Digestion of feed amino acids in the rumen and small intestine of dairy cows measured with nylon-bag techniques

BY W. M. VAN STRAALEN*, J. J. ODINGA AND W. MOSTERT

DLO-Institute for Animal Science and Health (ID-DLO), PO Box 65, 8200 AB Lelystad, The Netherlands, and
Department of Animal Nutrition, Wageningen Agricultural University, Marijkeweg 40, 6709 PG, Wageningen, The Netherlands

(*Received 2 February 1994 – Revised 1 December 1995 – Accepted 23 January 1996*)

The disappearance of total N, non-protein-N and amino acid-N after washing, rumen incubation and intestinal passage of sugarbeet pulp, maize-gluten feed, maize feed meal, palm kernel meal, soyabean hulls, soyabean meal, grass silage, maize silage and concentrate was measured in four dairy cows using nylon-bag techniques. Disappearance of amino acid-N after washing varied between feedstuffs from 14 to 69 % of feed amino acid-N, and was lower than disappearance of non-protein-N. For sugarbeet pulp, grass silage and maize silage, washing had a considerable effect on the amino acid profile. Disappearance of amino acid-N after rumen incubation was also lower than non-protein-N and varied between feedstuffs from 25 to 73 % of feed amino acid-N. Rumen incubation had only a small effect on the amino acid profile of the residue after washing. Disappearance of amino acid-N in the intestine varied between feedstuffs from 70 to 99 % of rumen undegraded amino acid-N, and was higher than the disappearance of non-protein-N. Intestinal incubation showed a considerable effect on the amino acid profile for all feedstuffs. It was concluded that protein that was assumed to escape rumen degradation and was absorbable in the intestine was higher in amino acids and methionine, and lower in non-amino acid-N and glutamic acid and proline compared with protein in the feedstuff.

Amino acid digestion: Nylon bags: Feedstuffs: Dairy cows

Modern protein evaluation systems for ruminants describe the supply and requirement of true protein that can be absorbed from the small intestine. To increase the efficiency by which this true protein is used for production purposes, research has been focused on the requirement of first limiting amino acids (Schwab *et al.* 1992), and results are being incorporated into protein evaluation systems (Rulquin & Vérité, 1993). To meet requirements, the supply of absorbable individual amino acids can be altered by changing the source of undegraded protein in the diet (Seymour *et al.* 1990). Before protein is absorbed in the small intestine, it is subjected to microbial fermentation in the rumen and enzymic digestion in the intestine. However, the effect of rumen fermentation on the amino acid profile of undegraded protein is not clear. Some authors found no effect (Varvikko *et al.* 1983; Weakley *et al.* 1983; Messman *et al.* 1992), while others reported different effects for different feedstuffs (Ganev *et al.* 1979; Hennessey *et al.* 1983; Rooke, 1985; Crooker *et al.* 1986; Susmel *et al.* 1989). Hardly any research has been carried out to study the effect of intestinal digestion on the amino acid profile of undegraded protein (Le Henaff *et al.* 1988).

*Present address: CLO Institute for Animal Nutrition, "De Schothorst", PO Box 533, 8200 AM Lelystad, The Netherlands.

The effect of rumen fermentation and small intestine digestion on the amino acid profile of feed protein can be studied using the rumen nylon-bag and mobile nylon-bag techniques (van Straalen *et al.* 1993). With these techniques dietary ingredients are generally incubated separately. Murphy & Kennelly (1987) and Stallings *et al.* (1991) showed that rumen degradation of protein in a concentrate or complete diet mixture could be predicted from the degradation of the ingredients of the concentrate or diet, but for intestinal digestion this was never tested.

The objective of the present study was to determine the effect of washing, rumen incubation and intestinal incubation on the amino acid profile of feedstuffs using nylon-bag techniques, in order to predict the supply of individual amino acids to the animal from rumen undegraded feed. Also, a comparison was made between measured residues after washing, rumen incubation and intestinal incubation of amino acids of a concentrate, and the estimated values from the ingredients of the concentrate.

MATERIALS AND METHODS

Experimental animals

Four dairy cows (Holstein-Friesian) were used, of which three were lactating and one was non-lactating. All animals were fitted with a permanent rumen cannula (Bar Diamond Inc., Pharma, ID, USA) and a T-piece cannula in the distal duodenum. Animals were housed in a tie-stall and fed twice daily (08.00 and 20.00 hours) on an equal portion of the daily allowance according to energy and protein requirements. The three lactating cows were fed on a diet consisting of (g/kg DM): maize silage 170, grass silage 230, concentrates 600. The dry cow received a mixture of (g/kg DM): grass silage 600, maize silage 400; with 1 kg concentrates.

Samples

Ten feedstuffs were obtained from a parallel feeding experiment: one concentrate, six concentrate ingredients and two roughages. The concentrate contained the following ingredients (g/kg) sugarbeet pulp (BP) 155, maize-gluten feed (MGF) 300, maize feed meal (MFM) 108, palm kernel expeller (PKE) 125, soyabean hulls (SBH) 50, soyabean meal (SBM) 148. The concentrate also contained (g/kg): palm kernels 10, molasses 80, minerals 24. Roughages were grass silage (GS) and maize silage (MS). Samples of concentrate ingredients were obtained from the feed factory before pelleting of the concentrate. Samples from the concentrate, grass silage and maize silage were taken during the feeding experiment. Concentrate ingredients and the concentrate were ground to pass a 3 mm screen. Roughages were chopped in a laboratory cutter to pieces of approximately 10 mm, and kept at $-20°$. A portion of each of the samples was dried at 70°, ground to pass a 1 mm sieve and used for chemical analysis.

Rumen and intestinal incubations

Nylon-bag incubations in rumen and intestine were carried out according to the procedures of van Straalen *et al.* (1993). Samples of each feedstuff corresponding to 5 g DM were weighed into nylon bags (90 × 180 mm, polyamide, pore size 41 μm, porosity 30%; Nybolt, Zurich, Switzerland). For each feedstuff eight bags were incubated for 12 h in the rumen of each cow. The length of the incubation time was chosen to have close agreement between the residue after 12 h incubation and the expected effective escape fraction, based

on rumen degradation characteristics of the same feedstuffs (van Straalen, unpublished results), and calculated according to Ørskov & McDonald (1979) with a passage rate of 6 % per h (van Straalen *et al.* 1993). After incubation, bags were rinsed under tap-water and subsequently washed in a domestic washing machine (using 60 litres water at 15° without spinning). An extra four bags for each feedstuff were not incubated in the rumen and only washed to determine the soluble fractions. Residues were lyophilized, pooled for each animal and feedstuff, and ground to pass a 3 mm screen. A portion of each of the residues was further ground (1 mm) and used for chemical analysis.

