

Secretion and reabsorption of endogenous protein along the small intestine of sheep: estimates derived from ^{15}N dilution of plasma non-protein-N

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Sheep fitted with a PVC cannula in the dorsal rumen, a silastic infusion tube in the abomasum, and single 'T'-shape PVC cannulas in the proximal duodenum (D₁), distal duodenum (D₂), mid-jejunum (J), and terminal ileum (I), were fed on diets varying in crude protein (N×6.25) and fibre contents. ^{15}N was administered to the sheep as a ^{15}N -labelled grass meal-beer yeast suspension which was continuously infused into the abomasum over a period of 1 week. Subsequently, over a period of 4 d, ^{15}N excess in the D₁, D₂, J and I digesta and faeces was measured and related to ^{15}N excess in blood plasma TCA-soluble N. Average daily intakes of DM and N were 1.12 kg and 26.3 g respectively. Average daily endogenous N (g) amounted to 3.1 at D₁, 10.2 at D₂, 6.1 at J, 5.0 at I, and 5.1 in the faeces (F). Corresponding percentage values for total N were: D₁ 12.4, D₂ 32.9, J 46.4, I 48.4 and F 50.7. The secretion, particularly of pancreatic juice and/or bile, varied. The apparent re-absorption of endogenous protein varied among experimental diets, on average by 50.4% between D₂ and I. Along the small intestine the percentage of ^{15}N in the TCA-precipitable fraction gradually changed, on average by 66.0, 63.0, 46.3 and 55.7 at D₁, D₂, J and I respectively. Because of differential rates of enrichment and turnover among protein pools which contribute towards endogenous secretion, care should be taken in interpreting these data as absolute values.

Endogenous protein: Protein conversion: Environmental N burden

Reducing the environmental burden of livestock production systems is a major objective for current nutrition research. To achieve this objective the first option is to replace the external inputs of concentrate feeds and by-products grown elsewhere by home-grown feeds of a comparable nature. If this is not a viable option, including a larger proportion of home-grown alternative feeds in the diet constitutes a second possibility. Hence, increasing the utilization of home-grown feeds has been identified as one of the keys. For ruminant production, particularly in Western Europe, this implies that a larger part of the diet should be fibrous feeds, such as grass or grass-maize silages. However, for higher levels of production, especially of milk, only part of the nutrients can be derived from roughages. Concentrate supplements are needed to meet energy requirements, to optimize rumen conditions and to manipulate the amount and composition of the nutrient mixture rendered available for intermediary metabolism.

Livestock production systems geared towards longer-term sustainability aim at the reduction of the environmental burden in terms of nutrient losses, particularly of N and P. In other words, they aim to optimize (1) the conversion of nutrients into product and (2) the

recycling of nutrients voided with faeces and urine. These aims are not necessarily complementary. Increasing the proportion of roughage in the diet will inevitably result in a lower level of production and an associated decrease in the overall efficiency of conversion of nutrients. However, such a system may be preferable as the most appropriate for recycling of nutrients within the soil-plant-animal system.

With increased proportions of roughage in ruminant diets, voluntary intake of feed may decrease and, hence, the level of production. It is necessary therefore to improve roughage quality through N fertilization and/or harvesting of forage crops at an earlier stage of growth. However, in such material rumen-degradable N is usually available to excess, relative to rumen-degradable organic matter. This excess N is absorbed as NH_3 , converted into urea in the liver and excreted in the urine. Alternatively, if roughages are harvested at a more mature stage, voluntary intake may decrease and fibre:protein ratio in the small intestine increase, so that apparent ileal amino acid digestibilities may decrease (Van Bruchem *et al.* 1989). This effect can be largely attributed to the endogenous protein added to the digesta in the course of the digestion process. This occurs particularly in the small intestine, in the form of pancreatic juice and bile, mucus glycoproteins, lymph and plasma proteins, and the shedding of epithelial cells which line the intestinal mucosa.

Only a minor proportion of the protein N entering the duodenum is of endogenous origin; 2.5 g N per kg DM intake in sheep (Siddons *et al.* 1982) and 3.6 g N/kg DM intake in cattle (Brandt *et al.* 1980). On the other hand, it has been shown that 50–75 % of the protein collected at the terminal ileum is of endogenous origin (Van Bruchem *et al.* 1989; Oosting *et al.* 1995). These multiple-regression-derived estimates showed a positive relationship with the small intestinal flow of non-protein DM, largely consisting of cell-wall constituents and ash.

Neither the extent to which the endogenous protein is reabsorbed, nor the efficiency of conversion of the salvaged amino acids into newly-synthesized endogenous protein are known. The objective of the present study was to obtain a more reliable estimate of the quantity of endogenous protein secreted along the small intestine of ruminants and its extent of apparent reabsorption.

MATERIALS AND METHODS

Animals and housing

Three Swifter (Flemish \times Texel) crossbred wether sheep (live weight approximately 55 kg) were surgically prepared with a PVC cannula (25 mm i.d.) in the dorsal rumen, a silastic infusion tube (3 mm i.d.) in the abomasal fundus, and single 'T'-shaped PVC cannulas (12 mm i.d.) in the proximal duodenum (D_1), the distal duodenum (D_2), the mid-jejunum (J) and the terminal ileum (I). The experiment started 2.5 months after surgery, allowing the animals to recover and fully adapt to the experimental routine.

During the experiment the animals were kept in metabolism cages and received equal portions of their ration at 04.00, 08.00, 12.00, 16.00, 20.00 and 24.00 hours. Water was freely available. For collection of blood samples, an indwelling catheter (Intraflon 2, V498; Vygon, Veenendaal, The Netherlands) was inserted into the jugular vein, just before the beginning of the experimental sample-collection periods.

Feeds

The experimental diets were based on grass hay. To vary protein and cell-wall contents the hay was partly replaced by two types of mixed concentrates, with higher (HP) and lower (LP) crude protein (N \times 6.25) content respectively. The experimental rations were

composed as follows: 1 approximately 1400 g hay; 2 approximately 700 g hay, approximately 700 g HP concentrates; 3 approximately 700 g hay, approximately 700 g LP concentrates. The diets were weighed out for each experimental period, including a preliminary period of 2 weeks and a sampling period of 2 weeks. Representative samples of hay and concentrates were oven dried, air equilibrated, ground to pass a 1 mm sieve, and stored until analysed. The composition of the dietary components is presented in Table 1. Feed residues were collected daily, pooled over the experimental period, sampled and prepared for analyses as indicated for the diet samples.

