

## Studies on the nitrogen compounds of the faeces of dairy cows fed urea as the sole or partial source of nitrogen

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**Abstract.** The nitrogen compounds of the faeces of dairy cows on purified, protein-free feed with urea and small amounts of ammonium salts as the sole source of nitrogen (0-feed), on low-protein feed in which part of the protein was replaced with urea (ULP-feed) and on normal protein-containing feed (NorP-feed) were studied.

The total nitrogen contents of the dry matter of the faeces were  $2.5 \pm 0.7\%$  on 0-feed,  $2.4 \pm 0.4\%$  on ULP-feed and  $2.3 \pm 0.6\%$  on NorP-feed. The proportion of urea- and ammonium nitrogen of the total nitrogen was  $3.2 \pm 2.4$ ,  $4.5 \pm 2.4$  and  $3.7 \pm 1.8\%$  respectively, and the proportion of nitrogen soluble in 70% ethanol  $10.9 \pm 5.7$ ,  $9.9 \pm 2.4$  and  $10.4 \pm 3.8\%$  of the total nitrogen; the proportion of nitrogen in the free amino acid fraction was  $4.1 \pm 2.9$  on 0-feed,  $1.6 \pm 1.4$  ( $P < 0.05$ ) on ULP-feed and  $3.2 \pm 2.9\%$  on NorP-feed.

The nitrogen insoluble in 70% ethanol as a proportion of the total nitrogen was  $82.3 \pm 7.2$  on 0-feed,  $85.4 \pm 5.0$  on ULP-feed and  $82.3 \pm 7.2\%$  on NorP-feed.

Of the total nitrogen of this insoluble fraction, the following proportions were released as amino acids by acid hydrolysis:  $62.3 \pm 8.5\%$  on 0-feed,  $61.6 \pm 10.7\%$  on ULP-feed and  $59.0 \pm 13.9\%$  on NorP-feed.  $\alpha$ ,  $\epsilon$ -diaminopimelic acid was  $1.7 \pm 0.6$ ,  $0.7 \pm 0.2$  ( $P < 0.05$ ) and  $1.2 \pm 0.4$  mol % respectively of the total amino acids of the hydrolyzate.

The soluble nitrogen fraction was separated into free amino acid and «peptide» fractions, and the amino acid composition of both was determined, the latter after acid hydrolysis.

### Introduction

The faeces and urine of ruminants contain a considerable amount of nitrogen, for example residue from metabolism, indigestible feed nitrogen and large amounts of microbial nitrogen synthesized in the rumen and to some extent also in the large intestine, which has passed undigested and unabsorbed through the alimentary canal.

It is difficult to determine what proportion of the nitrogen of the faeces of cows on normal feed is indigestible feed nitrogen and what is microbial nitrogen. With cows fed purified protein-free feed with urea (and a small amount of ammonium salts) as the sole source of nitrogen, all the protein available is produced by bacteria (VIRTANEN 1966). The digestibility of nitrogen

averaged 67 % in these feeding experiments, so that 33 % of the urea nitrogen fed was removed in the faeces. In the present study the composition of the nitrogenous fraction of the faeces was studied. Further, we studied the faeces of cows on a feed containing small amounts of protein, the protein deficiency being made good with urea. With these cows also the amount of nitrogen removed in the faeces was almost 30 % of the amount fed (KREULA and ETTALA 1977).

## Materials and methods

All the cows included in the test were Ayrshire dairy cows. Those fed protein-free feed with urea as the sole source of nitrogen are called 0-cows and the feed 0-feed. Those fed small amounts of protein and plenty of urea are called ULP-cows and the feed ULP-feed. Both of these feeds have been described in previous publications (VIRTANEN 1963, 1966, 1967, ETTALA and KREULA 1976). Samples of faeces were collected at different stages of lactation from both feeding groups during a period of several years. For comparison samples were also taken from cows on ordinary indoor and pasture feeds (NorP-feed, NorP-cows).

