Netherlands Journal of Agricultural Science 33 (1985) 273–284

Digestion of proteins of varying degradability in sheep. 2. Amount and composition of the protein entering the small intestine

J. van Bruchem, S. M. G. Rouwers, G. A. Bangma, S. C. W. Lammers-Wienhoven and P. W. M. van Adrichem

Department of Animal Physiology, Agricultural University, Haarweg 10, 6709 PJ Wageningen, Netherlands

Received 7 August 1984; accepted 24 April 1985

Key-words: diaminopimelic acid, DAPA, amino acid profile, microbial protein synthesis, solubility.

Summary

Mixed concentrates of which the protein degradability varied were fed to sheep provided with an infusion tube into the abomasum and with cannulas in the cranial duodenum and terminal ileum. Degradation of dietary protein in the rumen and synthesis of microbial protein were estimated based on diaminopimelic acid (DAPA) and amino acid profiles. Slight differences were found between these methods. The extent of protein degradation in the rumen was almost independent of dietary protein content but varied with the nature of the diet. Degradability decreased in the order: groundnut expeller and peas, breweries grains, potato protein.

Introduction

In ruminants, the ingested feed is subjected to an extensive microbial degradation in the forestomachs. Dietary protein is degraded for the major part and instead microbial protein is synthesized. The extent of degradation of dietary protein depends on both the degradability of the protein and the time interval the feed is retained in the forestomachs (Tamminga, 1983).

In general, intestinal protein supply is increasing with decreasing degradability of the dietary protein. This is caused by an increased quantity of dietary protein escaping rumen fermentation under conditions that microbial protein synthesis in the rumen is not affected negatively. Some findings even suggest that with less easily degradable rations the efficiency of microbial protein synthesis is increased, as long as rumen-degradable energy and protein are not limiting for microbial growth (Miller, 1982).

The susceptibility of protein to degradation in the rumen is determined by its nature and solubility (Wohlt et al., 1976). In general, an increasing solubility coincides with an increasing rate of degradation in the rumen. Protein degradability may also be decreased by technological treatment. Structural differences caused either by disulphide bridges or cross-linking of the protein are probably important determinants of degradability (Nugent & Mangan, 1978; Mahadevan et al., 1980).

The main protein sources contributing to intestinal protein supply are microbial protein, undegraded dietary protein and endogenous protein. In general, in vivo the undegraded dietary protein fraction is estimated by determining the microbial protein fraction. In the present experiments diaminopimelic acid (DAPA) was used as a marker. It has been shown that different markers may give different results (Siddons et al., 1982) and besides the estimation of the undegraded dietary protein is less accurate since the quantity of endogenous protein entering the small intestine cannot be estimated reliably.

For this reason in the present experiments, the quantity of dietary protein escaping rumen degradation was estimated by two other methods as well, by the regression method of Hvelplund et al. (1976) and based on the amino acid profiles of dietary, microbial and duodenal proteins.

In recent years, determination of the degradability of proteins with the nylon bag method of Mehrez & Ørskov (1977) became increasingly popular. Extrapolation of such in sacco degradabilities to the extent of degradation in vivo is not easy, among other factors because of the great variability of the data obtained so far. Therefore the estimation of the degradability of different protein sources in vivo offered with the diet remains necessary.

The present experiments were conducted with sheep. In quantitative terms protein degradability in sheep may differ from that obtained in high producing dairy cattle but in a qualitative sense rumen fermentation in cattle and sheep have much in common. This allows us to apply the results obtained with sheep to rank the proteins according to their rumen degradability.

Materials and methods

Two mature Texel wethers with a live weight of about 60 kg were equipped with a permanent silastic infusion tube (3 mm i.d.) into the abomasal fundus and with T-shaped hard plastic cannulas (12 mm i.d.) into the cranial duodenum and into the terminal ileum.

The details of the experimental conditions are described by van Bruchem et al. (1985). With groundnut expeller (GE), potato protein (PP), dried breweries grains (BG) and peas (PE) as the main protein sources concentrate mixtures were composed of which the rumen protein degradability varied. GE and PP were fed at a higher (2) and lower (1) protein level, BG and PE only at the lower (1) level. The composition of the concentrates and the experimental design were as given by van Bruchem et al. (1985).