Infusate ¹⁵N-labelled material

Perennial rye grass (*Lolium perenne*), fertilized at a level of 200 kg N/ha, was given 94.3 g (¹⁵NH₄)₂SO₄ (30 atom % excess ¹⁵N) per m² after the first cutting of forage (14 June). The ¹⁵N label was administered in two portions separated by a 3 d interval. Subsequently, the grass was cut twice, 28 June and 17 July respectively, freeze-dried and ground to pass a 1 mm sieve. The material contained 35.6 g N/kg on an air-dry-matter basis with 21.4 % ¹⁵N atom % excess.

Beer yeast was produced according to a method modified from the procedure described by Wutzke & Heine (1984). The growth medium contained 2.95 g (¹⁵NH₄)₂SO₄ (95 atom % excess ¹⁵N), inoculated with 50 mg yeast. After 4 d the yeast was collected by centrifugation (3000 rev./min; 15 min), washed with distilled water, freeze-dried and ground in a mortar. The material contained 57.4 g N/kg air-dry matter with 90.1 % ¹⁵N atom % excess in air-dry matter.

Of the ¹⁵N-labelled material (mixture of grass meal and beer yeast), 10 g was suspended in an aqueous medium containing (/l): 15 g carboxymethylcellulose and 9 g NaCl, and continuously infused into the abomasum at a rate of approximately 100 ml/h, during each experimental period, over 7 d. Hence, 24 g ¹⁵N-labelled material/d was infused, equivalent to 375 mg ¹⁵N.

Experimental design, time chart and routine

Experimental diets were allocated to sheep according to a 3 × 3 Latin square design, covering a total time interval of approximately 6 months. Between experimental periods a change-over period of about 1 month was observed. Each collection period was preceded by a preliminary period of 2 weeks, and started 7 d before infusions; (d₋₇) with daily application of a marker mixture containing 2.5 g Cr neutral-detergent fibre and 0.75 g Co EDTA, introduced into the rumen four times daily at 06.00, 12.00, 18.00 and 24.00 hours respectively. The liquid (Co) and particulate (Cr) marker complexes were prepared according to Udén *et al.* (1980). After 1 week (d₀; 18.00 hours) the continuous intra-

Table 1. *Organic matter (OM), neutral-detergent fibre (NDF), acid detergent fibre (ADF), acid-detergent lignin (ADL) and nitrogen contents (g/kg DM) of grass hay, high (HP)- and low (LP)-protein concentrates*

Experimental diet*	OM	NDF	ADF	ADL	N
Hay	899	574	311	30.1	20.3
HP concentrates	901	227	123	20.6	35.7
LP concentrates	911	428	241	34.8	22.4

* For details, see pp. 274–275.

abomasal infusion of the ^{15}N labelled material was started, and continued over a period of 7 d (until day 7 (d_7); 18.00 hours).

Blood plasma samples were taken twice daily (i.e. 08.00 and 20.00 hours) from day 2 (d_2) onwards, until d_7 and four times (i.e. 08.00, 12.00, 16.00 and 20.00 hours) from days 8–11 (d_8 – d_{11}). The samples were centrifuged (3000 rev./min; 15 min), and the plasma stored at -20° pending analyses.

From d_2 to d_{11} , rumen samples were collected at 08.30, 10.30, 14.30 and 18.30 hours. From the 08.30 and 10.30 hours samples, rumen microbes were isolated by a two-stage centrifugation, first at 550 g (4° , 15 min) using an Heraeus omnifuge 2.0 RS (Heraeus Sepatech GmbH, Osterode, Germany) and then at 70 000 g (4° , 20 min) using a MSE superspeed 65 centrifuge (Measuring and Scientific Equipment Ltd, Crawley, W. Sussex). The pellet was washed twice with a buffer, prepared according to Meyer *et al.* (1967), and freeze-dried. The supernatant rumen fluid fraction was stored at -20° until analysed for NH_3 and Co. In addition, the 14.30 and 18.30 hours samples were centrifuged and stored for NH_3 and Co analyses.

Samples from the cannulas along the small intestine (D_1 , D_2 , J, I) were collected from d_8 to d_{11} . For each day and per cannula a sample was collected at 09.00, 11.00, 13.00, 15.00, 17.00 and 19.00 hours. Samples collected at each period varied in amount from 30 (D_1 , D_2) to 20 g (J, I). The samples were freeze-dried, air equilibrated, ground over a 1 mm sieve and stored until analysed. A portion of the fresh samples was kept at -20° for the determination of NH_3 and the TCA-soluble and insoluble fractions.

Faeces and urine samples were collected quantitatively from d_0 to d_{12} . Urine was acidified with 3 M- H_2SO_4 to pH 2. Urine (12 h) and fresh faeces samples were kept at -20° , whereas pooled faecal samples were oven-dried and ground over a 1 mm sieve.

After the last application of the Co–Cr marker mixture on d_{11} , rumen Co concentration was followed until d_{13} . This procedure was followed for the determination of the volume and turnover of rumen liquids (Bosch *et al.* 1988).

Analyses

In diet, digesta and faecal samples, DM and organic matter were determined following standard procedures (Association of Official Analytical Chemists, 1990). For urine, fresh faeces and oven-dried diet and freeze-dried digesta samples, N was determined by the Kjeldahl method using K_2SO_4 and CuSO_4 as catalysts. The cell-wall constituents, i.e. cellulose, hemicellulose and lignin, were determined following the procedures of Goering & Van Soest (1970).

Amino acids, including diaminopimelic acid (DAPA) were determined as described by Van Bruchem *et al.* (1988b) with a Biotronic LC5001 automatic amino acid analyzer (Eppendorf-Netheler-Hinz GmbH, Maintal, Germany), after hydrolysing the samples under reflux with 6 M-HCl at 110° for 22 h. The S-containing amino acids, methionine and cystine, were assessed as methionine sulphone and cysteic acid respectively after performic acid oxidation (Moore, 1963).

RNA in rumen microbes and duodenal digesta was determined according to the method described by Schönhusen *et al.* (1990).

NH_3 in rumen fluid and small intestinal digesta was determined by distillation after rendering the solution alkaline.

Marker concentrations, i.e. Co for the liquid phase and Cr for the particulate phase were determined, after wet digestion, using an atomic absorption spectrophotometer

(Varian SpectrAA 300; Variantechtron Pty Ltd, Springvale, Australia), at wavelengths of 240.7 and 357.9 nm respectively.