The dry matter was determined by drying the samples at 100° C or *in vacuo* at 60° C. Nitrogen was determined according to the Kjeldahl method using  $\text{KMnO}_4$  (BEET 1955). Total nitrogen was determined on fresh faeces. Soluble nitrogen was extracted with 70 % ethanol from fresh faeces. The free amino acid fraction was isolated from the soluble nitrogen fraction with Amberlite IR 120 cation ion exchanger (in  $\text{H}^+$  form). The so-called peptide fraction (eluted with water from Amberlite IR 120) was hydrolyzed (6 M HCl, 20 h, 106° C, under  $\text{N}_2$ ) and the amino acids of the hydrolyzate were purified on Amberlite IR 120.

The fraction insoluble in 70 % ethanol was hydrolyzed (6 M HCl, 20 h, 106° C, under  $\text{N}_2$ ) and the amino acids were purified as above using Amberlite IR 120. Nitrogen was determined on all the fractions by the Kjeldahl method. The quantitative amino acid determinations were made with an amino acid analyzer, constructed in our laboratory (PIEZ and MORRIS 1960) or with a Bio Cal BC 100 amino acid analyzer.

Under these hydrolysis conditions tryptophan is decomposed completely and methionine, cystine and glucosamine partly. Further, the high carbohydrate content in the faeces fractions resulted in the formation of humin during hydrolysis, thus making quantitative recovery more difficult. Part of the tyrosine of the hydrolyzate was evidently retained in the humin, so that the contents of tyrosine obtained are too low. Without acid hydrolysis it was impossible to bring the entire insoluble nitrogen fraction of the faeces into solution. Using an ultrasonic disintegrator (MSE Ultrasonic Disintegrator, 150 W) a maximum of 80 % of the total nitrogen could be solubilized. Neither was it possible to recover the amino acids completely by enzyme hydrolysis. Amino acid analyses were made during a period of several years with two different amino acid analyzers. During this time the methods were developed, one of the results being that the number of identified amino acids increased.

## Results

The total nitrogen contents of the faeces and the distribution of nitrogen in the different fractions were determined in samples from 0-, ULP- and NorP-cows. The average results according to feeding group are given in Table 1. For 0-cows the total nitrogen content was  $2.5 \pm 0.7$ , ULP-cows  $2.4 \pm 0.4$  and NorP-cows  $2.3 \pm 0.6$  % of the dry matter. In each group there were large between sample differences, but the differences between the average values of the groups were not statistically significant, with the exception of the free amino acid nitrogen with ULP-cows, which, as a proportion of the total faecal nitrogen, showed an almost significant ( $P < 0.05$ ) difference from the corresponding values of 0- and NorP-cows.

In all of the groups most of the nitrogen of the faeces was insoluble in 70 % ethanol. The nitrogen of the amino acids released from this fraction by acid hydrolysis was about 60 % of the total nitrogen of the faeces. The amino acids of the hydrolyzate from the faeces of 0-, ULP- and NorP-cows are given in Table 2 as a mol % of the total identified amino acids. In all feeding groups  $\alpha, \epsilon$ -diaminopimelic acid and glucosamine were present in this fraction; with 0-cows their content as a proportion of the total amount of amino acids was greater than with the other feeding groups. The proportion of glycine, histidine and arginine of the total amino acids was smaller with 0- and ULP-cows than with NorP-cows, and the differences were statistically significant ( $P < 0.05$ ). In contrast, the proportion of glutamic acid, methionine, isoleucine and tyrosine of the total amino acids with 0- and ULP-cows was much greater than with NorP-cows, and the differences were significant.

The proportion of nitrogen soluble in 70 % ethanol in all the feeding groups was about 10 % of the total nitrogen. The free amino acids of this fraction from 0- and ULP-cows were determined (Fig. 1).