The sheep were adapted to the rations for a preliminary period of 3 weeks. The passage rate of digesta in the small intestine was determined with Cr-EDTA, pre-

pared as described by Binnerts et al. (1968). Starting 24 h before the beginning of the sampling period, 20 ml/h of an aqueous solution containing 0.86 g Cr/l were infused continuously into the abomasum. The passage rate of digesta was calculated as the quotient of the Cr infusion rate and the Cr concentration in the digesta supernatant fraction (Christ UJ3, 2000 *g*, 15 min) and subsequent correction for the dry matter content of the digesta.

Samples of the duodenal and ileal contents were collected for 3 weekly experimental periods of 3 consecutive days. Per day every hour samples of about 10 g were taken between 08h00 and 16h00. Samples were pooled per week and per sampling site and stored at −20 °C.

In these samples the following parameters were measured:
- dry matter content by drying to constant weight at 101 °C;
- N by the Kjeldahl method with K_2SO_4 and HgO as catalysts;
- α-NH_2-N according to the method described by van Slijke et al. (1941);
- ammonia after the method of Berthelot with phenol and hypochlorite. The extinction of the resulting blue colour was estimated at 623 nm with a Perkin Elmer 55E spectrophotometer;
- DAPA and the individual amino acids with a Biotronic LC2000 automatic amino acid analyser. The test sample was hydrolysed with HCl 6 mol/l at 110 °C for 22 h. The sulphur-containing amino acids methionine and cystine were determined as methionine sulphone and cysteic acid respectively, after performic acid oxidation (Moore, 1963);
- Cr after proper dilution with an atomic absorption spectrometer (Perkin Elmer 360) at 357.9 nm, following the method of standard addition.

From samples collected from the ventral rumen sac (van Bruchem et al., 1985) and the cranial duodenum, microbes were isolated by differential centrifugation (550-70 000 *g*) with a MSE superspeed 65 centrifuge at 4 °C. The pellet was washed twice with buffer solution according to the method described by Meyer et al. (1967). After freeze-drying the individual amino acids, including DAPA, were estimated in acid hydrolysates and N according to the micro Kjeldahl method.

The data were analysed statistically by a two-sample analysis of variance, taking into consideration differences between sheep. Student T values for testing significances can be calculated dividing contrasts between rations by SEM $\times \sqrt{2}$ (df. = 29).

Results

The rates of passage in the cranial duodenum of organic matter (OM), non-ammonia N (NAN), α-NH_2-N and NH_3-N are given in Table 1. With potato protein (PP) and breweries grains (BG) relative high passage rates of protein were obtained. NH_3-N was highest with groundnut expeller (GE) and peas (PE) containing the more easily degradable proteins, corresponding with the ammonia levels in the rumen. Of the microbes isolated from the rumen (van Bruchem et al., 1985) and duodenum scanning electron micrographs are presented in Fig. 1. The duodenal microbes looked smaller and more shrivelled in comparison with the rumen microbes. It

Table 1. Duodenal flow (g/h) of organic matter (OM), non-ammonia N (NAN), α-NH_2-N and NH_3-N, and extent of degradation (%) of dietary N (D) and efficiency of microbial N synthesis (E) in the rumen of sheep fed rations differing in protein degradability. (Mean values with their standard error.)

	GE (2)	PP (2)	GE (1)	PP (1)	PE (1)	BG (1)	SEM
OM	12.0	16.1	13.8	13.5	12.8	18.2	0.71
NAN	0.77	1.30	0.78	0.89	0.68	0.87	0.048
α-NH_2-N	0.59	1.01	0.62	0.70	0.53	0.67	0.038
NH_3-N	0.088	0.061	0.042	0.029	0.040	0.034	0.0030
DAPA D	85.9	57.0	83.3	54.3	82.2	60.5	4.11
E[1]	20.3	29.3	26.4	21.4	22.2	29.1	2.60
AAP D	97.1	48.7	85.2	50.3	96.5	80.6	3.52
E[1]	25.4	25.0	26.7	20.4	23.5	36.0	2.12

[1] g microbial N/kg OM truly digested.

Table 2. Amino acid profile (expressed as substance fraction in total amino acid, mol/100 mol) N content and DAPA-N/N ratio of microbes isolated from duodenal digesta.