To determine intestinal digestion, nylon bags (30 × 60 mm, the same material as the rumen bags) were filled with 0·5 g rumen residue DM. Pepsin (*EC* 3.4.23.1)–HCl incubation (1 g pepsin; Merck, Darmstadt, Germany; 2000 FIP units g, in 1 litre 0·1 M-HCl) was carried out for 1 h at 37° immediately before intestinal incubation. Bags were introduced into the duodenum (every 20 min) via the T-piece cannula (four bags three times hourly) and recovered from the faeces. Recovered bags were rinsed under tap-water and stored at −20° until all bags had been retrieved. Bags were washed in a domestic washing machine, using 100 litres water at 40° with spinning. This procedure was more intensive than the washing procedure of rumen nylon bags because with the latter method not all washable material may have been removed from the bags (van Straalen, unpublished results). Residues were dried at 70°, pooled for each animal and feedstuff, and ground to pass a 1 mm sieve.

Laboratory analysis

Feedstuff samples and residues after washing, rumen incubation and intestinal incubation were analysed for DM, ash and Kjeldahl N according to the standard procedures used at IVVO-DLO (Steg *et al.* 1990). Residues after washing, rumen incubation and intestinal incubation were pooled for each feedstuff before analysis of amino acids. A single amino acid analysis was carried out according to the *Proposed Official Method for Determination of Amino Acids in Animal Feed* European Commission 1977, 1979, as described by Van Vuuren *et al.* (1992). Threonine, serine, valine and isoleucine contents were corrected for incomplete recovery of amino acids after hydrolysis (Slump, 1969). Non-protein-N (NPN) was calculated as total N minus amino acid-N (AAN). The AAN was further separated into essential AAN (EAAN) and non-essential AAN (NEAAN). Feedstuffs were further analysed for crude fat, starch (Steg *et al.* 1990), neutral-detergent fibre (NDF), acid-detergent fibre (ADF) and acid-detergent-insoluble N (ADIN; Robertson & Van Soest, 1981). Apparent digestibility of organic matter was determined *in vitro* using rumen fluid followed by pepsin–HCl incubation according to Steg *et al.* (1990).

Comparison of measured and estimated disappearance of N in concentrate

The measured disappearance of the concentrate (Cm) after washing, rumen incubation and intestinal incubation was compared with the value estimated from the disappearance of the ingredients of the concentrate and their respective contents (Ce). Disappearance of palm kernel, molasses, and minerals and vitamins were based on tabulated and assumed values.

Statistical analysis

Differences between feedstuffs in rumen and intestinal disappearance of DM and total N were tested using ANOVA, with feedstuffs as a factor. Differences between feedstuffs for individual AAN disappearances could not be tested, because samples obtained from four

cows had been pooled per feedstuff before analysis of AAN, resulting in only one observation per amino acid and per feedstuff.

For the comparison of the amino acid profile of the protein in the feedstuff, residue after washing, rumen incubation and intestinal incubation, the method of Guilloteau *et al.* (1986) was used. In this method the similarity between two amino acid profiles is expressed as the distance in χ^2:

$$\text{distance in } \chi^2 = 17 \times \sum_{k=1}^{k=17} (\text{AAN}_{ik} - \text{AAN}_{jk})^2/((\text{AAN}_{ik} + \text{AAN}_{jk})/2).$$

In this equation AAN_{ik} and AAN_{jk} are the respective percentages of N in amino acid k in total AAN of proteins i and j (feedstuff, residue after washing, rumen incubation and intestinal incubation). The same procedure was used to compare the measured and estimated amino acid profiles of the concentrate. A higher distance in χ^2 corresponds with a larger difference between the amino acid profiles of the two proteins. Statistical calculations were carried out using the Genstat software (Genstat 5 Committee, 1987).

RESULTS

Composition of feedstuffs

The chemical composition and composition of total N of samples is given in Table 1 and Table 2 respectively. Total N content in the samples varied between 12·6 g/kg DM for MS and 82·2 g/kg DM for SBM. The content of ADIN in total N varied from 15 g/kg for SBM to 173 g/kg for PKE. The lowest contribution of AAN to total N was observed for BP (59 %) and the highest for SBM (84 %). The percentage of EAAN in AAN varied between 45 (MS) and 60 (PKE).

Compared with the other feedstuffs, BP had a relatively high content of glutamic acid-N, and a low content of arginine-N. PKE had very high proportion of arginine-N in total AAN. Within each class of feedstuffs, amino acid profiles were similar (MGF and MFM; SBH and SBM). Exceptions were the higher glycine-N and lower glutamic acid-N and arginine-N contents of SBH compared with those of SBM. The lysine-N and arginine-N contents of MS were low, and those of alanine-N and leucine-N high compared with values for the other feedstuffs.

Table 1. *Dry Matter (DM) (g/kg), organic matter (OM), nitrogen, crude fat, neutral-detergent fibre (NDF), acid-detergent fiber (ADF) and starch contents (g/kg DM) and* in vitro *digestibility coefficient of organic matter (DOM) of feedstuffs*

Feedstuff ...	BP	MGF	MFM	PKE	SBH	SBM	GS	MS	Cm	Ce
DM (g/kg)	920	901	899	922	902	892	620	339	906	893
OM	915	939	952	952	949	925	882	954	913	911
N	19·5	34·6	24·6	26·2	22·1	82·2	40·5	12·6	33·4	34·6
Crude fat	7	37	51	103	27	23	43	29	39	40
NDF	300	394	311	640	620	123	450	399	323	330
ADF	148	97	81	368	436	63	239	208	142	13
Starch	nd	200	335	4	8	8	nd	323	109	98
DOM (%)	89	86	85	70	82	90	79	73	85	85

BP, sugarbeet pulp; MGF, maize gluten feed; MFM, maize feed meal; PKE, palm kernel expeller; SBH, soyabean hulls; SBM, soyabean meal; GS, grass silage; MS, maize silage; Cm, concentrate measured; Ce, concentrate estimated from ingredient composition; nd, not determined.