Their total amount was on average 10.5 and 0.9 mg/g dry faeces with 0- and ULP-cows respectively. In both feeding groups the amount of alanine was the highest, the proportion of which in the total identified free amino acids averaged 25.7 with 0-cows and 19.7 mol % with ULP-cows. The total amount of amino acids liberated by hydrolysis of the peptide fraction was 1.4

Table 1. Nitrogen fractions of the faeces of cows on 0-, ULP- and NorP-feeds.

	0-cows n = 32	ULP-cows n = 19	NorP-cows n = 9
Dry matter %	$18.1 \pm 3.3$	$19.0 \pm 2.9$	$15.6 \pm 1.6$
Total N, % of dry matter	$2.5 \pm 0.7$	$2.4 \pm 0.4$	$2.3 \pm 0.6$
N soluble in 70 % ethanol, % of tot. N	$10.9 \pm 5.7$	$9.9 \pm 2.4$	$10.4 \pm 3.8$
Urea and $\text{NH}_4\text{N}$ , % of tot. N	$3.2 \pm 2.4$	$4.5 \pm 2.4$	$3.7 \pm 1.8$
Free amino acid N, % of tot. N	$4.1 \pm 2.9$	$1.6 \pm 1.4^*)$	$3.2 \pm 2.9$
«Peptide» fraction N, % of tot. N	$2.8 \pm 2.1$	$3.4 \pm 2.9$	$4.5 \pm 1.7$
N insoluble in 70 % ethanol, % of tot. N	$82.3 \pm 7.2$	$85.4 \pm 5.0$	$82.3 \pm 7.2$
«Protein» N, % of tot. N	$62.8 \pm 8.5$	$61.6 \pm 10.7$	$59.0 \pm 13.9$

\*)  $P < 0.05$

Table 2. Amino acid composition of the insoluble fraction hydrolyzate of the faeces (mol % of total amino acids).

Amino acid	0-cows n = 14	ULP-cows n = 9	NorP-cows n = 5
Asp	9.1 ± 1.4	10.6 ± 0.7*	8.1 ± 0.3
Thr	5.9 ± 0.3	6.2 ± 0.3*N	5.5 ± 0.6
Ser	5.9 ± 0.4*N	6.2 ± 0.4	6.4 ± 0.3
Glu	12.0 ± 1.5	11.9 ± 0.8	9.0 ± 0.5*
Pro	4.1 ± 0.4*	4.9 ± 0.6	5.4 ± 0.7
Gly	8.8 ± 1.1	10.1 ± 1.8	15.4 ± 1.9*
Ala	13.8 ± 1.2	12.3 ± 1.7*	13.9 ± 1.0
Cys	0.4 ± 0.3	0.6 ± 0.2	0.4 ± 0.1
Val	6.4 ± 0.7	6.6 ± 0.8	5.5 ± 0.7*
Met	1.4 ± 0.4	1.0 ± 0.3	0.4 ± 0.2*
A <sub>2</sub> pm	1.7 ± 0.6	0.7 ± 0.2*O	1.2 ± 0.4
Ile	5.0 ± 0.7	4.8 ± 0.4	3.6 ± 0.1*
Leu	7.3 ± 0.9	7.0 ± 0.4	6.2 ± 0.3*
Tyr	2.0 ± 0.5	2.2 ± 0.4	1.1 ± 0.2*
βAla	2.5 ± 2.1	2.4 ± 1.6	0
Phe	3.9 ± 0.9	4.0 ± 1.1	2.4 ± 0.1*
γAbu	0.6 ± 0.6	0.6 ± 0.5	0.6 ± 0.2
Orn	0.5 ± 0.5	0.3 ± 0.1	0.5 ± 0.5
Lys	5.8 ± 1.1	5.6 ± 1.2	6.8 ± 1.4
His	0.9 ± 0.2	1.0 ± 0.3	1.9 ± 0.5*
Arg	2.3 ± 0.6	2.2 ± 0.9	5.3 ± 1.1*
GlcN	2.9 ± 1.9	2.2 ± 0.3	1.3 ± 0.2