	GE (2)	PP (2)	GE (1)	PP (1)	PE (1)	BG (1)	SEM
Asp	11.15	11.40	10.91	11.08	10.94	10.66	0.043
Tre	6.31	6.42	6.21	6.25	6.16	6.11	0.042
Ser	6.11	6.33	6.19	6.40	6.25	6.37	0.065
Glu	11.09	10.74	11.26	11.01	11.08	11.31	0.100
Pro	3.98	3.98	4.01	3.77	3.98	4.87	0.101
Gly	9.67	9.46	9.89	9.67	9.86	9.11	0.179
Ala	10.67	10.37	10.34	10.49	10.38	10.07	0.094
Val	7.47	7.36	7.21	7.31	7.36	7.36	0.062
Ile	5.87	5.85	5.90	5.92	5.94	5.87	0.036
Leu	7.74	7.88	7.77	7.82	7.67	7.91	0.038
Tyr	4.23	4.34	4.29	4.44	4.35	4.23	0.041
Phe	3.98	4.15	4.11	4.14	4.10	4.33	0.032
Lys	6.31	6.43	6.35	6.32	6.45	6.03	0.079
His	1.69	1.69	1.77	1.71	1.71	1.82	0.018
Arg	3.73	3.60	3.79	3.66	3.76	3.90	0.034
N (% in DM)	7.75	8.34	7.62	7.77	7.46	7.07	0.156
DAPA-N/N (%)	0.64	0.59	0.69	0.73	0.69	0.56	0.049

seemed that of the duodenal microbes a part of the cytoplasmic matter was lost as was also shown by Mathers & Miller (1980) with rumen microbes after incubation with pepsin and hydrochloric acid.

Of the duodenal microbes the amino acid profiles, excluding the S-containing amino acids and tryptophane, are presented in Table 2. They showed slight though significant variations. Also the N content and the DAPA-N/N ratio varied slightly with the rations. After correcting the duodenal flow of NAN for an endogenous contribution of 2.5 g N per kg dry matter ingested (Siddons et al., 1982), the unde-