For the determination of the ^{15}N atom % excess in rumen NH_3 , rumen supernatant fraction samples were distilled after alkalization, the distillate taken up in 0.05 M- H_2SO_4 , titrated with 0.1 M-NaOH and concentrated to a sample size of 0.2 ml (approximately 40 μg N). NH_3 was isolated similarly from fresh digesta samples collected from the small intestine. Further, through the addition of an equal volume of a TCA solution (200 g/l) and centrifugation, a separation was made between the TCA-soluble (non-protein-N; NPN) and TCA-precipitable N (protein-N; PN) fractions. The precipitate was washed three times with the TCA solution.

Subsequently, after destruction of these samples according to the Kjeldahl procedure, the resultant NH_3 was distilled and prepared for ^{15}N analyses as described previously. In urine, ^{15}N in total N and urea-N was determined. A diluted urine sample was incubated with a urease-containing buffer at pH 7.0 for 2 h at 37°. The resultant NH_3 was separated by microdiffusion (Voigt & Steger, 1967), and prepared for analysis as described previously. Total N samples were prepared for ^{15}N analyses following digestion according to Kjeldahl. As for fresh digesta samples, plasma N was subdivided into PN and NPN by using TCA (200 g/l). Urea was isolated as described for urine, NH_3 being removed by microdiffusion and prepared for ^{15}N analyses as described previously. In rumen microbes and freeze-dried small intestine and oven-dried faeces samples, ^{15}N was determined in dry samples which had been thoroughly ground with a swing mill (Retsch MM 2000; Retsch GmbH & Co., Haan, Germany). The ^{15}N analyses were carried out using an automatic N and C analyser-isotope ratio mass spectrometer; Europe Scientific Ltd, Crewe, Ches.).

Calculations and statistical evaluation

Following the termination of marker application, the fractional rate of passage of the rumen liquids was derived from the decrease in rumen Co concentration as described by Bosch *et al.* (1988). The volume of rumen liquids was estimated from the following formula:

$$\text{Co-dosage (mg/d)} = V \times [\text{Co}] \times k_l \times 24,$$

where V is rumen volume (l), $[\text{Co}]$ is mean Co concentration during marker application (mg/l), and k_l is fractional rate of liquid flow from rumen (per h).

The quantity of microbial protein arriving in the duodenum was estimated using DAPA and RNA as internal markers. An additional estimate was derived from the differences in amino acid profiles of dietary, microbial and duodenal proteins, as described by Van Bruchem *et al.* (1985). The mean of these three estimates was taken as a measure for microbial N arriving in the duodenum. Subsequently, assuming 2.5 g endogenous N enters the duodenum (N_2D_1) per kg DM ingested (Siddons *et al.* 1982), an estimate was obtained for rumen-undegraded protein in the proximal duodenum and rumen protein degradability.

Digesta flows in the small intestine were based on Co and Cr contents relative to daily Co and Cr dosage. The average of the computed liquid (Co) and particulate (Cr) flows was used. Duodenal (D_1 , D_2) ^{15}N flows were corrected for ^{15}N contained in NH_3 and microbes flowing from the rumen. Based on the course of protein digestion in the small intestine described by Van Bruchem *et al.* (1988a), jejunal and ileal ^{15}N flows were corrected for 35 and 15 % of rumen microbial ^{15}N flows respectively. The endogenous N along the small

intestine was based on the corrected ^{15}N flows relative to ^{15}N excess in the blood plasma NPN precursor pool including urea:

$$\text{flow-N}_e \text{ (g/d)} = \frac{\text{flow-N (g/d)} \times {}^{15}\text{N}_{\text{digesta}}}{{}^{15}\text{N}_{\text{plasma-NPN}}}$$

where ^{15}N is ^{15}N excess (atom %) and N_e is endogenous N.

The results were analysed statistically using ANOVA (Brouwer, 1990) appropriate for a 3×3 Latin square taking into account differences between animals and periods. Differences were tested using a two-tailed Student's *t* test, and $P < 0.05$ was taken as the level of significance.

RESULTS

In Table 2 a summary is presented of the composition of the ingested feed. There was a highly significant difference for N and NDF intake.

The ^{15}N excess in the TCA-soluble blood plasma NPN pool gradually increased to a plateau at d_5 after the start of the intra-abomasal infusion of ^{15}N -labelled protein. During infusion the ^{15}N excess in blood plasma urea was about 10 % higher than that in the blood plasma NPN fraction. After cessation of the intra-abomasal infusion, during the period that small intestinal digesta samples were collected, ^{15}N excess in plasma NPN was similar to that in urea and that excreted with the urine (results not shown). The ^{15}N excess in blood plasma PN increased at a considerably slower rate, reaching a plateau at 7–8 d after the onset of the intra-abomasal infusion of the ^{15}N label. Fig. 1 shows an overview of ^{15}N excess in plasma urea, NPN and PN. After termination of the intra-abomasal ^{15}N infusion, the ^{15}N excess decreased at a fractional rate of about 15 %/d.

Under all dietary conditions, the mean rumen NH_3 concentrations exceeded the minimal level required for maximal growth rate of rumen microbes (Hoover, 1986). For hay, HP and LP diets mean values were 133, 236 and 180 (least significant difference (LSD)_{0.05} 86.4) mg/l respectively. Nevertheless, the ^{15}N excess in rumen NH_3 was considerably higher than that anticipated from the results reviewed by Cheng & Costerton (1980) and Egan *et al.* (1986), suggesting that due to a decreased urease activity in the micro-organisms adherent to the rumen wall, the influx of plasma urea by diffusion through the rumen wall was drastically reduced at such levels of rumen NH_3 . However, alternatively, urea enters the rumen with saliva. The dynamics of ^{15}N excess in rumen NH_3 and rumen micro-organisms are also presented in Fig. 1. During the phase of intra-

Table 2. *Nutrients (g/d) ingested with grass hay and hay and concentrate high (HP)- and low (LP)-protein diets fed to sheep*

Experimental diet* ...	Hay	HP	LP	SEM
DM	1088	1103	1177	38.3
N	22.2 ^a	31.6 ^b	25.1 ^a	0.92
Neutral-detergent fibre	629 ^b	443 ^a	590 ^b	20.0
Acid-detergent fibre	347 ^b	243 ^a	328 ^b	10.7
Acid-detergent lignin	32.8 ^a	29.1 ^a	39.2 ^b	1.19
Cellulose	314 ^b	214 ^a	288 ^b	10.6
Hemicellulose	283 ^b	200 ^a	262 ^b	12.2

^{a,b} Mean in rows with unlike superscript letters were significantly different ($P < 0.05$).

