due to ^{15}N leaching and accumulation of ^{14}N in the bags (which was also observed by Varvikko and Vanhatalo, 1990), estimates based on recovery of total N seem to be more realistic than esti-

mates based on ^{15}N recovery. Hence the size of pool 1 (indigestible feed N + microbial N from rumen) is assumed to be 26% + 17% = 43% of the faecal N, of which the 17% are residues of microbial N from the rumen. The ^{15}N enrichment in pool 1 is assumed to be the same as for the feed.

The remaining 57% of the total faecal N is assumed to be in pool 2. The relationship between ^{15}N atom % enrichments in faecal total N, of N in pool 1 and of N in pool 2 can be expressed by:

$$\begin{aligned}&\text{Atom \% } ^{15}\text{N enrichment in total N} = \\ &\quad((\text{atom \% } ^{15}\text{N enrichment})_{\text{pool 1}} \\ &\qquad \times N_{\text{pool 1}}/\text{total N}) \\ &\quad +((\text{atom \% } ^{15}\text{N enrichment})_{\text{pool 2}} \\ &\qquad \times N_{\text{pool 2}}/\text{total N})\end{aligned}$$

Solving this equation for $(\text{atom \% } ^{15}\text{N enrichment})_{\text{pool 2}}$, the atom % ^{15}N enrichment in pool 2 is found to be 2.82.

This estimate corroborates the view that N in pool 2 includes endogenous N originating from the animal. Consequently, N in pool 2 is not labelled to the same extent as N in pool 1 after 9 days feeding on ^{15}N-labelled ryegrass. The ^{15}N enrichment of mineralized N was 2.89 ± 0.09 during the incubation period, showing that pool 2 apparently is much more decomposable than pool 1. Indeed, the mineralization of N from pool 1 was negligible during 32 weeks of incubation of faeces in quartz sand.

Conclusions

Nitrogen mineralized from ^{15}N-labelled manure was at constant ^{15}N enrichment during several months of decomposition, despite being lower enriched than that of the manure total N. Throughout the period of incubation there was no detectable mineralization from pool 1 of faecal N, which consisted of indigestible feed N and microbial N from the rumen (assumed to have the same ^{15}N enrichment as N in the feed). If the manure is used in long-term decomposition experiments, the ^{15}N enrichment of mineralized N will gradually increase, due to mineralization from this slowly-decomposable N pool. The recovery of organic ^{15}N gives only an approximate measure of the actual recovery of manure N.

The ^{15}N-labelled manure can be a very useful tool in ^{15}N balance studies of the fate of manure N in soil-plant-atmosphere systems. It can also be used in studies of short-term gross mineralization of manure N. In cross-labelling experiments it will be possible to distinguish between mineralized soil N, manure N and immobilized N. However, it is important to consider possible heterogeneous ^{15}N enrichment of faecal N pools, and more work is needed to improve methods for labelling ruminant manure with ^{15}N (Sørensen et al., 1994).

Acknowledgements

We thank the staff on The Danish Institute of Plant and Soil Science, Department of Roughage for performing the feeding and the faeces sampling, C Jespersen and M Brink Jensen for skilled technical assistance and Dr A Andersen for useful criticism of the manuscript.

References

Ayoma M 1985 Properties of fine and water-soluble fractions of several composts. 1. Micromorphology, elemental composition and nitrogen mineralization of fractions. Soil Sci. Plant Nutr. 31, 189–198.

Castellanos J Z and Pratt P F 1981 Mineralization of manure nitrogen-correlation with laboratory indexes. Soil Sci. Soc. Am. J. 45, 354–357.

Danfær A 1990 A Dynamic Model of Nutrient Digestion and Metabolism in Lactating Dairy Cows. 671 Report from the National Institute of Animal Science, Denmark. Landhusholdningsselskabets Forlag, Frederiksberg, Denmark. 511 p.

Herbst F, Aziz F and Garz J 1987 Stickstoffumsetzungen im Boden nach Zugabe von ^{15}N-markierter Gülle. Arch. Acker- Pflanzenbau Bodenkd. 31, 169–175.