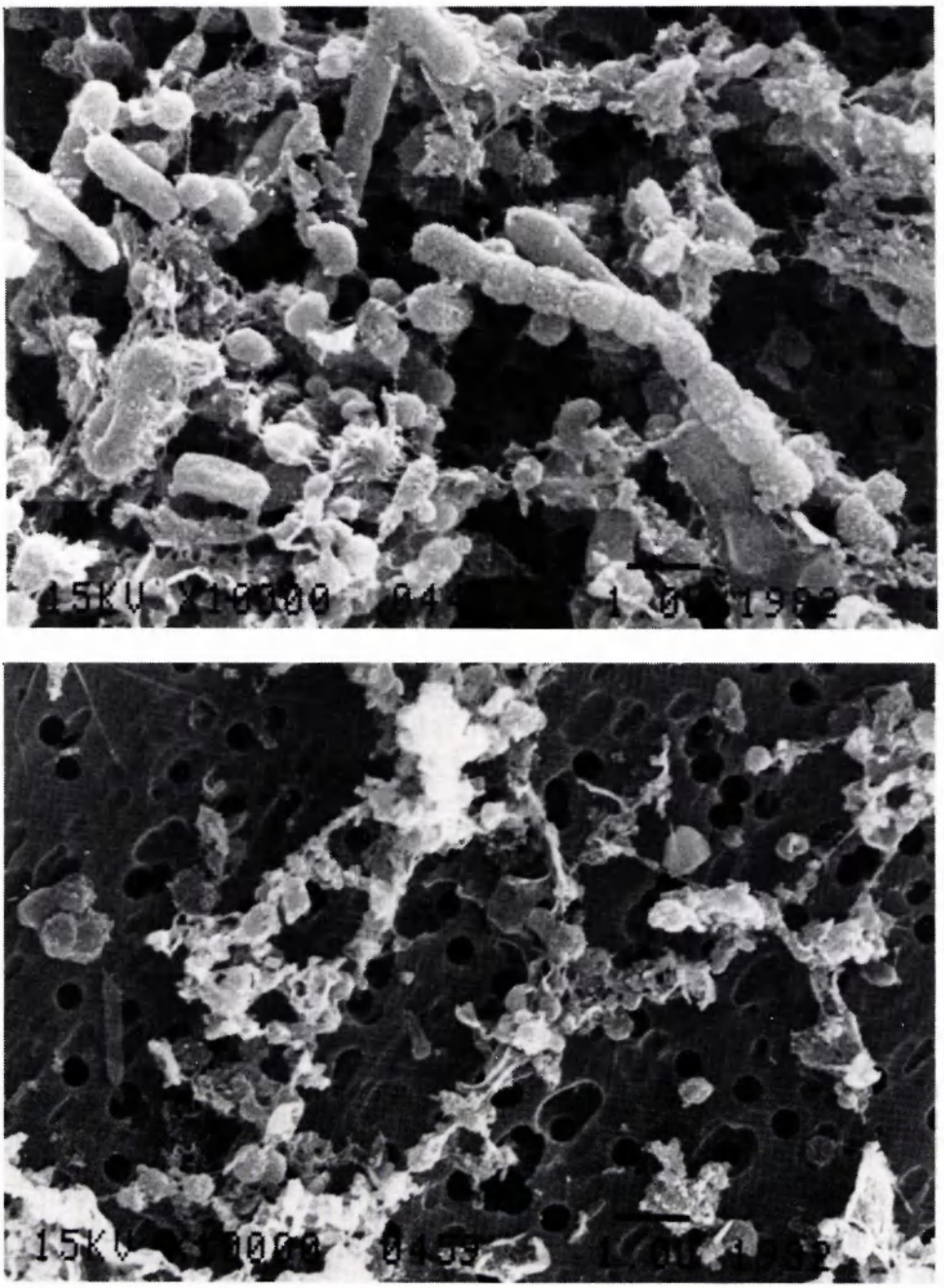

Fig. 1. Scanning electron micrographs of bacteria isolated from rumen fluid (top) and duodenal digesta (bottom), isolated by fractionated centrifugation (× 20 000). The dark spotted background is the grid on which the bacteria were isolated. The crystalline material contains buffer salt.

Table 3. Quantity (Q, μmol/h) and amino acid profile (AAP, expressed as substance fraction in total amino acid, mol/100 mol) of the protein ingested with the rations.

	GE (2)		PP (2)		GE (1)		PP (1)		PE (1)		BG (1)	
	Q	AAP	Q	AAP	Q	AAP	Q	AAP	Q	AAP	Q	AAP
Cys	575	0.84	918	1.27	227	0.68	298	0.80	248	0.80	345	1.07
Asp	8093	11.77	8282	11.42	3820	11.47	4095	11.02	3444	11.02	2613	8.10
Met	815	1.18	1262	1.74	405	1.22	606	1.63	347	1.11	551	1.71
Tre	2830	4.12	4484	6.18	1555	4.67	2270	6.11	1490	4.77	1509	4.68
Ser	4884	7.10	5072	7.00	2482	7.45	2716	7.31	2201	7.04	2162	6.70
Glu	10690	15.55	7251	10.00	4938	14.82	3971	10.69	4914	15.72	5328	16.52
Pro	3653	5.32	3742	5.16	1881	5.65	2348	6.32	1803	5.77	3250	10.08
Gly	6539	9.52	6037	8.33	3139	9.42	3162	8.51	2603	8.33	2658	8.24
Ala	4521	6.58	5203	7.17	2385	7.16	2800	7.53	2278	7.29	2443	7.59
Val	4027	5.86	5349	7.38	2095	6.29	2745	7.39	1920	6.14	2154	6.68
Ile	2801	4.08	3849	5.31	1388	4.17	1880	5.06	1311	4.19	1359	4.21
Leu	4834	7.03	6460	8.91	2361	7.09	3170	8.53	2218	7.10	2389	7.41
Tyr	2202	3.20	2636	3.64	1050	3.15	1269	3.41	808	2.59	856	2.65
Phe	2997	4.36	3379	4.66	1388	4.17	1651	4.44	1176	3.77	1350	4.19
Lys	2713	3.95	4487	6.19	1438	4.32	2093	5.63	1803	5.77	1290	4.00
His	1413	2.06	1344	1.85	690	2.07	707	1.90	667	2.13	602	1.87
Arg	5147	7.49	2753	3.80	2070	6.22	1382	3.72	2022	6.47	1383	4.29
N (mg/h)	1440		1440		710		770		680		690	

Table 4. Quantity (Q, μmol/h) and amino acid profile (AAP, expressed as substance fraction in total amino acid, mol/100 mol) of the protein (NAN, α-NH_2-N, mg/h) entering the duodenum (mean values with their standard error).