Table 2. *Acid-detergent-insoluble nitrogen (ADIN), non-protein-N (NPN), amino acid-N (AAN), essential AAN (EAAN), non-essential AAN (NEAAN) and N for individual amino acids in feedstuffs (g/kg total N)*

Feedstuff*...	BP	MGF	MFM	PKE	SBH	SBM	GS	MS	Cm	Ce
ADIN	47	26	59	173	63	15	22	25	45	41
NPN	411	269	271	204	210	158	400	389	272	232
AAN	589	731	729	796	790	842	600	611	728	768
EAAN	284	378	384	475	406	476	304	278	391	417
NEAAN	305	353	344	321	384	366	296	333	337	351
EAAN:										
Thr	25	27	26	22	28	29	27	25	24	27
Val	33	38	35	38	36	37	37	36	35	37
Ile	20	21	21	23	24	31	23	23	24	25
Leu	26	55	55	40	38	50	39	61	47	48
Tyr	19	14	14	12	22	18	12	10	15	16
Phe	12	19	20	20	21	26	21	19	20	21
His	40	52	49	32	52	47	26	34	43	47
Lys	51	36	39	29	73	72	38	25	47	52
Arg	53	108	117	250	107	158	73	35	128	137
Met	6	10	9	10	6	7	7	9	8	8
NEAAN:										
Asp	43	40	41	49	63	72	55	40	52	54
Ser	37	40	39	37	54	47	33	35	40	42
Glu	116	89	86	107	78	106	49	80	95	99
Pro	26	57	54	28	38	40	54	54	42	44
Gly	38	51	50	50	94	48	45	41	48	50
Ala	37	60	60	39	44	42	52	72	48	48
Cys	7	16	16	10	13	11	6	11	13	13

BP, sugarbeet pulp; MGF, maize gluten feed; MFM, maize feed meal; PKE, palm kernel expeller; SBH, soyabean hulls; SBM, soyabean meal; GS, grass silage; MS, maize silage; Cm, concentrate measured; Ce, concentrate estimated from ingredient composition.

*For details of composition, see Table 1.

Except for the higher NPN content, the composition of Cm was almost identical to that of Ce (Tables 1 and 2).

Disappearance after washing

Disappearance after washing varied between 17% for SBH and 45% for Cm (Table 3). The disappearance of N varied between feedstuffs from 11% for SBM to 78% for MS. For all feedstuffs (except SBM) there was a higher disappearance of NPN after washing than that of AAN. For BP and GS the value for EAAN was considerably lower than that for NEAAN.

The distance in χ^2 indicated a large difference in the amino acid profile of the feedstuff and residue after washing for BP, GS and MS (Table 4). This was mainly due to glutamic acid in BP, proline in GS and arginine in MS (Table 3). For the other feedstuffs washing had only a minor effect on the amino acid profile. In general, for threonine and cysteine the levels of disappearance after washing were relatively low, and for proline and histidine they were relatively high. Of the first limiting amino acids, there was a relatively low disappearance of methionine for most feedstuffs while that of lysine was variable.

Table 3. *Disappearance after washing of DM, nitrogen (N), non-protein-N (NPN), amino acid-N (AAN), essential AAN (EAAN), non-essential AAN (NEAAN) and N in individual amino acids in feedstuffs (% of content in feed)**

Feedstuff† ...	BP	MGF	MFM	PKE	SBH	SBM	GS	MS	Cm	Ce
DM	44	35	40	19	17	31	35	42	45	41
N	50	48	41	22	31	11	54	78	47	30
NPN	69	60	100	44	80	−2	75	93	81	49
AAN	38	44	18	17	17	14	39	69	35	25
EAAN	22	43	17	17	17	12	33	66	33	23
NEAAN	52	46	19	16	18	15	46	71	37	27
EAAN:										
Thr	25	41	8	14	17	6	37	66	26	19
Val	22	38	6	13	9	10	39	66	31	19
Ile	31	37	7	11	12	6	32	71	30	17
Leu	22	40	13	15	16	10	30	74	35	22
Tyr	26	36	5	6	21	10	24	57	26	19
Phe	17	37	9	14	25	8	26	64	29	18
His	29	45	21	28	33	16	50	76	39	29
Lys	24	47	28	13	9	10	36	64	30	21
Arg	10	47	24	19	17	18	28	42	35	26
Met	18	43	21	−15	19	−7	4	63	15	15
NEAAN:										
Asp	44	45	20	14	27	10	49	68	33	19
Ser	37	50	14	17	19	11	46	70	31	25
Glu	76	46	19	18	24	29	40	74	42	35
Pro	35	46	23	24	18	13	69	77	44	29
Gly	32	41	14	14	9	9	29	61	31	22
Ala	39	46	22	15	7	11	43	76	40	27
Cys	29	43	24	8	37	−2	9	43	30	23

BP, sugarbeet pulp; MGF, maize gluten feed; MFM, maize feed meal; PKE, palm kernel expeller; SBH, soyabean hulls; SBM, soyabean meal; GS, grass silage; MS, maize silage; Cm, concentrate measured; Ce, concentrate estimated from ingredient composition.

*For details of procedures, see pp. 84–85.

†For details of composition, see Table 1 and 2.

Table 4. *Distance in χ^2 (similarity between two amino acid profiles; Guilloteau* et al. *1986) between amino acid-nitrogen (AAN) profiles of feedstuff (F) residue after washing (S), rumen incubation (R) and intestinal (I) incubation**

Feedstuff†...	BP	MGF	MFM	PKE	SBH	SBM	GS	MS	Cm
F *v.* S	239	7	10	7	16	13	82	119	11
F *v.* R	249	20	27	8	44	18	85	226	11
S *v.* R	22	11	16	4	35	7	16	71	4
F *v.* I	748	668	224	209	474	772	171	586	234
S *v.* I	550	634	223	188	442	717	72	703	209
R *v.* I	542	617	216	178	262	675	66	677	211

BP, sugarbeet pulp; MGF, maize gluten feed; MFM, maize feed meal; PKE, palm kernel expeller; SBH, soyabean hulls; SBM, soyabean meal; GS, grass silage; MS, maize silage; Cm, concentrate measured.

*For details of procedures, see pp. 84–86.

†For details of composition, see Tables 1 and 2.

The levels of disappearance after washing for N, NPN and AAN of Ce were lower than that for Cm (Table 3). The estimated amino acid profile of the residue was comparable with that of the measured profile; the distance in χ^2 was 4.

Disappearance after rumen incubation

Values for the disappearance of DM and N after 12 h of rumen incubation for each feedstuff are given in Table 5. There were significant differences between levels of disappearance for both DM and N between feedstuffs. The lowest level of DM disappearance was observed for PKE (39%) and the highest for BP (78%). For N the level of disappearance varied from 24% for PKE to 76% for GS. For PKE the disappearance of AAN was higher than that of NPN; for MGF, MFM and SBH the differences were small, and for the remaining feedstuffs the disappearance of AAN was lower than that of NPN. For BP, PKE, GS, MS and Cm, NEAAN was degraded further in the rumen than EAAN, while differences were small for the other feedstuffs.

