Vol. 11 (2002): 79–91.

Concentration and estimated flow of soluble non-ammonia nitrogen entering the omasum of dairy cows as influenced by different protein supplements

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Four ruminally fistulated Finnish Ayrshire cows were used to study the effects of different protein supplements on concentration and flow of soluble non-ammonia N (SNAN) into the omasum. The treatments in a 4 × 4 Latin square design were a basal diet of grass silage and barley and the basal diet supplemented with fishmeal, soybean meal and maize gluten meal. Protein supplements significantly increased concentrations of peptide N (P = 0.009) and total SNAN (P = 0.03) fractions in omasal digesta. Peptide constituted the largest proportion of SNAN flow into the omasum indicating that hydrolysis of peptides to amino acids is the most limiting step in rumen proteolysis. The microbial contribution to SNAN was on an average 0.64 indicating that a large proportion of SNAN flow leaving the rumen was of microbial origin. The estimated SNAN flow per kg dry matter intake from the basal diet and protein supplemented diets indicated that approximately 49, 22 and 37 g kg^{-1} of fishmeal, soybean meal and maize gluten meal protein, respectively, escaped from ruminal degradation as SNAN.

Key words: soluble non-ammonia nitrogen, protein supplements, omasum, dairy cows, peptides

Introduction

Ruminal protein degradability is typically assessed by in situ method, which assumes that the rapidly degradable nitrogen (N) (*a-fraction*) is degraded at an infinite rate, and consequently that only insoluble feed N can escape ruminal degradation. However, relatively high concentrations of soluble non-ammonia N (SNAN) consisting of free amino acid (AA), peptide and soluble protein, in rumen fluid (Chen et al. 1987a, Robinson and McQueen 1994) or omasal digesta (Choi et al. 2002a, b) suggest that a proportion of protein can escape rumen degradation in the liquid phase. Broderick (1987) reported using in vitro system that the degradation rate of casein N was 0.40 – 0.60 h^{-1}, also suggesting that a considerable portion of casein N can escape ruminal degradation. Consequently, the assumption of in situ method may be invalid. When skimmed milk powder was used as a protein supplement for dairy cows, the SNAN concentration in omasal digesta was 110 mg N l^{-1} (Choi et

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al. 2002b). Assuming a rumen volume of 80 litres and a liquid passage rate of 0.15 h^{-1}, approximately 32 g N d^{-1} of SNAN could potentially escape the rumen. It was calculated that 116 g kg^{-1} of skimmed milk powder N escaped ruminal degradation as SNAN in dairy cows fed a grass silage based diet (Choi et al. 2002b). However, the value was not corrected for microbial contamination.

Few dietary data on the concentration and the estimated flow of SNAN from the rumen and/or the omasal canal of ruminant animals have been reported. When dairy cows were given grass silage based diet, inclusion of rapeseed meal increased the concentration of SNAN in the liquid phase of the omasal digesta (Choi et al. 2002a). Chen et al. (1987b) reported that high concentration of peptide N accumulated in the rumen when untreated soybean meal was given. However, when treated soybean meal was given the peptide N concentration decreased even though the concentration was still relatively high (Chen et al. 1987b). Peptide N concentration varied when different protein supplements were given to steers, but it was poorly correlated with degradability and solubility of the supplements (Williams and Cockburn 1991). More recently, our study (Choi et al. 2002b) also showed that omasal SNAN did not depend on the type of protein supplements although it was increased by all protein supplements. However, some of the supplements used in the experiment (skimmed milk powder and wet distiller's solubles) are not commonly used in practice.

The present experiment was designed to study effects of more widely used protein supplements on the concentration and the estimated flow of SNAN escaping ruminal degradation in dairy cows fed grass silage based diets. Furthermore, because a proportion of SNAN in the liquid phase was suggested to be of microbial origin (Choi et al. 2002a), we also determined the potential microbial contamination using ^{15}N as a microbial marker. Data on nutrient flows and subsequent animal responses have been reported elsewhere (Korhonen et al. 2002).