	GE (2)		PP (2)		GE (1)		PP (1)		PE (1)		BG (1)		SEM	
	Q	AAP	Q	AAP	Q	AAP	Q	AAP	Q	AAP	Q	AAP	Q	AAP
Cys	300	0.89	557	0.93	285	0.86	385	0.95	338	1.14	383	1.00	26.6	0.060
Asp	3685	10.95	6552	10.97	3737	11.22	4465	11.05	3208	10.80	3838	10.02	243.8	0.104
Met	555	1.65	1032	1.73	495	1.49	663	1.64	467	1.57	617	1.61	44.3	0.031
Tre	2158	6.41	3945	6.60	2140	6.43	2596	6.42	1900	6.40	2333	6.09	146.1	0.047
Ser	2245	6.67	4055	6.79	2308	6.93	2813	6.96	1953	6.58	2485	6.49	142.7	0.068
Glu	3395	10.09	5500	9.20	3392	10.19	3923	9.71	3130	10.54	4460	11.64	208.9	0.106
Pro	1464	4.35	3011	5.04	1537	4.62	1949	4.82	1262	4.25	2114	5.52	144.9	0.125
Gly	3183	9.46	5213	8.72	3185	9.56	3610	8.93	2806	9.45	3483	9.09	202.7	0.071
Ala	3082	9.16	4962	8.30	3222	9.68	3599	8.91	2955	9.95	3670	9.58	186.6	0.065
Val	2500	7.43	4627	7.74	2312	6.94	3067	7.59	2178	7.33	2858	7.46	176.3	0.141
Ile	1870	5.56	3362	5.63	1830	5.50	2228	5.51	1635	5.50	2020	5.27	121.0	0.045
Leu	2685	7.98	5195	8.69	2562	7.69	3310	8.19	2240	7.54	3033	7.92	190.0	0.039
Tyr	1245	3.70	2302	3.85	1182	3.55	1480	3.66	1067	3.59	1322	3.45	80.5	0.036
Phe	1332	3.96	2615	4.38	1252	3.76	1629	4.03	1099	3.70	1510	3.94	96.1	0.035
Lys	2115	6.28	3673	6.15	2077	6.24	2560	6.34	1942	6.54	2152	5.62	124.8	0.065
His	655	1.95	1127	1.89	663	1.99	792	1.96	547	1.84	717	1.87	40.7	0.025
Arg	1181	3.51	2023	3.39	1123	3.37	1343	3.32	971	3.27	1321	3.45	76.3	0.028
NAN	768		1300		780		893		681		869		48.0	
α-NH_2-N	592		1007		625		695		532		678		38.4	

graded dietary N was estimated from the difference between the flow of total NAN and microbial N, the latter being estimated with DAPA as a marker. The extent of degradation of dietary N in the rumen is given in Table 1. PP and BG showed lower degradabilities without a significant effect on the efficiency of microbial protein synthesis, calculated as the quantity of microbial N passing in the duodenum per kg OM truly fermented in the rumen. Microbial OM was estimated for this purpose as N × 6.25 × 1.57 (McAllen & Smith, 1969).