\* significantly different from the other two values ( $P < 0.05$ ) as indicated by analysis of variance and Tukey's t-test

\*O significantly different from 0-cow value ( $P < 0.05$ )

\*N significantly different from NorP-cow value ( $P < 0.05$ )

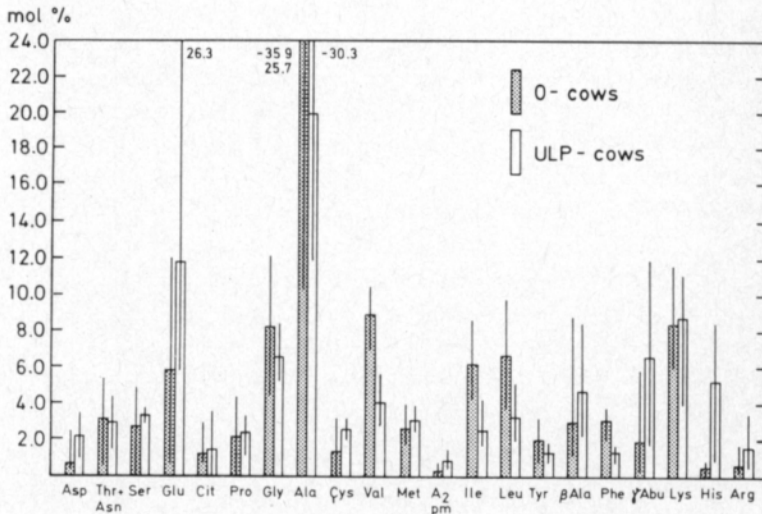


Figure 1. Free amino acids of the faeces, as mol % of the amount of all the free amino acids with 0- and ULP-cows.

and 1.6 mg per g dry faeces with 0- and ULP-cows respectively (Fig. 2). Of these amino acids the proportion of glutamic acid with 0-cows was on the average  $19.1 \pm 5.6$ , that of glucosamine  $9.1 \pm 6.1$  and  $\beta$ -alanine  $13.3 \pm 4.1$ ; the corresponding figures with ULP-cows were 12.9, 11.1 and 16.1 mol %.

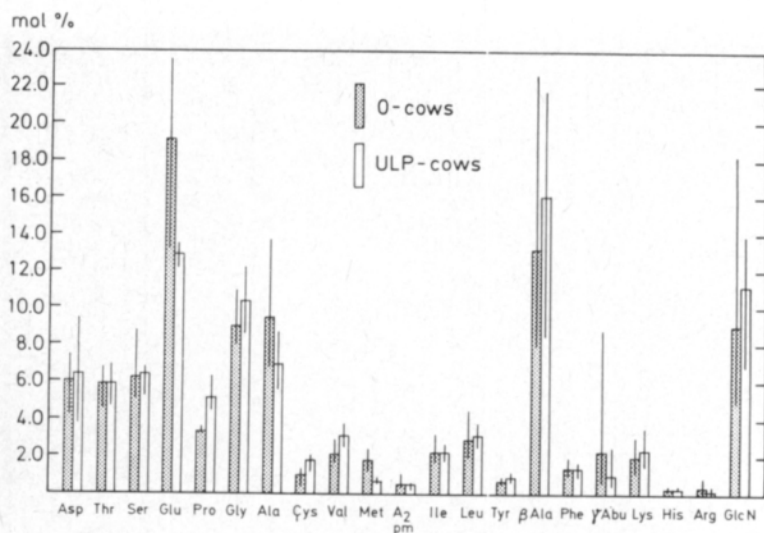


Figure 2. Amino acids of the «peptide» fraction hydrolyzate of the faeces as mol % of the total amount of the amino acids with 0- and ULP-cows.

Besides the amino acids in Fig. 1 and 2, unidentified ninhydrin-positive compounds were present in the free amino acid fractions and «peptide» fractions.