Table 5. *Disappearance after 12 h rumen incubation of DM, nitrogen (N), non-protein-N (NPN), amino acid-N (AAN), essential AAN (EAAN), non-essential AAN (NEAAN) and N in individual amino acids in feedstuffs (% of content in feed)**

Feedstuff† ...	BP	MGF	MFM	PKE	SBH	SBM	GS	MS	Cm	Ce
DM‡	78^{f}	64de	61^{cd}	39^{a}	41^{a}	69^{e}	54^{b}	57^{bc}	69^{e}	66
N‡	74de	73^{cd}	61^{b}	24^{a}	62^{b}	59^{b}	76^{e}	74de	69^{c}	62
NPN	89	73	60	18	61	72	83	79	83	69
AAN	63	73	61	25	62	57	72	71	64	60
EAAN	55	72	60	24	63	57	68	66	63	58
NEAAN	71	73	61	27	62	57	76	75	66	63
EAAN:										
Thr	60	70	56	19	66	53	73	66	60	58
Val	60	72	59	23	60	52	73	74	63	58
Ile	56	67	52	17	58	52	67	69	59	54
Leu	49	68	51	20	62	54	68	80	62	56
Tyr	66	68	52	14	60	52	67	63	60	56
Phe	49	69	53	22	66	55	70	69	61	56
His	65	78	68	27	59	56	67	73	67	64
Lys	58	71	62	13	57	59	66	40	60	59
Arg	40	76	67	28	69	62	66	41	64	59
Met	45	66	51	1	55	45	60	69	61	49
NEAAN:										
Asp	63	71	59	24	70	58	77	65	64	64
Ser	68	73	61	25	57	51	75	71	63	59
Glu	85	74	61	32	70	63	72	80	69	67
Pro	57	75	62	24	59	60	86	80	67	64
Gly	57	72	63	26	53	52	70	66	62	58
Ala	62	72	59	22	63	52	74	81	66	61
Cys	55	72	66	21	69	51	60	64	69	60

^{a,b,c,d,e,f}Values in the same row with different superscript letters were significantly different ($P < 0{\cdot}05$).

BP, sugarbeet pulp; MGF, maize gluten feed; MFM, maize feed meal; PKE, palm kernel expeller; SBH, soyabean hulls; SBM, soyabean meal; GS, grass silage; MS, maize silage; Cm, concentrate measured; Ce, concentrate estimated from ingredient composition.

*For details of procedures, see pp. 84–85.

†For details of composition, see Tables 1 and 2.

‡No. of replicates 4; SED: DM 2·4, N 3·3 (excluded Ce).

The amino acid profiles of the residues after rumen incubation were different from those of the original feed for BP, GS and MS, but comparable to those after washing, as indicated by the distance in χ^2 (Table 4). In general, glutamic acid showed high levels of disappearance (Table 5). For arginine the levels of disappearance were relatively low for BP and MS, but relatively high for MGF, MFM and SBM. Of the first limiting amino acids, for all feedstuffs, especially PKE, methionine was relatively undegradable, while the residual lysine was relatively high in PKE and MS.

For Ce the levels of disappearance of total N, NPN and AAN in the rumen were lower than those for Cm (Table 5), but the amino acid profiles of the rumen residues were comparable (distance in χ^2: 2).

Intestinal disappearance

The disappearance of DM and N, expressed as a percentage of the rumen residue, differed significantly between feedstuffs and varied from 20 % for MS to 88 % for SBM and from 64 % for MS and SBH to 99 % in SBM, respectively (Table 6). The disappearance of NPN varied between 38 % for SBH and 97 % for SBM, and for all feedstuffs was lower than that of AAN, which varied between 70 % for SBH and 99 % for SBM. EAAN had a higher level of disappearance in the intestine than NEAAN.

Judged by the distance in χ^2, intestinal incubation of rumen residues resulted in a considerable change in amino acid profile (Table 4). This difference was highest for MS, SBM, MGF and BP. For most feedstuffs arginine had a relatively high level of disappearance, and glycine and cysteine a relatively low level of disappearance, compared with other amino acids (Table 6). The disappearance of methionine was relatively high in MFM and PKE, while lysine disappearance was relatively low in MGF, MFM and SBH. In MS an extremely low level of histidine disappearance was observed.

The intestinal levels of disappearance of the different components of total N for Ce were lower than those for Cm, but the amino acid profiles were similar (Table 6; distance in χ^2 was 25).

DISCUSSION

Feedstuff composition

The NPN values for concentrate ingredients were generally higher than those given in the Dutch Feedstuff Table (Centraal Veevoederbureau, 1991). Only part of this difference could be attributed to tryptophan, which was not determined in our experiment. There were no differences in EAAN:NEAAN and the profile of individual amino acids between tabulated values and values measured in our experiment. For BP only there was a lower NPN, higher glutamic acid-N and lower aspartic acid-N content than that given by the Centraal Veevoederbureau (1991). The NPN content in BP increases with the inclusion of molasses. The N in molasses consists of about 70 % NPN and 30 % AAN, which is mainly aspartic acid-N and glutamic acid-N (Centraal Veevoederbureau 1991). The level of inclusion of molasses in the BP in our experiment was not known, but based on NDF content it was comparable with the quality of BP with the highest sugar content and, thus, highest inclusion of molasses.

The AA profile of GS observed in our experiment differed from that published by Rooke *et al.* (1984) and Syrjälä-Qvist *et al.* (1984 *a*,*b*). The total and individual EAAN in MS was lower than observed by Le Henaff *et al.* (1988). One can speculate that these differences may be due to differences in the contents of different types of protein, related to species, fertilization and maturity.