* For details, see pp. 274–275.

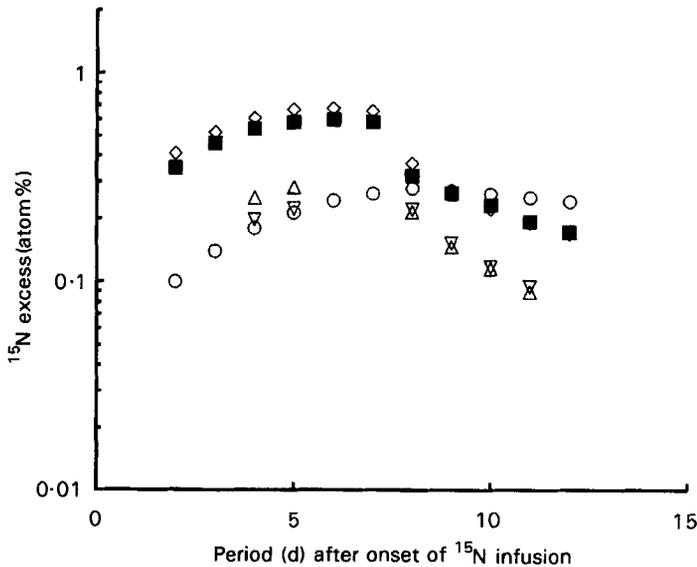


Fig. 1. ^{15}N excess in plasma non-protein-N (■), urea (◇) and protein-N (○), and rumen NH_3 (△) and microbes (▽) relative to the time period of ^{15}N -labelled protein infusion into the abomasum of sheep. For details of procedures, see pp. 274–278.

abomasal infusion, while ^{15}N excess in rumen fluid was gradually increasing, ^{15}N excess in NH_3 exceeded that in rumen micro-organisms. The results indicate that under the three dietary treatments more than 90 % of microbial N was derived from rumen NH_3 . The fractional rate of decrease in ^{15}N enrichment in NH_3 and rumen microbes was of a similar magnitude to that in urinary urea, 27.9 (SE 3.3), 26.6 (SE 2.2) and 29.0 (SE 3.3) % per d respectively, although slightly different from plasma urea (21.4 (SE 3.1) % per day).

Table 3 shows the flow of N along the small intestine and the quantity excreted with the faeces. A considerable part of the protein in the HP concentrates was apparently degraded in the rumen. As a result, the variation in duodenal N flow was only marginally different between diets. In part, this was also related to the fact that with the hay and LP diets a net influx of N into the rumen was observed, leading to duodenal N flows slightly higher than the respective quantities ingested.

Fig. 2 shows the dynamics of ^{15}N excess in small intestinal digesta, corrected for ^{15}N in NH_3 and microbes originating from the rumen. In line with ^{15}N excess in plasma, ^{15}N excess in the proximal duodenum (D_1), the distal duodenum (D_2) and the terminal ileum (I), gradually decreased from d_8 to d_{11} , after cessation of the abomasal infusion of ^{15}N .

From the ^{15}N flows, corrected for ^{15}N in NH_3 and micro-organisms flowing from the rumen, relative to ^{15}N excess in plasma NPN, daily estimates were derived of endogenous N passing at various sites in the small intestine and, finally, the quantity excreted with the faeces. The average values over d_8 – d_{11} are presented in Table 3. A considerable net re-absorption was observed between D_2 and I. The extent differed slightly among diets, with the highest value (57.3 %) for diet LP. Despite the observed partial re-absorption, endogenous protein as a proportion of total protein gradually increased towards the ileum. Approximately 50 % of the N voided with the faeces was of endogenous origin.

Net apparent re-absorption is the balance between true reabsorption and the addition of endogenous N. Hence, as illustrated by Van Bruchem *et al.* (1996), as the result of true

Table 3. Rumen N degradation, total N flow and/or excretion, endogenous N (N_e) flow and/or excretion and apparent reabsorption in small intestine, and proportion of ^{15}N enrichment in TCA-precipitable fraction (TCA \downarrow) for sheep fed on grass hay and hay and concentrate (high (HP)- and low (LP)-protein) diets*

Experimental diet* ...	Hay	HP	LP	SEM
Rumen N degradation (%)	80.2 ^b	56.7 ^a	73.8 ^{ab}	4.17
Total N flow (g/d)				
D ₁	23.7	25.5	26.3	0.68
Rumen microbial (D ₁)	14.6	15.1	15.8	1.32
D ₂	28.1 ^a	31.6 ^b	33.1 ^b	0.52
J	11.5	12.7	15.6	†
I	10.1	10.1	11.0	0.16
F	10.0	9.8	10.6	0.51
N_e flow (g/d; % total N in parentheses)				
D ₁	3.4 (14.6)	3.1 (12.2)	2.9 (10.5)	0.29 (1.03)
D ₂	8.9 ^a (32.2)	10.1 ^{ab} (31.4)	11.7 ^b (35.1)	0.43 (1.05)
J	5.9 (51.4)	5.6 (44.3)	6.8 (43.5)	†
I	5.2 (51.6)	4.8 (48.0)	5.0 (45.7)	0.21 (1.30)
F	5.2 (53.5)	5.2 (52.8)	4.8 (45.7)	0.20 (4.68)
Apparent reabsorption N_e D ₂ -I (%)	41.6 ^a	52.4 ^b	57.3 ^c	0.49
^{15}N in TCA \downarrow fraction (%)				
D ₁	71.4	61.5	65.0	4.15
D ₂	68.5	60.7	59.9	2.12
J	49.8	51.4	37.7	†
I	61.1	49.9	56.1	2.48

^{a,b,c} Mean values in rows with unlike superscript letters were significantly different ($P < 0.05$).

D₁, proximal duodenum; D₂, distal duodenum; J, jejunum; I, ileum; F, faeces.

* For details of diets and procedures, see Tables 1 and 2 and pp. 274–278.

† Two sheep only.