## Discussion

The average composition and relative proportion of the nitrogen fractions of the faeces of the cows in the different feeding groups differed surprisingly little, even though the 0-cows' sole source of feed nitrogen was urea, the ULP-cows received both urea and feed protein, and the NorP-cows consumed feed protein and no urea at all.

With all the feeding groups most of the nitrogen of the faeces was insoluble in 70 % ethanol, the proportion of the total nitrogen being  $82.3 \pm 7.2$  with 0-cows,  $85.4 \pm 5.0$  with ULP-cows and  $82.3 \pm 7.2$  % with NorP-cows. MÄKINEN (1972) found that the proportion of insoluble nitrogen in the rumen was greater with 0- and ULP-cows than with NorP-cows. The amino acid composition of the hydrolyzate of the insoluble fraction differed to some extent in the various groups.

The amount of  $\alpha$ ,  $\epsilon$ -diaminopimelic acid in the faeces of 0-cows was  $1.7 \pm 0.6$  mol %, and in that of ULP-cows  $0.7 \pm 0.2$  mol %. In the faeces of 0- and ULP-cows the contents of glutamic acid, methionine, isoleucine and tyrosine were greater than with NorP-cows, whereas the amounts of proline, glycine, histidine and arginine were smaller. In a feeding experiment with

$^{15}\text{NH}_4\text{Cl}$  the slowest incorporation of ammonium nitrogen was into histidine, proline, arginine and glycine with both bacteria and protozoa in the rumen of the ULP-cows (SYVÄÖJA and KREULA 1979). The amino acid composition of NorP-cows' faeces was almost the same as that in the studies of TAMMINGA (1975). Exceptions are found in the larger amounts of tyrosine, alanine and glutamic acid and the smaller amounts of histidine and methionine in the present studies. TAMMINGA determined only the amino acids present in protein.

Compared with the hydrolyzate of the insoluble fraction of the rumen, the proportion of  $\alpha$ ,  $\epsilon$ -diaminopimelic acid, alanine, glycine and valine of the total identified amino acids in the hydrolyzate of the faeces of 0-cows was high and that of arginine low. The proportion of glucosamine of the total amount of amino acids was  $2.9 \pm 1.9\%$  in the faeces of 0-cows,  $2.2 \pm 0.3\%$  in that of ULP-cows and  $1.3 \pm 0.2\%$  in the faeces of NorP-cows,  $0.1\%$  in rumen hydrolyzate from 0-cows (MÄKINEN 1972) and  $0.2\%$  in that from ULP- and NorP-cows. The fact that the amounts of  $\alpha$ ,  $\epsilon$ -diaminopimelic acid and glucosamine in the faeces are greater than in the rumen seems to show that there are large amounts of bacterial cell wall in the faeces. According to HOOGENRAAD *et al.* (1970), glutamic acid, alanine and diaminopimelic acid along with muramic acid and glucosamine make up the peptidoglycan portion of the bacterial cell (*B. subtilis*). In successive hydrolyses performed with papain and pepsin it was possible to liberate 37 and 30% respectively of the amino acids of the insoluble fraction of the faeces of ULP-cows. HOOGENRAAD and HIRD (1970) were able to release 45% of the total amount of amino acids of the bacterial cell walls with pepsin, and 30% with chymotrypsin. According to an unpublished study of our laboratory there were  $1.6\text{--}4.0 \times 10^8$  living anaerobic bacteria and  $3.4\text{--}7.9 \times 10^6$  aerobic bacteria per g fresh faeces with 0-cows; the corresponding figures with ULP-cows were  $3.0 \times 10^6\text{--}3.9 \times 10^8$  and  $4.2 \times 10^6\text{--}2.6 \times 10^8$ .