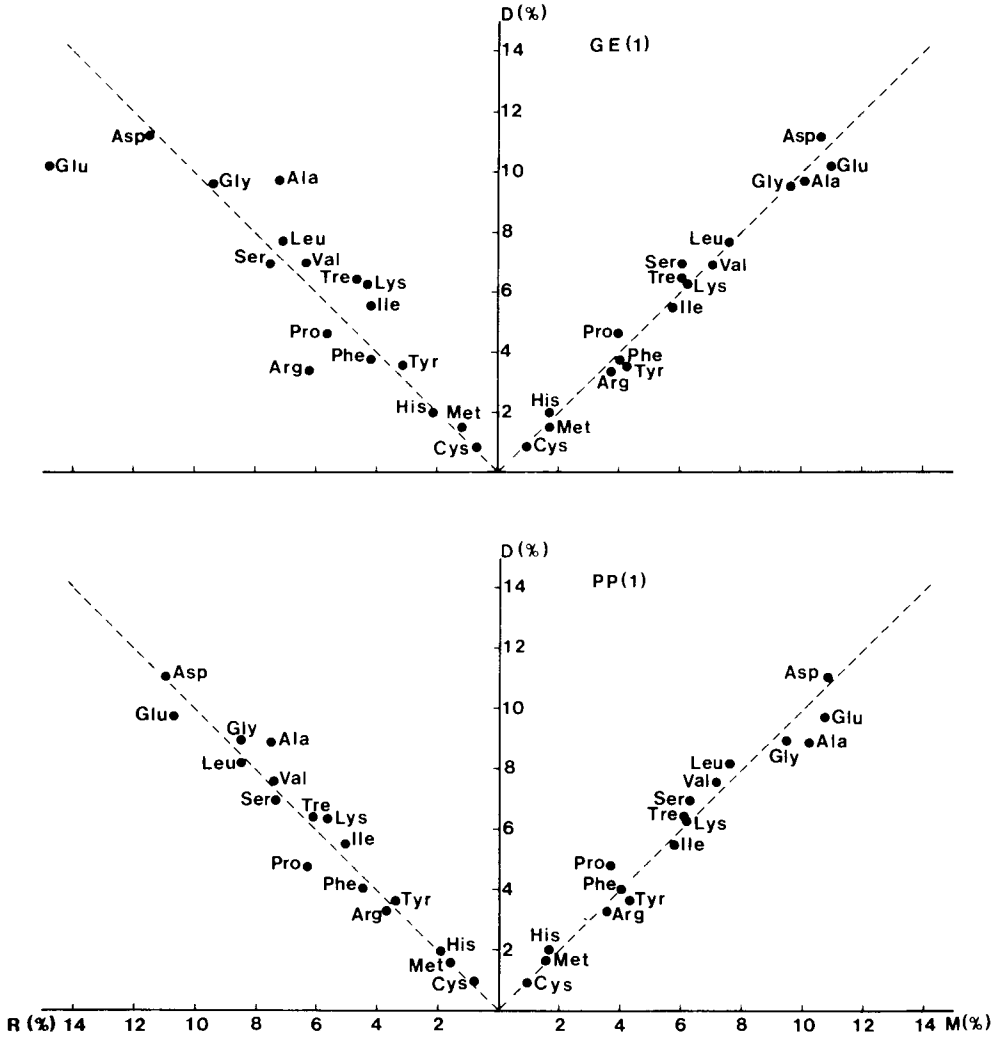

Fig. 2. The amino acid profiles of duodenal (D) protein relative to those of dietary (R) and duodenal microbial (M) protein after feeding GE (1) and PP (1). The broken lines represent the line of identity.

The proportion of microbial protein in duodenal digesta was also estimated based on amino acid profiles (AAP). Dietary and microbial proteins (Tables 3 and 4) were mixed by a computerized iterative procedure in such proportions that the computed AAP matched best to the actual AAP of duodenal protein. This was tested by minimizing the objective function:

$$\sum_{AA=1}^{17} (1-AA_{computed}/AA_{actual})^2$$

The resulting degradabilities and efficiencies of microbial protein synthesis are presented in Table 1. PP and GE were supplied at two levels of N intake. Based on the ratio of duodenal NAN to dietary N (y) the extent of degradation of these proteins was also estimated by regression according to Hvelplund et al. (1976). In the regression equation of the form:

$y = a + b\,(1/x)$

a represents the proportion of dietary N entering the small intestine undegraded, b the amount of microbial N synthesized per kg dry matter (DM) ingested and x the N content of the ration (g N/kg DM). For GE and PP a values were obtained of −0.04 (SE = 0.10) and 0.60 (SE = 0.16), respectively. For b the results were 24.5 (SE = 2.6) and 13.1 (SE = 4.6).

The degradabilities obtained varied with the method applied. In comparison with GE and PE, PP and BG were less easily degradable. Thus with GE and PE, the duodenal AAP was closely related to the microbial AAP. Especially with PP a more close relationship was found to the dietary AAP. This is examplified for GE (1) and PP (1) in Fig. 2.

Discussion

Protein flow to the ruminant small intestine depends on the degradability of the dietary protein and the efficiency of microbial protein synthesis in the rumen. The extent of degradation is determined by degradability and the time interval the proteins are subjected to rumen fermentation. Under the present experimental conditions (van Bruchem et al., 1985) the less easily degradable rations PP and BG were retained in the forestomachs slightly longer. Dilution rates of the liquid phase amounted to about 0.10 h^{-1}. The insoluble proteins are retained in the particulate phase of which the rate of emptying is considerably lower, under the present experimental conditions probably not higher than 0.03 h^{-1} (van Bruchem et al., 1984). This explains why the extent of protein degradation was hardly related to the N content of the rations. Assuming no major effects on retention time of the feed particles in the rumen, the variability of dietary protein degradation is primarily determined by dietary protein degradability. With the protein sources applied degradability showed to increase with increasing solubility determined according to Wohlt et al. (1973) as given by van Bruchem et al. (1985). The order of degradation found

in the present experiments was in line with that determined with nylon bags in the rumen of cattle (S. Tamminga, personal communication).