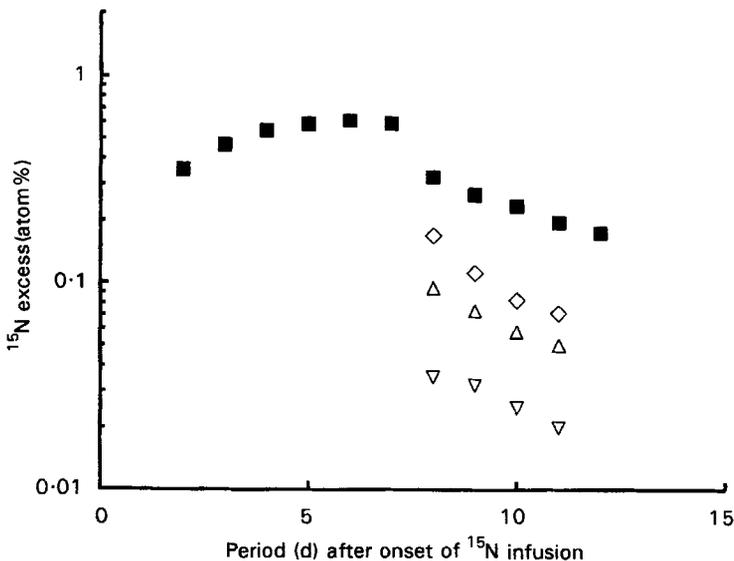


Fig. 2. ^{15}N excess in plasma non-protein-N (■), proximal duodenal (▽), distal duodenal (△) and ileal (◇) digesta, v. time period of ^{15}N -labelled protein infusion in sheep. For details of procedures, see pp. 274–278.

absorption and continuing endogenous contribution, the amino acid profile gradually changes from the duodenum towards the ileum. This is also illustrated by the present results in Table 3. The proportion of ^{15}N enrichment contained in TCA-precipitable protein showed a tendency to decrease towards the ileum.

DISCUSSION

Modern high-input animal production systems aim to optimize the production of protein with a minimum of fat (Tamminga & Verstegen, 1991). This requires diets which are balanced in terms of proteins and other nutrients, in ruminants particularly with respect to the volatile fatty acids produced in the rumen and the amino acids absorbed from the small intestine. In intensive production systems in Western European countries, the amount of N excreted in waste has become a burden for the environment.

A considerable part of the N excreted in the faeces is of an endogenous nature. Urinary N originates from inevitable losses related to maintenance, losses associated with the deposition of amino acids into skeletal muscle tissue, losses due to an imbalance between energy and protein supply in the diets, and purine derivatives, which are degradation products of microbial nucleic acids absorbed from the small intestine (Chen *et al.* 1990). The actual excretion of endogenous protein (N) is considerably higher than the ileal flow or the quantity excreted in the faeces. These fractions constitute only part of the total quantity of endogenous protein produced. Based on results derived from sheep fitted with cannulas along the small intestine, Van Bruchem *et al.* (1988a) suggested that a major part of the endogenous protein is reabsorbed. This finding is supported by the present results. Nevertheless, the proportion of endogenous protein increased towards the ileum. This is caused by the steady addition of endogenous protein, and the fact that endogenous proteins are more resistant to proteolytic degradation, as suggested by Ben-Ghedalia *et al.* (1974) for digestive enzymes, and by Fuller & Cadenhead (1991) for mucus.

It has been suggested also that an increased production of endogenous protein, despite partial reabsorption, is associated with a less-efficient utilization of the absorbed amino acids. In piglets this was shown by Makkink (1993), with increasing endogenous protein losses related to anti-nutritional factors in the diet. Based on a conceptual analysis, Huisman *et al.* (1993) arrived at a similar conclusion for pigs. It is of utmost importance therefore to assess reliably the factors governing the kinetics of endogenous protein production, the extent of reabsorption, and the efficiency of reutilization. The present paper describes the results of a preliminary assessment of production and reabsorption. Without doubt, the observed quantities of endogenous protein passing into D_2 are underestimates of the whole tract secretion of endogenous protein.

Thus far, the ^{15}N dilution technique has been well documented in pigs (Bergner *et al.* 1984; De Lange *et al.* 1990; Souffrant, 1991; Huisman *et al.* 1992). There has also been extensive discussion as to whether the TCA-soluble fraction of plasma represents the best precursor pool to determine the endogenous ^{15}N appearing in the lumen of the gastrointestinal tract. However, in previous experiments, usually a single amino acid, e.g. [^{15}N]leucine, was infused intravenously. This method is subject to criticism relating to whether the ^{15}N excess in blood plasma NPN, including urea, is representative of the plasma amino acid precursor pool. In the present experiment a universally-labelled mixture of amino acids was applied. This explains why ^{15}N excess in urinary urea was comparable with ^{15}N excess in plasma NPN, excluding urea. This approach therefore, gives more reliable results, although endogenous N consists only partly of amino acid N.

The epithelial cells lining the villus membrane have been shown to be able to utilize lumen amino acids for protein turnover and mucus synthesis (Alpers, 1972); presumably

the crypt cells, the pancreas and liver must derive their amino acids from the blood plasma amino acid pool. In the present experimental design this may have resulted in a slight overestimation of endogenous N, particularly during the first period after cessation of the intra-abomasal infusion of ^{15}N -labelled protein. This would explain why in small intestinal digesta the fractional decrease in ^{15}N excess in the period d_8 - d_{11} was higher than that in plasma. As a result, the estimated endogenous N flows gradually decreased, on average 6.2 %/d in D_1 , 6.7 %/d in D_2 , 11.0 %/d in J and 10.9 %/d in I respectively. Therefore, the last two sampling days, when a plateau was reached, may give more reliable estimates (Fig. 3). Alternatively, it could be argued that these latter estimates may underestimate endogenous N flow, as part of the protein would originate from lumen amino acids with, in the latter period, ^{15}N excess lower than that in the blood plasma precursor pool.

The endogenous protein added to the digesta actually consists of a mixture of proteins of different origin. The secretion of bile salts and synthesis and/or storage of pancreatic proteins is in the order of minutes to hours respectively, whereas the life cycle of the epithelial cells before shedding from the villus top lasts approximately 3 d. Thus, the ^{15}N contained in the endogenous protein mixture should actually be related to an earlier ^{15}N excess in the plasma precursor pool. Taking into account the fact that protein turnover also takes place within the epithelial cells, including the membrane-associated enzymes, and that a major proportion of the intestinal secretions consists of mucus, the average time lag amounts to at least a couple of hours. Thus a correction factor could be defined, based on the average proportional decrease in plasma ^{15}NPN of 15% per day. Hence, the estimated levels of endogenous N should be corrected by not more than 10 %.