It is clear that on 0-feed in which urea was the sole source of nitrogen the faeces contained both microbial and endogenic nitrogen, but on the two other feeds the faeces probably contained also indigestible feed nitrogen. MASON (1969) reported that the indigestible feed nitrogen was 3.2–28.3% of the total nitrogen of the faeces, with sheep. As for the present studies, it seems that much of the feed nitrogen was converted to microbial nitrogen. It is likely that on all but nitrogen-free rations a variable but important part of the bacterial nitrogen in the faeces is derived directly from the feed (MASON 1969, 1971).

The peptide fraction also contained  $\alpha$ ,  $\epsilon$ -diaminopimelic acid and glucosamine, and with some cows  $\alpha$ ,  $\epsilon$ -diaminopimelic acid was present even in the free amino acid fraction. MASON and co-workers (MASON and WHITE 1971, MASON and MILNE 1971) found evidence that the sheep is unable to digest diaminopimelic acid-containing mucopeptide with its own enzyme systems. A decreased amount of diaminopimelic acid passing through the rectum indicated partial removal or destruction of this amino acid in the hindgut, probably as a result of microbial activity. These authors point out that though much of the water-soluble nitrogen of faeces is of bacterial origin, a large proportion must be degraded pigments, enzymes and other secretions.

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Ms received July 4, 1979

## Lannan typpiyhdisteistä lypsylehmillä, joilla urea on ollut ainoana tai osittaisena typenlähteenä

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Lypsylehmien lannan typpiyhdisteitä on tutkittu puhdistetulla proteiinittomalla ruokinnalla, jossa ainoana typenlähteenä on ollut urea ja vähäisessä määrässä ammoniumsuolat (0-ruokinta), vähän proteiinia sisältävällä ruokinnalla, jossa puuttuva proteiini on korvattu urealla (ULP-ruokinta) ja tavanomaisella proteiinia sisältävällä ruokinnalla (NorP-ruokinta).

Lannan kokonaistyyppipitoisuudet ovat olleet 0-ruokinnalla  $2,5 \pm 0,7$  %, ULP-ruokinnalla  $2,4 \pm 0,4$  ja NorP-ruokinnalla  $2,3 \pm 0,6$  % kuiva-aineesta. Urea- ja ammoniumtypen osuus on vastaavasti ollut  $3,2 \pm 2,4$ ,  $4,5 \pm 2,4$  ja  $3,7 \pm 1,8$  % kokonaistypestä, 70 %:seen etanoliin liukenevan typen osuus  $10,9 \pm 5,7$ ,  $9,9 \pm 2,4$  ja  $10,4 \pm 3,8$  % kokonaistypestä. Erot eivät ole olleet tilastollisesti merkitseviä. Vapaiden aminohappojen typen osuus on ollut 0-ruokinnalla  $4,1 \pm 2,9$ , ULP-ruokinnalla  $1,6 \pm 1,4$  ( $P < 0,05$ ) ja NorP-ruokinnalla  $3,2 \pm 2,9$  % kokonaistypestä.

70 %:seen etanoliin liukenematonta tyyppiä on ollut 0-ruokinnalla  $82,3 \pm 7,2$ , ULP-ruokinnalla  $85,4 \pm 5,0$  ja NorP-ruokinnalla  $82,3 \pm 7,2$  % kokonaistypestä.

Liukenemattoman typen fraktiosta on happohydrolyysillä vapautunut aminohappotyyppiä 0-ruokinnalla  $62,8 \pm 8,5$  %, ULP-ruokinnalla  $61,6 \pm 10,7$  % ja NorP-ruokinnalla  $59,0 \pm 13,9$  % kokonaistypestä. Vapautuneista aminohapoista on  $\alpha$ ,  $\epsilon$ -diaminopimeliinihapon osuus ollut vastaavasti  $1,7 \pm 0,6$ ,  $0,7 \pm 0,2$  ( $P < 0,05$ ) ja  $1,2 \pm 0,4$  mooliprosenttia.

Liukenevan typen fraktiosta on määritetty vapaat ja happohydrolyysillä vapautuneet peptidi-fraktion aminohapot.