Material and methods

Experimental procedures

Four Finnish-Ayrshire dairy cows (mean live weight, 668 ± 109 kg; days in milk, 51 ± 6 days) fitted with 10-cm i.d. ruminal cannulas were used in a 4 × 4 Latin-square experiment with periods of 28 d including 4 d for omasal sampling. During an adaptation period (14 d) dry matter (DM) intake of each cow was recorded. On day 15, DM intake was then restricted to 0.95 of the *ad libitum* intakes. The basal diet (kg kg^{-1} DM) contained grass silage (0.55) and rolled barley concentrate (0.45) (Table 1). Part of DM intake of the basal diet (control) was isonitrogenously replaced by one of protein supplements as follows; fishmeal (0.06) (diet FM), soybean meal (0.09) (diet SBM) or maize gluten meal (0.06) (diet MGM). Each protein supplement replaced portions of both silage and barley such that the ratio of grass silage to barley (55:45) remained constant for all treatments. Mineral and vitamin mixture was given at a rate of 300 g d^{-1}. Grass prepared from secondary growth of swards containing predominately timothy (*Phleum pratense*) and meadow fescue (*Festuca pratensis*) and cocksfoot (*Dactylis glomerata*) was ensiled in a tower silo with a formic acid-based additive (AIV-2+; Kemira-Agro, Helsinki, Finland) at a rate of 5 l t^{-1} of the grass. Barley was coarsely milled using a roller mill prior to feeding, and all protein supplements and mineral and vitamin mixture were purchased from commercial sources (Rehuraisio Ltd., Raisio; Suomen Rehu Ltd., Helsinki, Finland). The cows had a free access to water and salt block throughout the experiment. Feeds were offered twice daily at 0600 and 1800 and the cows were milked at 0700 and 1700.

Sampling and chemical analyses

Representative samples of grass silage and concentrate were collected over the last 10 d of each

Vol. 11 (2002): 79–91.

Table 1. Proportion of dietary ingredients in experimental diet.

	Diet[a]			
	Control	FM	SBM	MGM
Grass silage, kg kg^{-1} dry matter	0.55	0.52	0.50	0.52
Barley concentrate, kg kg^{-1} dry matter	0.45	0.42	0.41	0.42
Fishmeal, kg kg^{-1} dry matter		0.06		
Soybean meal, kg kg^{-1} dry matter			0.09	
Maize gluten meal, kg kg^{-1} dry matter				0.06

[a] Control = grass silage + barley; FM = grass silage + barley + fishmeal; SBM = grass silage + barley + soybean meal; MGM = grass silage + barley + maize gluten meal. Each diet contained 300 g d^{-1} of mineral and vitamin mixture (16% of Ca, 6.4% of P, 9.0% of Na, 8.0% of Mg, 150,000 IU kg^{-1} of vitamin A, 100,000 IU kg^{-1} of vitamin D, 950 mg kg^{-1} of vitamin E, 530 mg kg^{-1} of Cu, 20 mg kg^{-1} of Se, 4200 mg kg^{-1} of Zn, 20 mg kg^{-1} of Mo, 15 mg kg^{-1} of Co, 2250 mg kg^{-1} of Mn and 140 mg kg^{-1} of I).

experimental period pooled over the period and stored at – 20ºC until analysis. Details of analysis for chemical composition of feeds have previously been described by Ahvenjärvi et al. (2000). In brief, crude protein of feeds was determined using a Dumas-type N analyser (Leco FP-428; Leco Corporation, St Joseph, MI, USA). Soluble N content of silage was analysed using Kjeldahl method (Method No. 984.13; AOAC 1990). Neutral detergent fibre of feeds was assayed with α-amylase and sodium sulphite, and was expressed without residual ash (Van Soest et al. 1991). Soluble N fractions (non-protein N (NPN) and soluble true protein N) of feeds were prepared and analysed as described by Licitra et al. (1996). In the fractionation of the feed NPN, free AA and peptide N were determined using ninhydrin (Choi et al. 2002a) while ammonia N was analysed using a colorimetric method (McCullough 1967).

Digesta flow into the omasum was estimated with a triple marker method (France and Siddons 1986) using indigestible neutral-detergent fibre, Yb-acetate and LiCo-EDTA as markers for large particle, small particle and liquid phase, respectively. Doses of LiCo-EDTA (18 g) and Yb-acetate (6 g) were given at 60 h before the first sampling time, and then the markers were continuously infused into the rumen (12 g d^{-1} of LiCo-EDTA and 4 g d^{-1} Yb-acetate). Microbial contribution to omasal SNAN was estimated using ammonium sulfate (Isotec Inc., Miamisburg, OH, USA) with 10% enrichment of ^{15}N (250 mg ^{15}N d^{-1} per cow) as a microbial marker. Infusion of the ^{15}N-enriched ammonium sulfate was started at 48 h before the first sampling.