Table 6. *Disappearance in the intestine of DM, nitrogen (N), non-protein-N (NPN), amino acid-N (AAN), essential AAN (EAAN), non-essential AAN (NEAAN) and N in individual amino acids in feedstuffs (% of 12 h rumen residue)**

Feedstuff†...	BP	MGF	MFM	PKE	SBH	SBM	GS	MS	Cm	Ce
DM‡	70[b]	39[e]	47[d]	52[c]	30[f]	88[a]	43[de]	20[g]	51[cd]	52
N‡	86[c]	84[b]	86[cd]	85[cd]	64[e]	99[a]	87[bc]	64[e]	89[b]	85
NPN	64	73	83	70	38	97	78	50	71	67
AAN	91	87	87	90	70	99	91	72	92	89
EAAN	92	89	88	91	74	99	91	74	93	91
NEAAN	90	86	85	88	67	99	90	69	91	88
EAAN:										
Thr	90	79	79	88	81	100	90	72	90	86
Val	99	88	88	89	74	99	90	71	92	92
Ile	91	89	90	88	82	100	91	75	94	91
Leu	91	90	92	88	83	100	91	75	94	91
Tyr	89	86	88	88	61	99	92	86	91	87
Phe	91	82	90	89	83	100	91	72	94	89
His	86	81	85	88	62	99	89	5	91	85
Lys	93	81	82	90	63	99	92	77	91	88
Arg	93	100	90	94	85	100	92	100	95	94
Met	93	89	92	92	82	100	92	77	93	91
NEAAN:										
Asp	92	86	87	89	82	100	91	73	94	89
Ser	86	86	86	88	65	99	90	73	91	87
Glu	92	91	92	90	80	100	91	76	95	92
Pro	89	81	85	86	59	99	93	65	89	85
Gly	82	79	79	81	53	98	87	63	84	79
Ala	100	89	77	88	75	99	90	69	92	91
Cys	84	82	82	86	67	99	78	53	88	84

[a,b,c,d,e,f,g]Values in the same row with different superscript letters were significantly different ($P < 0{\cdot}05$).

BP, sugarbeet pulp; MGF, maize gluten feed; MFM, maize feed meal; PKE, palm kernel expeller; SBH, soyabean hulls; SBM, soyabean meal; GS, grass silage; MS, maize silage; Cm, concentrate measured; Ce, concentrate estimated from ingredient composition.

*For details of procedures, see pp. 84–85.

†For details of composition, see Tables 1 and 2.

‡No. of replicates 4; SED: DM 3·8, N 2·4 (excluded Ce).

Solubility

For BP only there was a large difference in the soluble N fraction when comparing our values with those of van Straalen & Tamminga (1990). This difference can be explained by the high level of molasses included. For all feedstuffs (except SBM), NPN was relatively more soluble than AAN. Furthermore, MacGregor *et al.* (1978) observed a higher solubility of NPN in a modified Burroughs mineral buffer compared with AAN for a large range of feedstuffs. Depending on the feedstuff, NPN consists of NO_3, NH_3, purine, RNA and DNA, which are relatively soluble or easily degradable in the rumen (Greife, 1984; Tamminga, 1986). There was a positive relationship between the NPN content and the soluble N (SN) fraction: SN (g/kg N) $= -76 + 1{\cdot}74 \times$ NPN (g/kg N) (r^2 0·74; n 9). In addition to NPN, some of the AAN was also washed out of the nylon bags. The solubility of total AAN in BP was higher than that observed by MacGregor *et al.* (1978), who also reported a higher solubility for glutamic acid compared with the other amino acids. The high solubility of glutamic acid in BP can be explained by the difference in amino acid

profile and solubility between BP and added molasses, as discussed previously. For MFM and SBM the solubilities of total AAN were comparable with those of MacGregor *et al.* (1978), but those for individual AAN were different.

Rumen degradation

For most feedstuffs the residual N after 12 h rumen incubation was comparable with the effectively-fermented fraction calculation from degradation characteristics in the rumen (W.M. van Straalen, unpublished results) and an assumed passage rate of 6 % per h (%; BP 73, MGF 70, MFM 63, PKE 45, SBH 57, SBM 56, GS 78, MS 78, Cm 67). These values were also similar to the effective escaped N given by Centraal Veevoederbureau (1991). For PKE a higher value was observed; this could be due to the relatively long lag-time before the onset of the degradation, resulting in a large residue after 12 h incubation time in the present experiment. (A. Steg, unpublished results).

The further disappearance of N after rumen incubation (Table 5) when compared with that after washing (Table 3) varied between feedstuffs. For MS no extra disappearance was measured (−4 %), while for SBM another 48 % disappeared after rumen incubation. For NPN this variation ranged from −40 % for MFM to 74 % in SBM, and for AAN, 2 % for MS and 45 % for SBH. From the higher distance in χ^2 between the feedstuff and residue after washing, compared with that for the residue after washing and rumen incubation, it can be concluded that depending on the feedstuff the amino acid profile is more influenced by washing than by rumen degradation. This agrees with the conclusion of MacGregor *et al.* (1978) that the amino acid profile of the insoluble residue can be regarded as more representative of that of escaped protein than that of the feedstuff.

In our study methionine in SBM was relatively undegradable compared with other amino acids, which is in agreement with results obtained by Crooker *et al.* (1987), Le Henaff *et al.* (1988) and Mir *et al.* (1984), but contrasts with those of Susmel *et al.* (1989). Relatively high degradability of arginine and glutamic acid in SBM was also reported by Crooker *et al.* (1986,1987). According to the literature, tyrosine in SBM was relatively undegradable (Ganev *et al.* 1979; Hennessy *et al.* 1983; Varvikko *et al.* 1983), and histidine relatively degradable (Varvikko *et al.* 1983; Mir *et al.* 1984); we were unable to confirm these observations in our experiment. Also, our finding that methionine in GS was relatively undegradable disagreed with the findings of Rooke *et al.* (1984), although the low degradation of proline was similar. Degradation of individual amino acids in MS was in line with the findings of Le Henaff *et al.* (1988).

Changes in the amino acid profile after rumen fermentation were mainly observed with BP and MS (Table 4). Since the total N content of BP and MS is low, microbial contamination of rumen residues can have a large effect on the amino acid profile of the residues. The high residues of lysine and arginine in both BP and MS are an indication that these residues contained microbial protein, which has a relatively high content of those amino acids (Rooke *et al.* 1984). Diaminopimelic acid (DAPA) was used to estimate microbial contamination. However, the DAPA content of both feedstuffs and residues was generally below the detection level of 0·50 g/kg DM, and if it was higher a large variation was observed. The DAPA content, therefore, was assumed to be unsuited for the estimation of microbial contamination. An attempt was made to estimate microbial contamination according to van Bruchem *et al.* (1985), by means of comparison of amino acid profiles of the feed, microbial protein (Rooke *et al.* 1984) and rumen residue. The method of van Bruchem *et al.* (1985) incorrectly assumes that the amino acid profile of feed does not change during rumen incubation and, therefore, the amino acid profile of the insoluble

residue was used instead of that of the feed. Values for estimated microbial contamination were (%): 27, 0, 10, 4, 0, 5, 12, 36 and 0 of total AAN for BP, MGF, MFM, PKE, SBH, SBM, GS, MS and Cm respectively. Estimated values for contamination agreed with those observed for concentrate ingredients (Crooker *et al.* 1986, 1987) but were lower than those reported for roughages (Nocek & Grant, 1985; Messman *et al.* 1992). If the amino acid profile of the feed was used, estimated contamination values for all feedstuffs were higher, especially for BP, GS and MS (78, 53 and 58% respectively). The latter feedstuffs also showed high differences between the amino acid profile of the feedstuff and residue after washing. This indicates the importance of using the amino acid profile of the insoluble residue rather than that of the feed. It was concluded that microbial contamination cannot be neglected, at least for BP, GS and MS.