The present data set does not allow the study of the relationship between endogenous N secretions at the various sites and duodenal N and non-protein DM passage in D_1 , D_2 , J and I respectively. This can be attributed to the limited number of data and, at the level of the small intestine, the limited variation in protein and non-protein DM flow. On average, ileal endogenous N flow amounted to 11.9 g/kg non-protein DM. This estimate is of a

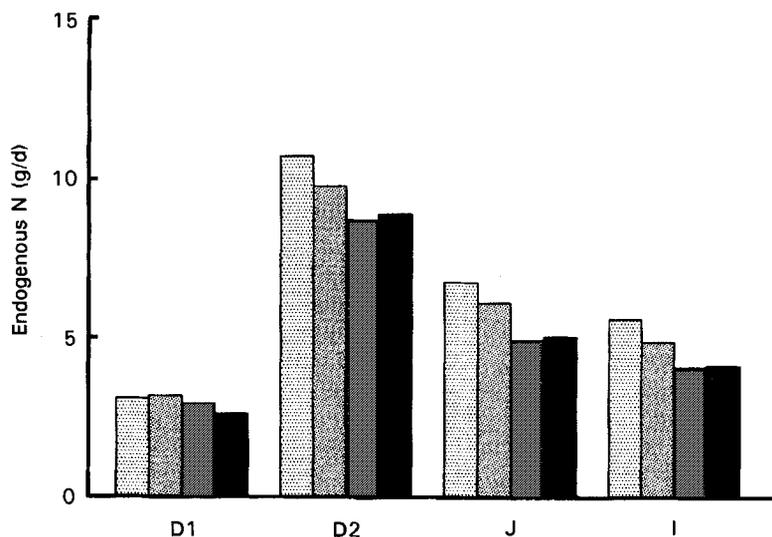


Fig. 3. Estimated quantities of endogenous nitrogen from day 8 to day 11 of the experimental period in sheep. (□), Day 8; (▤), day 9; (▥), day 10; (■), day 11. For details of procedures, see pp. 274-278. D_1 , proximal duodenum; D_2 , distal duodenum; J, jejunum; I, ileum.

different magnitude to the value of 6–8 g ileal endogenous amino acid-N derived previously by regression analysis of ileal v. duodenal amino acid-N (Van Bruchem *et al.* 1989; Oosting *et al.* 1995). However, a considerable part of the endogenous N consists of NPN, particularly in the form of the amino sugars contained in mucin. Further research is needed therefore, at the level of the small intestine, using more variable flows of (non)-protein DM. Such research should also focus on the various components included in the endogenous fraction, e.g. pancreatic secretions and mucin. In this respect, it has been suggested that amino sugars could be used as a marker for the mucus fraction (Fuller & Cadenhead, 1991).

In conclusion, the method described in the present paper, has two major shortcomings, hence, the results need to be interpreted with care. The first, a consequence of the prolonged labelling, is that absorption of ^{15}N products from the lumen and (partial) replacement by ^{15}N metabolites of endogenous origin create underestimates of endogenous input. As a consequence the estimated values may be lower than the actual values. Second, due to intracellular turnover, proteinaceous (and other N source) secretions will have a lower enrichment than ^{15}N -labelled plasma NPN and, thus, the values obtained will again be underestimated by the equation used. The present results should, therefore, probably be regarded as minimum values. In this respect the appropriateness of the corrections for microbial N for 35 and 15 % residues in the jejunum and ileum respectively, needs consideration. Ileal N consisting of approximately 50% of endogenous N is in agreement with previous experimentation (Van Bruchem *et al.* 1989). However, when corrected for endogenous N the present average ileal N amounts to approximately 75 % of duodenal N, suggesting a true digestibility of only approximately 75 %. To a limited extent this could be related to a bias in the correction for the ^{15}N label in microbial N. On average, a correction of ± 1 % is equivalent to approximately ± 0.15 g N_e /d in J or I. With only 10 % of microbial N left in the ileum, N_e would, on average, amount to 5.8 g/d and, consequently, a true N digestibility of approximately 80 %.

Based on a set of regression equations, Van Bruchem *et al.* (1988a) suggested that the rate of digestion of endogenous N was about comparable with that of exogenous N, i.e. dietary and microbial N. Dividing the jejunum into two segments (D_2 -J, J-I), and assuming protein digestion and/or absorption to follow an exponential model with a true digestion of 1-SQRT (0.15), where SQRT is the square root, in segment D_2 -J and 1-SQRT(0.15) in segment J-I, would give residual fractions of duodenal endogenous N ($N_e D_2$) of 0.39 at J and 0.15 at I respectively. Quantities (g/d) of endogenous N secreted in duodenum ($S_{D_1-D_2}$) and jejunum (S_{D_2-J} and S_{J-I}) can then be approximated as follows:

$$S_{D_1-D_2} = N_e D_2 - N_e D_1 = 7.1 \text{ g/d,}$$

with residues of $0.39 \times 7.1(2.7)$ at J and $0.15 \times 7.1(1.1)$ at I;

$$S_{D_2-J} = (N_e J - 2.7)/\text{SQRT}(0.39) = (6.1 - 2.7)/0.62 = 5.4 \text{ g/d,}$$

with a residue of $5.4 \times 0.39^{1.5}(1.3)$ at I;

$$S_{J-I} = (N_e I - 1.1 - 1.3)/\text{SQRT}(0.39) = (5.0 - 1.1 - 1.3)/0.62 = 4.2 \text{ g/d,}$$

with a residue of $4.2 \times 0.39^{1.5}(2.6)$ at I.