To estimate the flow of SNAN fractions in the liquid phase of digesta, digesta entering the omasum was sampled according to the procedure described by Choi et al. (2002b). In brief, approximately 30 ml of digesta was collected at 4-h intervals during a 12-h feeding cycle starting on day 25 of each period. On subsequent sampling days, the time of sampling was advanced by 1 h relative to the previous sampling day (i.e. totally 12 samples during 4 days). Details of the sample preparation have been described previously (Choi et al. 2002a) with a modification that a portion of each supernatant of the omasal digesta deproteonised with trichloroacetic acid was prepared with 10 N NaOH to increase pH above 10 and incubated at 60ºC for 10 min to eliminate ammonia. Residual ammonia in the omasal digesta was analysed using a colorimetric method (McCullough 1967). Different fractions (free AA, peptide and soluble protein) of SNAN within the omasal digesta were assessed using ninhydrin (Choi et al. 2002a). In brief, each frac-

tion of SNAN in the omasal sample was estimated as follows: i) free AA as N from supernatant without acid-hydrolysis, ii) peptide as N from difference between hydrolysed supernatant (6 M HCl at 110ºC for 24 h) and free AA N and iii) protein as N from the hydrolysis of trichloroacetic acid-precipitate.

The ^{15}N-enrichement in the liquid phase of digesta entering the omasal canal was analysed as previously described by Choi et al. (2002a) with an exception that an oven (60ºC for 48 h) instead of a freeze-drying was used.

Calculation and statistical analysis

Since the ^{15}N-enrichment of ammonia was not measured, a calculation for ^{15}N-enrichment of the liquid phase was estimated assuming 0.70 of liquid associated bacteria (LAB)-NAN derived from ammonia N (Firkins et al. 1987). The details of calculations have been described by Choi et al. (2002a).

The rumen-escape of SNAN in the liquid phase of omasal digesta (eSNAN) from each protein supplement was calculated as follows:

$$(1)\ eSNAN = (SNAN_{supp} - SNAN_{basal}) / (NI_{supp} - NI_{basal})$$

where $SNAN_{supp}$ and NI_{supp} are the flow of SNAN in the liquid phase of omasal digesta and N intake for the protein supplemented diet, respectively, and $SNAN_{basal}$ and NI_{basal} are the flow of SNAN in the liquid phase of omasal digesta and N intake for grass silage and barley concentrate in the protein supplemented diet, respectively. These are calculated as follows:

$$(2)\ SNAN_{basal} = SNAN_{cont} \times DMI_{basal} / DMI_{cont}$$

where $SNAN_{cont}$, DMI_{basal} and DMI_{cont} are the flow of SNAN in the liquid phase of omasal digesta for control diet, DM intake as grass silage and barley concentrate in the protein supplemented diet and DM intake in control diet excluding mixture of vitamin and mineral, respectively. Finally,

$$(3)\ NI_{basal} = NI_{cont} \times DMI_{basal} / DMI_{cont}$$

where NI_{cont} is N intake in control diet.

Data for feed N intake, liquid flow and total NAN were analysed with the GLM procedure of SAS (1996) according to the following statistical model:

$$(4)\ Y_{ijk} = \mu + A_i + P_j + D_k + e_{ijk}$$

where A, P and D are animal, period, diet effects, respectively.

Data obtained from ammonia N and concentration and flow of SNAN determined at each sampling interval were analysed with the MIXED procedure of SAS (1996) for repeated measures according to the following statistical model:

$$(5)\ Y_{ijkl} = \mu + A_i + P_j + D_k + e_{ijk} + T_l + (A\times T)_{il} + (P\times T)_{jl} + (D\times T)_{kl} + e_{ijkl}$$

where T is time effect, and A×T, P×T and D×T are animal by time, period by time and diets by time interactions, respectively. Animal effect, animal by time interaction and error terms (e_{ijk} defined as between unit error and e_{ijkl} as within unit error) are multivariate normally distributed random effects with AR (1) covariance structure. Orthogonal contrasts used in post-ANOVA comparisons were as follows; 1) effect of protein supplement (control versus protein supplements), 2) comparison between animal and plant proteins (FM versus SBM + MGM) and 3) comparison between plant proteins (SBM versus MGM).

Results

Feed composition, intake and digesta flow into the omasal canal

The chemical composition and the soluble N fractions of experimental feeds are shown in Table 2. Fishmeal had relatively high free AA, peptide and soluble protein N but extremely low

Vol. 11 (2002): 79–91.

Table 2. Chemical composition of experimental feeds.