In addition to the differences in solubility and microbial contamination, changes in the amino acid profile after rumen incubation can also be caused by the difference in degradation rates between individual amino acids. Messman *et al.* (1992) observed only small differences between individual amino acid degradation rates with bromegrass (*Bromis inermis*) hay, while with grass silage, Rooke *et al.* (1984) found consistent differences in degradation rates between individual amino acids. Differences in the degradation rates of individual amino acids might be dependent on the content of the different protein classes (albumin, globulin etc.) in the feedstuff and their respective physical properties (solubility, structure) and amino acid composition (Messman *et al.* 1992).

Intestinal disappearance

Because mobile nylon bags were recovered from the faeces, the disappearance of N is the sum of its disappearance in the small intestine and large intestine. Although the latter was not measured in our experiment, other studies indicate that large-intestine fermentation has only a limited effect on total intestinal disappearance, both in nylon bag (Hvelplund, 1985; Voigt *et al.* 1985) and in *in vivo* experiments (van Straalen & Tamminga, 1990).

Total N disappearance from mobile nylon bags containing concentrate ingredients were similar to the digestibility coefficients in the small intestine given by Centraal Veevoederbureau (1991). The disappearance of total AAN in the intestine was higher than that of total N and showed a close relationship: disappearance of AAN (g/g) = $0{\cdot}18 + 0{\cdot}82 \times$ disappearance of total N (g/g; r^2 0·98; n 9). With this equation data from grass silage and hay from Varvikko & Vanhatalo (1991) showed a reasonable fit (r^2 between predicted and measured disappearance of AAN was 0·77). If this relationship is valid also for other feedstuffs, it can be used to predict the AAN disappearance from total N disappearance in mobile nylon bags.

The disappearance of NPN was generally lower than the intestinal digestibility of nucleic acids mentioned by Greife (1984). This finding indicates that NPN in rumen-undegraded N is mainly linked to components that are indigestible in the intestine. Combined with the observations for NPN disappearance in the rumen, this leads to the conclusion that NPN in feedstuffs consists of two fractions: one that is highly soluble in the rumen, and one that is linked to cell walls and is indigestible. Furthermore, Hof *et al.* (1990) observed a decrease of NPN in rumen residues with short incubation periods and an increase with long incubation periods. The ADIN content as a measure of indigestible N was proposed by Robertson & Van Soest (1981). However, the relationships between the indigestible N or indigestible NPN measured with mobile nylon bags and the ADIN content were poor (Fig. 1). Webster *et al.* (1984) proposed the estimation of small intestinal

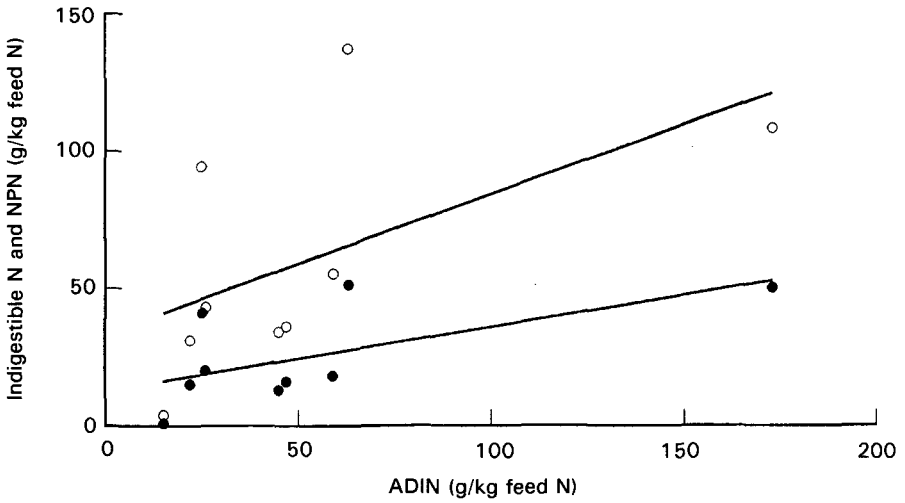

Fig. 1. Relationship between indigestible nitrogen (○), indigestible non-protein-N (NPN; ●) measured with nylon bag incubations and acid-detergent-insoluble N (ADIN; all expressed as g/kg feed-N). Regression equations: IN = 33·5 + 0·505 × ADIN (r^2 0·32, SE of estimate 38·1; NPN = 12·8 + 0·230 × ADIN (r^2 0·39, SE of estimate 14·9).

digestibility of total N (dUN) from ADIN and undegraded dietary N (UDN): dUN = 0·9 × (UDN − ADIN)/UDN. This approach was adopted in the metabolizable protein system (Agricultural and Food Research Council, 1992). There was no relationship between dUN calculated for feedstuffs in our experiment (from Tables 2 and 5) and the disappearance of total N from nylon bags in the intestine (Table 6; r^2 0·19). For most samples the disappearance of N from nylon bags was higher than dUN (except for SBH and MS). Using mobile-nylon-bag data will, therefore, result in a higher predicted supply of absorbable feed N compared with the approach of Webster *et al.* (1984). This conclusion confirms the comparison of these methods that can be carried out using data from other feedstuffs as presented by Agricultural and Food Research Council (1992).

For all feedstuffs intestinal incubation had a considerable effect on the amino acid profile. For most feedstuffs this could be explained by the relatively high disappearance of arginine and low disappearance of glycine. Thus, these amino acids accounted for the difference between the intestinal disappearance of EAAN and NEAAN. The high disappearance of arginine could be the result of the action of trypsin (*EC* 3.4.21.4), which is an endopeptidase that hydrolyses only lysine and arginine-bonds (Stryer, 1988). Le Henaff *et al.* (1988), however, did not find a higher arginine disappearance in the intestine. The theory also does not explain the variable disappearances obtained for lysine. The low disappearance of glycine might be an indication of contamination with endogenous protein which has a high glycine content (Laplace *et al.* 1985).