Averaged over treatments, for the small intestine this model would arrive at a secretion of endogenous N of 16.7 g/d, approximately 75 % of the quantities of microbial and dietary N arriving in the duodenum. This estimate has to be regarded as an underestimate of whole gastrointestinal tract protein turnover, because (1) the compound stomach and large

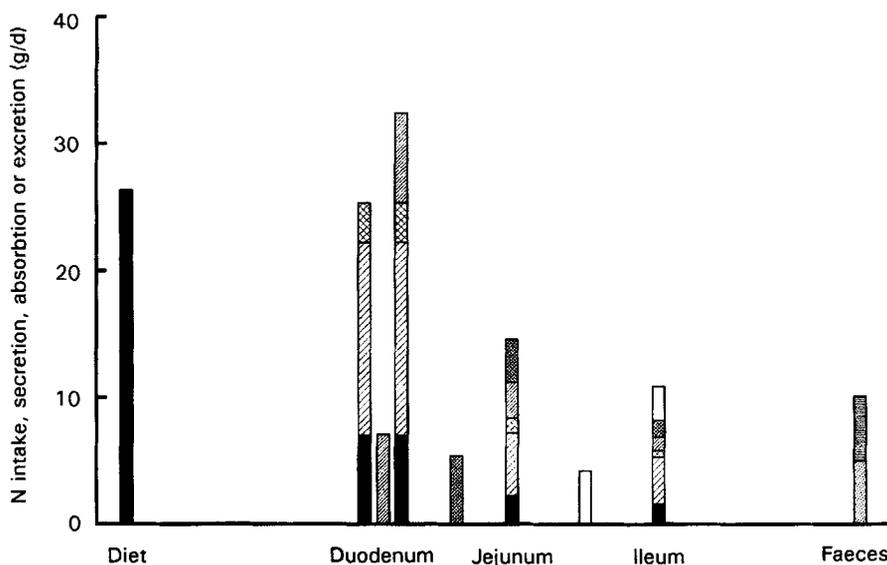


Fig. 4. Mean estimates for N intake (diet, ■), rumen N degradation and incorporation into microbes (microbial; ▨), endogenous N (N_e) secretion and N absorption along the small (SI) and large intestine (LI) and faecal N excretion of sheep. (▩, ▨), N_e for proximal (D_1) and distal duodenum respectively; (▨), N_3 for jejunum; (□), N_e for ileum (I); (▩), diet and microbial; (■), N_e for SI (D_1 to I) and LI. For details of procedures, see pp. 274–278.

intestine have not been accounted for, and (2) intracellular protein turnover has been disregarded. However, considering the dynamics of protein at a higher level of integration, both intracellularly and extracellularly, the secretion and partial reabsorption of endogenous protein (N) are part of whole-body protein turnover, with unavoidable losses included in body protein requirements for maintenance; this puts an upper limit to the endogenous protein losses. A summary of the processes related to N intake, conversion, secretion, absorption and excretion is presented in Fig. 4.

In the previously described conceptual model it is assumed that the rate of true digestion of endogenous protein is similar to that of microbial and dietary protein. If true digestibility is 85% (Van Bruchem *et al.* 1989) and the small intestine is divided in n segments, the extent of digestion per segment amounts to $(1 - 0.15^{1/n})$. If the rate of digestion of endogenous protein is lower, taking instead 0.15, 0.25 or 0.35, the model would arrive at a secretion of endogenous protein of 13.5 or 11.4 g/d.

Since there are many unknown factors, e.g. the differential rates of enrichment and turnover among protein pools which contribute to endogenous secretion, care should be taken to interpret the present results as absolute values. However, in a relative sense they indicate the importance and the dynamics of endogenous protein in relation to diet composition and site of digestion. The present results unequivocally show that separation of N into TCA-soluble and -precipitable fractions does not give any reliable quantitative information on endogenous protein secretion.

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REFERENCES

- Alpers, D. H. (1972). Protein synthesis in intestinal mucosa: the effect of route of administration of precursor amino acids. *Journal of Clinical Investigation* **51**, 167–173.
- Association of Official Analytical Chemists (1990). *Official Methods of Analysis*, 15th ed. Washington, D.C.: Association of Official Analytical Chemists.
- Ben-Ghedalia, D., Tagari, H. & Bondi, A. (1974). Protein digestion in the intestine of sheep. *British Journal of Nutrition* **31**, 125–142.
- Bergner, U., Bergner, H. & Simon, O. (1984). Untersuchungen zu endogenen N-Umsatzprozessen an ¹⁵N-markierten Schweinen. 2. Mitt.: Fäkale Exkretion von Aminosäuren und ¹⁵N-markierten Aminosäuren bei unterschiedlichen Rohfasergehalt der Diäten (Investigations on endogenous N conversions in ¹⁵N-labelled pigs. 2. Faecal excretion of amino acids in relation to dietary fibre content). *Archives of Animal Nutrition* **34**, 505–517.
- Bosch, M. W., Janssen, I. M., Van Bruchem, J., Boer, H. & Hof, G. (1988). Digestion of alfalfa and grass silages in sheep. 1. Rates of fermentation in and passage from the reticulo-rumen. *Netherlands Journal of Agricultural Science* **36**, 175–185.
- Brandt, M., Rohr, K. & Lebziern, P. (1980). Estimation of postruminally secreted protein-N in duodenal digesta of cows by means of ¹⁵N. In *Proceedings Vth International Symposium on Amino Acids*, Serock, Poland, pp. 1–5. [T Zebrowska, L. Buraczewska, S. Buraczewski, J. Kowalczyk and B. Pastuszewska, editors]. Warsaw: Polish Scientific Publishers.
- Brouwer, B. O. (1990). *DBSTAT User's Guide*. Wageningen, The Netherlands: Department of Animal Husbandry, Agricultural University.
- Chen, X. B., Ørskov, E. R. & DeB. Hovell, F. D. (1990). Excretion of purine derivatives by ruminants: endogenous excretion, differences between cattle and sheep. *British Journal of Nutrition* **63**, 121–129.
- Cheng, K. J. & Costerton, J. W. (1980). Adherent bacteria in ruminant digestion. In *Digestive Physiology and Metabolism in Ruminants*, pp. 227–250 [Y. Ruckebush and P. Thivend, editors]. Lancaster: MTP Press Limited.
- De Lange, C. F. M., Souffrant, W. B. & Sauer, W. C. (1990). Real ileal protein and amino acid digestibilities in feedstuffs for growing pigs as determined with the ¹⁵N-isotope dilution technique. *Journal of Animal Science* **78**, 409–418.
- Egan, A. R., Boda, K. & Varady, J. (1986). Regulation of nitrogen metabolism and recycling. In *Control of Digestion and Metabolism in Ruminants*, pp. 386–402 [L. P. Milligan, W. L. Grovum and A. Dobson, editors]. Englewood Cliffs, NJ: Prentice-Hall.
- Fuller, M. F. & Cadenhead, A. (1991). Effect of the amount and composition of the diet on galactosamine flow from the small intestine. In *Digestive Physiology in Pigs. Proceedings of Vth International Symposium on Digestive Physiology in Pigs, European Association of Animal Production, Publication no 54*, pp. 331–333 [M. W. A. Verstegen, J. Huisman and L. A. den Hartog, editors]. Wageningen: Pudoc.
- Goering, H. K. & Van Soest, P. J. (1970). Forage fiber analysis. *Agricultural Handbook* no. 379, pp. 1–12. Washington, D.C.: Agricultural Research Service, US Department of Agriculture.
- Hoover, W. H. (1986). Chemical factors involved in ruminal fiber digestion. *Journal of Dairy Science* **69**, 2755–2766.
- Huisman, J., Heinz, Th., Van der Poel, A. F. D., Van Leeuwen, P., Souffrant, W. B. & Verstegen, M. W. A. (1992). True protein digestibility and amounts of endogenous protein measured with the ¹⁵N technique in piglets fed on peas (*Pisum sativum*) and common beans (*Phaseolus vulgaris*). *British Journal of Nutrition* **68**, 101–110.
- Huisman, J., Verstegen, M. W. A., Van Leeuwen, P. & Tamminga, S. (1993). Reduction of N pollution by decrease of the excretion of endogenous N in pigs. In *Nitrogen Flow in Pig Production and Environmental Consequences*, pp. 55–61 [M. W. A. Verstegen, L. A. den Hartog, G. J. M. van Kempen and J. H. M. Metz, editors]. Wageningen, The Netherlands: Pudoc Scientific Publishers.
- Makkink, C. A. (1993). Of piglets, dietary proteins, and pancreatic proteases. Doctorate Thesis, Wageningen Agricultural University, The Netherlands.
- Meyer, R. M., Bartley, E. E., Deyoe, C. W. & Colenbrander, V. F. (1967). Feed processing. 1. Ration effects on rumen microbial protein synthesis and amino acid composition. *Journal of Dairy Science* **50**, 1327–1331.
- Moore, S. (1963). On the determination of cystin and cysteic acid. *Journal of Biological Chemistry* **238**, 235–237.
- Oosting, S. J., Van Bruchem, J. & Chen, X. B. (1995). Intake, digestion and small intestinal protein availability in sheep in relation to ammoniation of wheat straw with or without protein supplementation. *British Journal of Nutrition* **75**, 347–368.
- Schönhusen, U., Voigt, J., Piatkowski, B. & Kreienbring, F. (1990). Untersuchungen zur Nutzung von Ribonukleinsäure als Marker für die Messung des mikrobiellen Proteinertages in Pansen. 2. Einfluss der Probenbehandlung, des Probenahmezeitpunktes und der Rationszusammensetzung auf das RNS:N-Verhältnis in den Pansenmikroben (Studies on ribonucleic acid as a marker for microbial protein yield in the rumen. 2. Effect of sample treatment, sampling time and diet composition on RNA:N ratio in rumen microbes). *Archives of Animal Nutrition* **40**, 75–84.

