

The effect of various protein supplements on the flow of soluble non-ammonia nitrogen in the forestomach of dairy cows fed grass silage-based diet

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The soluble 'a-fraction' of feeds assessed using *in situ* method is assumed to be degraded at infinite rate in the rumen. This assumption consequently leads to a conclusion that only insoluble feed N can escape rumen degradation. However, a previous *in vivo* study (Choi et al., 2002) showed that a considerable proportion of soluble non-ammonia N (SNAN) flows into the omasal canal of dairy cows.

Five ruminally fistulated dairy cows (mean 534 ± 42.5 kg) were used to study effects of protein supplements on SNAN flow in the forestomach. Treatments in a 5 × 5 Latin square design were proportionately 0.50 grass silage and 0.50 barley concentrate (Control), and a part of meal on dry matter basis was isonitrogenously replaced with one of following protein supplements; skimmed milk powder (SMP), wet distiller's solubles (WDS), untreated rapeseed meal (URSM) or treated rapeseed meal (TRSM). Ruminal (RD) and omasal digesta (OD) were taken at 1 h sampling interval during a 12 h feeding cycle, and immediately acidified with H₂SO₄. The digesta were sequentially centrifuged at low- (1000 × g, 10 min, 4 °C) and at high-speed (10000 × g, 30 min, 4 °C), and precipitated with trichloroacetic acid followed by the high-centrifugation. Ruminal liquid flow (mean 339 l/d) and rumen outflow rate (0.17 /h) were estimated using a dose of LiCo-EDTA. Different N fractions within digesta were assessed using ninhydrin: free amino acids (AA) present in the supernatant, peptide in the supernatant hydrolysed with 6 M HCl (110 °C, 24 h) minus free AA and protein from hydrolysis of the precipitate. Fractions were corrected for ammonia N.

Daily flow of free AA, peptide, protein and total SNAN in RD and OD is shown in Table 1. Protein supplements significantly increased the flow of free AA and total SNAN both in RD and OD. With protein supplementation, mean flow of SNAN in RD was 10.5, 14.6 and 0.4 g N/d for free AA, peptide and protein, respectively, while that in OD was 14.5, 20.1 and 0.5 g N/d in the same order. Different type of protein supplements did not significantly affect SNAN flow. Mean flows of SNAN fractions in OD were higher than that in RD (P < 0.10) except for protein. Peptides constituted the largest proportion of SNAN flow suggesting that hydrolysis of peptides to AA rather than hydrolysis of soluble proteins to peptides or deamination of AA to ammonia is the most limiting step in rumen proteolysis. The present data raise questions concerning the validity of the assumptions of dacron bag study to assess ruminal protein degradability.

Table 1. Effect of protein supplements on the flow of N fractions of SNAN in the forestomach

	Diet					SEM	Orthogonal contrasts ¹			
	Control	SMP	WDS	URSM	TRSM		C1	C2	C3	C4
Ruminal, g N/d										
Free AA	6.2	10.1	9.0	11.0	11.7	1.66	0.01	0.22	0.59	0.75
Peptide	13.2	15.1	13.8	16.3	13.2	1.76	0.27	0.77	0.42	0.08
Protein	0.2	0.4	0.2	0.5	0.4	0.07	0.12	0.02	0.12	0.08
Total	19.7	25.5	23.0	27.8	25.3	2.91	0.03	0.28	0.39	0.38
Omasal, g N/d										
Free AA	7.6	15.8	11.4	16.5	14.2	3.02	0.009	0.43	0.17	0.46
Peptide	16.8	20.7	19.9	22.5	17.3	2.89	0.27	0.87	0.83	0.17
Protein	0.3	0.5	0.3	0.5	0.5	0.09	0.09	0.38	0.34	0.97
Total	24.6	36.9	31.7	39.4	32.0	4.54	0.03	0.71	0.33	0.18

¹ C1 = Control vs protein supplemented diets, C2 = SMP + WDS vs URSM + TRSM, C3 = SMP vs WDS, C4 = URSM vs TRSM

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