Predicted supply of absorbable feed amino acids to the animal

From the disappearance of AAN, in terms of the individual amino acids, from nylon bags in the rumen (Table 4) and intestine (Table 6), the supply to the animal of AAN in terms of the individual amino acids in the feed that are absorbed in the small intestine can be predicted. It was assumed that the residue after rumen incubation was representative of the escape fraction and all AAN that disappeared from the mobile nylon bags was absorbed in the small intestine. The predicted supply of AAN (expressed as a percentage of AAN in the

feed) varied between feedstuffs from 21 to 67 % and for all feedstuffs was higher than the supply of NPN (7–57 %). The predicted supply of EAAN (25–69 %) was higher than that of NEAAN (17–64 %). The predicted supply of methionine was higher than values for other amino acids for all feedstuffs but CS. For BP, GS and CS lysine had the second highest supply. Also, isoleucine, leucine, tyrosine and arginine had a high supply for several feedstuffs. Of the EAAN, histidine showed the lowest supply for MGF, MFM and CS. For most feedstuffs the supply of glutamic acid and proline was the lowest of the NEAAN. Also, other NEAAN showed poor supply compared with EAAN.

Measured and estimated AAN fermentation and digestion of concentrate

No explanation can be found for the higher AAN content of Ce compared with that of Cm. The lower disappearance after rumen incubation of all N fractions of Ce compared with those of Cm was mainly due to the lower soluble N fraction. A reason for this can be the treatment (grinding, mixing and pelleting) of concentrate ingredients during the manufacturing process of the concentrate, which might have reduced particle sizes, resulting in a higher disappearance of N from nylon bags in the washing machine. Results are in contrast to those of Murphy & Kennelly (1987), who observed similar measured and estimated protein degradation in the rumen: this can be explained by the absence of pelleting of concentrate mixtures. In contrast to the rumen residues, intestinal disappearance of all N fractions in the concentrate could be estimated from the values obtained with ingredients. Since the rumen residues were higher, the estimated absorption of AAN in terms of the individual amino acid were higher than the measured values. The amino acid profile of undegraded AAN or small intestine-digestible AAN in concentrates could be estimated very well from that of the individual concentrate ingredients.

Conclusions

Although differences between feedstuffs do exist, it can be concluded that AAN was less degradable in the rumen than NPN, and that EAAN was less degradable than NEAAN. Dependent on the feedstuff, rumen incubation has a pronounced effect on the amino acid profile, which was mainly due to the difference in solubility of the amino acids. Intestinal incubation resulted in a considerable change in the amino acid profile of the indigestible residue. It can be concluded that feed protein that is assumed to be absorbed from the small intestine is higher in EAAN, especially methionine-N and lysine-N, and lower in NEAAN, especially glutamine-N and proline-N, than protein in the feedstuff.

The authors are grateful to S. Tamminga (Agricultural University Wageningen) and A. M. van Vuuren for their contribution to the discussion and critically reading the manuscript. They also greatly appreciate the assistance of R. Terluin and his team during the experiment. This study was financed partly by Financieringsoverleg Mest- en Ammoniak-onderzoek (Fund for Manure and Ammonia Research).

REFERENCES

Agricultural and Food Research Council (1992). Nutritive requirements of ruminant animals: protein. *Nutrition Abstracts and Reviews* **62B**, 788–835.

Centraal Veevoederbureau. (1991). *Veevoedertabel (Dutch Feeding Tables)*. Lelystad: Centraal Veevoederbureau.