Hvelplund T 1985 Digestibility of rumen microbial protein and undegraded protein estimated in the small intestine of sheep and by in sacco procedure. Acta Agric. Scand., Suppl. 25, 132–144.

Jensen E S 1991 Evaluation of automated analysis of ^{15}N and total N in plant material and soil. Plant and Soil 133, 83–92.

Kamphake L J, Hannah S A and Cohen J M 1967 Automated analysis for nitrate by hydrazine reduction. Water Res. 1, 205–216.

Keeney D R and Nelson D W 1982 Nitrogen - inorganic forms. *In* Methods of Soil Analysis, Part 2. Chemical and Microbiological Properties. Agronomy Monographs No 9, 2nd edn. Ed. AL Page. pp 643–698. ASA-SSSA, Madison.

Kirchmann H 1985 Losses, plant uptake and utilisation of manure nitrogen during a production cycle. Acta Agric. Scand. Suppl. 24, 1–77.

Nolan J V 1975 Quantitative models of nitrogen metabolism in sheep. *In* Digestion and Metabolism in the Ruminant. Proceedings of the IV International Symposium on Ruminant Physiology. Sydney, Australia. Eds IW McDonald and ACI Warner. pp 416–431. The University of New England Publishing Unit, Armidale.

Peschke H 1982 Gezielte ^{15}N-Anreicherung von organischen Düngemitteln für Versuchszwecke. 2. Mitt.: Herstellung von ^{15}N-markierter Gülle. Isotopenpraxis 18, 104–106.

Rauhe K and Bornhak H 1970 Die Wirkung von ^{15}N-markiertem Rinderkot mit verschiedenen Zusätzen im Feldversuch unter besonderer Berücksichtigung der Reproduction der organischen Substanz im Boden. Albrecht-Thaer-Archiv 14, 937–948.

Rauhe K, Fichtner E, Fichtner F Knappe E and Drauschke W 1973 Quantifizierung der Wirkung organischer und mineralischer Stickstoffdünger auf Pflanze und Boden unter besonderer Berücksichtigung ^{15}N-markierter tierischer Exkremente. Arch. Acker-Pflanzenbau Bodenkd. 17, 907–916.

SAS 1989 SAS Institute Inc., User's Guide, Version 6, Fourth Edition, Vol. 2, Cary, North Carolina.

Sørensen P and Jensen E S 1991 Sequential diffusion of ammonium and nitrate from soil extracts to a polytetrafluoroethylene trap for ^{15}N determination. Anal. Chim. Acta 252, 201–203.

Sørensen P, Jensen E S and Nielsen N E 1994 The fate of ^{15}N-labelled organic nitrogen in sheep manure applied to soils of different texture under field conditions. Plant and Soil 162, 39–47.

Technicon 1974 Technichon Auto Analyzer II Method No. 345–74 W. Ammonia in Water and Waste Water. Technichon Industrial Systems, Tarrytown, New York.

Thompson R B, Ryden J C and Lockyer DR 1987 Fate of nitrogen in cattle slurry following surface application or injection to grassland. J. Soil Sci. 38, 689–700.

Van Faassen H G and Van Dijk H 1987 Manure as a source of nitrogen and phosphorus in soils. *In* Animal Manure on Grassland and Fodder Crops. Eds HG van der Meer, RJ Unwin, TA van Dijk and GC Ennik. pp 27–45. Martinus Nijhoff Publ, Dordrecht.

Varvikko T and Vanhatalo A 1990 The effect of differing types of cloth and of contamination by non-feed nitrogen on intestinal digestion estimates using porous synthetic-fibre bags in a cow. British J. Nutr. 63, 221–229.

Vilsmeyer K and Gutser R 1990 N-Ausnutzung nach Mineral- und Gülledüngung (^{15}N) im Weihenstephaner Lysimeter. Proceedings of the 101st VDLUFA Congress. pp 175–180. VDLUFA-Schriftenreihe, 30, VDLUFA-Verlag, Darmstadt, Germany.

Section editor: G R Stewart