In the present experiments sheep were fed at maintenance level and the ration was supplied in six portions over the day. Relatively to dairy cows, the lower level of feeding and in consequence a lower rate of emptying of the particulate phase from the reticulorumen will coincide with a higher extent of degradation of dietary protein. Also the increased frequency of feeding causes a smaller proportion of dietary protein to escape microbial degration in the rumen (Tamminga, 1981). Thus the degradability figures obtained under the present experimental conditions may be applied ranking the proteins according to their rumen degradability. In quantitative terms, however, the extent of protein degradation in the rumen of dairy cows may be lower and possibly the differences in degradability greater (Ørskov & McDonald, 1979).

With increasing extent of protein degradation the DAPA method produced lower estimates relative to our AAP method. This phenomenon that DAPA may lead to lower estimates of microbial protein is well known from literature (Ling & Buttery, 1978; Tamminga, 1981). This has been attributed to the absence of DAPA in protozoa. In contrast to this, Siddons et al. (1982) obtained with DAPA higher estimates of microbial protein in comparison with ^{35}S and ^{15}N as markers or based on the AAP method of Evans et al. (1975).

The present AAP method used does not take into account the quantity and composition of the duodenal endogenous protein fraction. This fraction probably amounts to about 10 % of the protein passing in the duodenum, which allows no accurate determination and its AAP is certainly not that of bovine pepsinogen as suggested by Evans et al. (1975). Probably the major part originates from desquamated epithelial cells and mucus.

A prerequisite for an accurate determination of the quantity of dietary protein escaping rumen fermentation by the AAP method is that the amino acid profiles of dietary and microbial proteins are appreciably different. For this reason we expressed our AAP on a molar basis, since then greater differences of the AAPs are obtained in comparison with AAPs expressed on a w/w basis. In contrast to the DAPA method it discriminated between GE (2) and GE (1). This difference is probably real because in the GE (1) ration sugar-beet and citrus pulp contribute relatively more to the dietary protein. Of both protein sources the degradability is considerably lower in comparison with GE protein (S. Tamminga, W. A. G. Veen, personal communication). With the present AAP method and with the present rations the estimates of protein degradability were least variable. In this sense the least accurate estimates were obtained with the regression method of Hvelplund et al. (1976). Unfortunately, it is difficult to judge whether the more accurate estimates resulting from our AAP method are also more reliable, since under certain conditions it has been shown that the AAP of the undegraded dietary protein residue may differ from that of the degraded proportion (MacGregor et al., 1978).

The efficiencies of microbial protein synthesis, obtained under the present experimental conditions, were of a magnitude comparable to those cited in literature (Czerkawski, 1978). It has been suggested that the efficiency of microbial protein

synthesis should decrease with increasing degradability of the ration, caused by a less steady availability of substrates for fermentation. This was not confirmed by the present results, probably because the frequent feeding regimen through a more stabilized fermentation caused easily degradable proteins to be transformed into microbial protein quite efficiently as well. With less frequent feeding, spillage of energy and protein possibly plays a more significant role.

Acknowledgements

The authors gratefully acknowledge Mr G. van Gelderen for collecting samples and taking care of the cannulated sheep, Messrs L. J. G. M. Bongers, C. P. Leffering, W. Onck and J. D. van Walsem for skilful analytical assistance, and Prof. S. Tamminga and Dr W. A. G. Veen for advice and discussion in the course of the experiments. The scanning electron micrographs of bacteria were prepared by the 'Technical and Physical Engineering Research Service (TFDL)' at Wageningen.

References

Binnerts, W. T., A. Th. van 't Klooster & A. M. Frens, 1968. Soluble chromium indicator measured by atomic absorption in digestion experiments. *Veterinary Records* 82: 470.

Bruchem, J. van, S. M. G. Rouwers, G. A. Bangma, C. P. Leffering & P. W. M. van Adrichem, 1985. Digestion of proteins of varying degradability in sheep. 1. Fermentation in and rate of passage from the reticulorumen. *Netherlands Journal of Agricultural Science* 33: 263-272.