Crooker, B. A., Clark, J. H., Shanks, R. D. & Fahey, G. C. (1987). Effects of ruminal exposure on the amino acid profile of feeds. *Canadian Journal of Animal Science* **67**, 1143–1148.
Crooker, B. A., Clark, J. H. Shanks, R. D. & Hatfield, E. E. (1986). Effects of ruminal exposure on the amino acid profile of non treated and formaldehyde treated soybean meal. *Journal of Dairy Science* **69**, 2628–2657.
European Commission (1977). *Proposed Official Method for Determination of Amino Acids in Animal Feed. Dir 77/101/EEC.* Strasbourg: European Commission.
European Commission (1979). *Proposed Official Method for Determination of Amino Acids in Animal Feed. Dir 79/373/EEC.* Strasbourg: European Commission.
Ganev, G., Ørskov, E. R. & Smart, R. (1979). The effect of roughage or concentrate feeding and rumen retention time on total degradation of protein in the rumen. *Journal of Agricultural Science, Cambridge* **94**, 651–656.
Genstat 5 Committee (1987). *Genstat 5 Reference Manual.* Oxford: Clarendon Press.
Greife, H. A. (1984). Enteraler und intermediärer Nukleinsäurenstoffwechsel (Nucleic acids in the intestine and metabolism). *Ubersicht zur Tierernährung* **12**, 1–44.
Guilloteau, P., Toullec, R. & Grongnet, J. F. (1986). Digestion of milk, fish and soya-bean protein in the preruminant calf: flow of digesta, apparent digestibility at the end of the ileum and amino acid composition of ileal digesta. *British Journal of Nutrition* **55**, 571–592.
Hennessy, D. W., Lee, G. J. & Williamson, P. J. (1983). Nitrogen loss from protein meals held in terylene bags in the rumen of cattle and the nutritive value of the residues. *Australian Journal of Agricultural Research* **34**, 453–467.
Hof, G., Kouwenberg, W. J. A. & Tamminga, S. (1990). The effect of washing procedure on the estimation of the in situ disappearance of amino acids from feed protein. *Netherlands Journal of Agricultural Science* **38**, 719–742.
Hvelplund, T. (1985). Digestibility of rumen microbial and undegraded dietary protein estimated in the small intestine of sheep and by the in sacco procedure. *Acta Agriculturae Scandinavica* Suppl. **25**, 132–144.
Laplace, J.-P., Darcy-Vrillon, B., Duval-Iflah, Y. & Raibaud, P. (1985). Proteins in the digesta of the pig: amino acid composition of endogenous, bacterial and fecal fractions. *Reproduction Nutrition Développement* **6**, 1083–1099.
Le Henaff, L., Rulquin, H. & Peyraud, J. L. (1988). Etude de la disparition des acides amines alimentaires dans le rumen et dans l'intestin a l'aide de la methode des sachets nylon (A study of amino acid disappearance from feedstuffs in the rumen and intestine measured with the nylon bag method). *Reproduction Nutrition Développement* **28**, Suppl 1, 133–134.
MacGregor, C. A., Sniffen, C. J. & Hoover, W. H. (1978). Amino acid profiles of total and soluble protein in feedstuffs commonly fed to ruminants. *Journal of Dairy Science* **61**, 566–573.
Messman, M. A., Weiss, W. P. & Erickson, D. O. (1992). Effects of nitrogen fertilization and maturity of bromegrass on nitrogen and amino acid utilization by cows. *Journal of Animal Science* **70**, 566–575.
Mir, Z., MacLeod, G. K., Buchanan-Smith, J. G., Grieve, D. G. & Grovum, W. L. (1984). Methods for protecting soybean and canola proteins from degradation in the rumen. *Canadian Journal of Animal Science* **64**, 853–864.
Murphy, J. J. & Kennelly, J. J. (1987). Effect of protein concentration and protein source on the degradability of dry matter and protein in situ. *Journal of Dairy Science* **70**, 1841–1849.
Nocek, J. E. & Grant, A. L. (1985). Characterisation of in situ nitrogen and fiber digestion and bacterial nitrogen contamination of hay crop forages preserved at different dry matter percentages. *Journal of Animal Science* **64**, 552–564.
Ørskov, E. R. & McDonald, I. (1979). The estimation of protein degradability in the rumen from incubation measurements weighted according to rate of passage. *Journal of Agricultural Science, Cambridge* **92**, 499–503.
Robertson, J. B. & Van Soest, P. J. (1981). The detergent system of analysis and its application to human foods. In *The Analysis of Dietary Fiber in Food*, pp. 123–158 [W. P. T. James and O. Theander, editors]. New York: Marcel Dekker Incorporated.
Rooke, J. A. (1985). The nutritive value of feed proteins and feed protein residues resistant to degradation by rumen microorganisms. *Journal of Science of Food and Agriculture* **36**, 629–637.
Rooke, J. A. Greife, H. A. & Armstrong, D. G. (1984). The effect of in sacco rumen incubation of a grass silage upon the total and D-amino acid composition of the residual silage dry matter. *Journal of Agricultural Science, Cambridge* **102**, 695–702.
Rulquin, H. & Vérité, R. (1993). Amino acid nutrition of dairy cows: Productive effects and animal requirements. In *Recent Advances in Animal Nutrition*, pp. 55–77 [P. C. Garnsworthy and D. J. A. Cole, editors]. Loughborough: Nottingham University Press.
Schwab, C. G., Bozak, C. K., Whitehouse, N. L. & Mesbah, M. M. A. (1992). Amino acid limitation and flow to duodenum at four stages of lactation. 1. Sequence of lysine and methionine limitation. *Journal of Dairy Science* **74**, 3486–3502.
Seymour, W. M., Polan, C. E. & Herbein, J. H. (1990). Effects of dietary protein degradability and casein or amino acid infusion on production and plasma amino acid in dairy cows. *Journal of Dairy Science* **73**, 735–748.

Slump, P. (1969). Characterization of the nutritive value of food proteins by amino acid composition and the effect of heat and alkali treatment on the availablity of amino acids, Thesis, University of Amsterdam, The Netherlands.
Stallings, C. C., Acosta, Y. M. & Polan, C. E. (1991). Predicting diet protein degradability from individual ingredient estimations in the diets containing barley silages. *Journal of Dairy Science* **74**, 3486–3491.
Steg, A., Spoelstra, S. F., Van der Meer, J. M. & Hindle, V. A. (1990). Digestibility of grass silage. *The Netherlands Journal of Agricultural Science* **38**, 407–422.
Stryer, L. (1988). *Biochemistry*. New York: Freeman and Company.
Susmel, P., Stefanon, B., Mills, C. R. & Candido, M. (1989). Change in amino acid composition of different protein sources after rumen incubation. *Animal Production* **49**, 375–385.
Syrjälä-Qvist, L., Pekkarinen, E., Setälä, J. & Kangasmaki, T. (1984*a*). Effect of red clover/timothy ratio on the protein value and the quality of silage. *Journal of Agricultural Science in Finland* **56**, 183–191.
Syrjälä-Qvist, L., Pekkarinen, E., Setälä, J. & Kangasmaki, T. (1984*b*). Effect of nitrogen fertilization on the protein quality of timothy grass and silage. *Journal of Agricultural Science in Finland* **56**, 193–199.
Tamminga, S. (1986). Utilization of naturally occurring NPN-compounds by ruminants. *Archives of Animal Nutrition* **36**, 169–176.
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 Straalen, W. M., Dooper, F. M. H., Antoniewicz, A. M., Kosmala, I. & van Vuuren, A. M. (1993). Intestinal digestibility of protein from grass and clover in dairy cows measured with the mobile nylon bag and other methods. *Journal of Dairy Science* **76**, 2970–2979.
van Straalen, W. M. & Tamminga, S. (1990). Protein degradation of ruminant diets. In *Feedstuff Evaluation*, pp. 55–72 [J. Wiseman and D. J. A. Cole, editors]. London: Butterworths.
Van Vuuren, A. M., Krol-Kramer, F., Van der Lee, R. A. & Corbijn, H. (1992). Protein digestion and intestinal amino acids in dairy cows fed fresh Lolium perenne with different nitrogen contents. *Journal of Dairy Science* **75**, 2215–2225.
Varvikko, T., Lindberg, J. E., Setälä, J. & Syrjälä-Qvist, L. (1983). The effect of formaldehyde treatment of soya-bean meal and rapeseed meal on amino acid profiles and acid-pepsin solubility of rumen undegraded protein. *Journal of Agricultural Science, Cambridge* **101**, 603–612.
Varvikko, T. & Vanhatalo, A. (1991). Intestinal nitrogen degradation of hay and grass silage estimated by the mobile nylon bag technique. *World Review of Animal Production* **16**, 73–76.
Voigt, J., Piatkowski, B., Engelman, H. & Rudolph, E. (1985). Measurement of postruminal digestibility of crude protein by the bag technique in cows. *Archive für Tierernährung* **35**, 555–562.
Webster, A. J. F., Kitcherside, M. A., Keirby, J. R. & Hall, P. A. (1984). Evaluation of protein feeds for dairy cows. *Animal Production* **38**, 548.
Weakley, D. C., Stern, M. D. & Satter, L. D. (1983). Factors affecting disappearance of feedstuffs from bags suspended in the rumen. *Journal of Animal Science* **56**, 493–507.