- Siddons, R. C., Beever, D. E. & Nolan, J. V. (1982). A comparison of methods for the estimation of microbial nitrogen in duodenal digesta of sheep. *British Journal of Nutrition* **48**, 377–389.
- Souffrant, W. B. (1991). Endogenous nitrogen losses during digestion in pigs. In *Digestive Physiology in Pigs. Proceedings of 11th International Symposium on Digestive Physiology in Pigs*, Wageningen. *European Association of Animal Production Publication* no. 54, pp. 147–166 [M. W. A. Verstegen, J. Huisman and L. A. den Hartog, editors]. Wageningen: Pudoc.
- Tamminga, S. & Verstegen, M. W. A. (1991). Protein nutrition and animal production: Consequences for environment and some possible recommendations. In *Proceedings 6th International Symposium on Protein Metabolism and Nutrition*, pp. 23–36 [B. O. Eggum, S. Boisen, A. Danfaer, C. Borsting and T. Hvelplund, editors]. Foulum, Denmark: National Institute of Animal Science.
- Udén, P., Colluci, P. E. & Van Soest, P. J. (1980). Investigation of chromium, cerium and cobalt as markers in digesta. *Journal of the Science of Food and Agriculture* **31**, 625–632.
- Van Bruchem, J., Bongers, L. J. G. M., Lammers-Wienhoven, S. C. W., Bangma, G. A. & Van Adrichem, P. W. M. (1989). Apparent and true digestibility of proteins in and losses of endogenous proteins from the small intestine in sheep as related to dry matter intake and digestibility. *Livestock Production Science* **23**, 317–327.
- Van Bruchem, J., Bosch, M. W., Bongers, L. J. G. M., Bangma, G. A. & Van Adrichem, P. W. M. (1988a). Apparent and true digestibility of amino acids in the small intestine of sheep – Effect of dry matter intake and digestibility. *Proceedings of 5th EAAP Symposium on Protein Metabolism and Nutrition, Rostock, European Association for Animal Production Publication* no. 35. *Wissenschaftliches Zeitschrift der Universität Rostock, N-Reihe* **37**, pp. 13–14. Rostock: Universität Rostock.
- Van Bruchem, J., Kies, A. K., Bremmers, R., Bosch, M. W., Boer, H. & Van Adrichem, P. W. M. (1988b). Digestion of alfalfa and grass silages in sheep. 2. Digestion of protein in the reticulo-rumen and intestines. *Netherlands Journal of Agricultural Science* **36**, 365–374.
- Van Bruchem, J., Rouwers, S. M. G., Bangma, G. A., Lammers-Wienhoven, S. C. W. & Van Adrichem, P. W. M. (1985). Digestion of proteins of varying degradability in sheep. 2. Amount and composition of the protein entering the small intestine. *Netherlands Journal of Agricultural Science* **33**, 273–284.
- Van Bruchem, J., Voigt, J., Bongers, L. J. G. M. & Tamminga, S. (1996). Production and re-absorption of endogenous protein along the small intestine of sheep – Relation to gradually changing amino acid profile. *Netherlands Journal of Agriculture Science* (In the Press).
- Voigt, J. & Steger, H. (1967). Zur quantitativen Bestimmung von Ammoniak, Harnstoff und Ketokörpern in biologischem Material mit Hilfe eines modifizierten Mikrodifusionsgefäßes (About the determination of ammonia, urea and ketone bodies in biological samples by a modified microdiffusion method). *Archives für Tierernährung* **17**, 289–293.
- Wutzke, K. & Heine, W. (1984). Präparative Darstellung von hochangereicherterem ¹⁵N-markiertem Hefeprotein (Preparation of high enrichment ¹⁵N-labelled yeast protein). *Isotopenpraxis* **20**, 90–93.