Bruchem, J. van, A. J. Schutte, G. A. Bangma, C. P. Leffering & P. W. M. van Adrichem, 1984. Dilution rate of a liquid and particulate phase marker from the reticulorumen of sheep. *Canadian Journal of Animal Science* 64: 72-73.

Czerkawski, J. W., 1978. Reassessment of efficiency of synthesis of microbial matter in the rumen. *Journal of Dairy Science* 61: 1261-1273.

Evans, R. A., R. F. E. Axford & N. W. Offer, 1975. A method for estimating the quantities of microbial and dietary proteins flowing in the duodenal digesta of ruminants. *Proceedings of the Nutrition Society* 34: 65A.

Hvelplund, T., P. D. Moller, J. Madsen & M. Hesselholt, 1976. Flow of digesta through the gastro-intestinal tract in the bovine with special reference to nitrogen. In: Yearbook 1976. Royal Veterinary and Agricultural University, Copenhagen, p. 173-192.

Ling, J. R. & P. J. Buttery, 1978. The simultaneous use of ribonucleic acid, ^{35}S, 2,6-diaminopimelic acid and 2-aminoethylphosphonic acid as markers of microbial nitrogen entering the duodenum of sheep. *British Journal of Nutrition* 39: 165-179.

MacGregor, C. A., C. J. Sniffen & W. H. Hoover, 1978. Amino acid profiles of total and soluble protein in feedstuffs commonly fed to ruminants. *Journal of Dairy Science* 61: 566-573.

Mahadevan, S., J. D. Erfle & F. D. Sauer, 1980. Degradation of soluble and insoluble proteins by bacteroides amylophilus proteases and by rumen microorganisms. *Journal of Animal Science* 50: 723-728.

Mathers, J. C. & E. L. Miller, 1980. A simple procedure using ^{35}S incorporation for the measurement of microbial and undegraded food protein in ruminant digesta. *British Journal of Nutrition* 43: 503-514.

McAllen, A. B. & R. H. Smith, 1969. Nucleic acid metabolism in the ruminant. *British Journal of Nutrition* 23: 671-682.

Mehrez, A. Z. & E. R. Ørskov, 1977. A study of the artificial fibre bag technique for determining the digestibility of feeds in the rumen. *Journal of Agricultural Science* 88: 645-650.

Meyer, R. M., E. E. Bartley, C. W. Deyoe & V. F. Colenbrander, 1967. Feed processing. I. Ration effects on rumen microbial protein synthesis and amino acid composition. *Journal of Dairy Science* 50: 1327-1331.

Miller, E. L., 1982. In: D. J. Thompson, D. E. Beever & R. G. Gunn, Forage protein in ruminant animal production. BSAP Occasional Publication No 6, p. 79-88.

Moore, S., 1963. On the determination of cystine as cysteic acid. *Journal of Biological Chemistry* 238: 235-237.

Nugent, J. H. A. & J. L. Mangan, 1978. Rumen proteolysis of fraction I leaf protein, casein and bovine serum albumin. *Proceedings of the Nutrition Society* 37: 48A.

Ørskov, E. R. & I. McDonald, 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.

Siddons, R. C., D. E. Beever & J. V. Nolan, 1982. A comparison of methods for the estimation of microbial nitrogen in duodenal digesta of sheep. *British Journal of Nutrition* 48: 377-389.

Slijke, D. D. van, R. T. Dillon, D. A. MacFadyen & P. Hamilton, 1941. Gasometric determination of carboxyl groups in free amino acids. *Journal of Biological Chemistry* 141: 627-669.

Tamminga, S., 1981. Nitrogen and amino acid metabolism in dairy cows. Thesis, Agricultural University, Wageningen.

Tamminga, S., 1983. Recent advances in our knowledge on protein digestion and absorption in ruminants. In M. Arnal, R. Pion & D. Bonin, Proceedings 4th EAAP Symposium on Protein Metabolism and Nutrition (Clermont-Ferrand, 5-9 September 1983). EAAP Publication No 31, Vol. I: 263-288.

Wohlt, J. E., C. J. Sniffen & W. H. Hoover, 1973. Measurement of protein solubility in common feedstuffs. *Journal of Dairy Science* 56: 1052-1057.