	Grass silage	Barley	Fishmeal	Soybean meal	Maize gluten meal
Component, g kg^{-1} dry matter (DM)					
DM, g kg^{-1}	222	888	909	912	917
Organic matter	914	973	881	940	969
Nitrogen	21	22	122	75	108
Neutral detergent fibre	506	189	134	112	60
Acid detergent fibre	257	39	0	59	7
Feed soluble N fractions[a], g kg^{-1} total N					
NPN	453	126	107	42	61
Ammonia	40.0	1.2	1.0	0.6	3.7
Free amino acid	219	20	60	6	17
Peptide	194	105	47	35	40
Soluble true protein N	15	151	80	163	9
Silage fermentation quality					
pH	4.00				
Lactic acid, g kg^{-1} DM	12.2				
Acetic acid, g kg^{-1} DM	9.77				
Water soluble carbohydrate, g kg^{-1} DM	146				
Soluble N, g kg^{-1} total N	458				

[a] Non-protein N (NPN) and soluble true protein N of feeds were determined according to Licitra et al. (1996), and each fraction of NPN was analysed using ninhydrin assay (Choi et al. 2002a).

ammonia N concentration. Peptide N in soybean meal and maize gluten meal was rather similar, but soybean meal contained more soluble protein N than maize gluten meal. Proportions of free AA in soluble N (NPN + soluble true protein N) of feeds were 0.47, 0.07, 0.32, 0.03 and 0.24 for grass silage, barley, fishmeal, soybean meal and maize gluten meal, respectively, whereas peptide proportions were 0.41, 0.38, 0.25, 0.17 and 0.57, respectively. Over half of the feed soluble N was in the form of free AA and peptide except for barley and soybean meal. Grass silage was restrictively fermented as indicated by relatively low concentrations of total acids and a low proportion of ammonia N in total N.

Table 3 shows the DM intake of experimental feeds and liquid and total NAN flows into the omasal canal. The liquid flow into the omasal canal was not affected by dietary treatment (mean 200 l d^{-1}). However, the liquid flow tended to be higher for MGM than that for SBM ($P = 0.08$). Protein supplements increased total NAN flow entering the omasal canal ($P = 0.05$) mainly as a result of increased total dietary NAN flow ($P < 0.001$). Total NAN ($P = 0.04$) and total dietary NAN flow ($P < 0.001$) were higher in cows fed diet MGM than diet SBM.

Soluble N entering the omasal canal

Concentration

Protein supplements significantly increased the concentration of ammonia N in omasal digesta ($P = 0.006$) (Table 4). The ammonia N concentration peaked at 2 h post-feeding (data not shown). Protein supplementation increased the ammonia N concentration compared to the control diet throughout the feeding cycle. Protein supplements significantly increased the concentrations of peptide ($P = 0.009$) and total SNAN ($P = 0.03$) fractions in omasal digesta. However,

Table 3. Intake of dietary ingredients and effect of protein supplements on flow measurements into the omasal canal.

	Diet[a]					Orthogonal contrasts[c]		
	Control	FM	SBM	MGM	SEM[b]	C1	C2	C3
Dry matter intake, kg d^{-1}								
Grass silage	10.9	10.7	10.2	10.5				
Barley concentrate	8.2	7.3	7.6	7.5				
Fishmeal		1.0						
Soybean meal			1.8					
Maize gluten				1.1				
Mineral and vitamin mixture	0.3	0.3	0.3	0.3				
Total	19.4	19.4	19.9	19.5	0.23			
Nitrogen, g d^{-1}	403	501	511	496	6.9	<0.001	0.69	0.17
Omasal canal flow								
Liquid, l d^{-1}	210	199	182	209	9.1	0.25	0.75	0.08
Total NAN[d], g N d^{-1}	358	396	368	413	11.2	0.05	0.95	0.04
Microbial NAN	246	232	226	221	9.5	0.12	0.46	0.75
Dietary NAN	112	164	142	192	4.6	<0.001	0.55	<0.001

[a] Control = grass silage + barley; FM = grass silage + barley + fishmeal; SBM = grass silage + barley + soybean meal; MGM = grass silage + barley + maize gluten meal.
[b] SEM = standard error of the mean.
[c] C1 = control vs. other diets; C2 = FM vs. SBM + MGM; C3 = SBM vs. MGM.
[d] Total NAN = total non-ammonia nitrogen.

Table 4. Effect of protein supplements on concentration (mg N l^{-1}) of ammonia N, soluble non-ammonia N (SNAN), soluble microbial non-ammonia N (SMNAN) and soluble dietary non-ammonia N (SDNAN) in the liquid phase of digesta entering the omasal canal.

	Diet[a]					Orthogonal contrasts[c]		
	Control	FM	SBM	MGM	SEM[b]	C1	C2	C3
Ammonia N	61.1	91.4	92.5	84.2	10.58	0.006	0.69	0.35
SNAN								
Free amino acids	13.5	16.9	16.8	18.9	3.73	0.37	0.84	0.69
Peptide	56.0	81.0	78.4	72.8	9.74	0.009	0.40	0.44
Protein	0.21	0.87	0.32	0.28	0.298	0.44	0.15	0.93
Total	69.8	98.8	95.5	92.0	11.30	0.03	0.61	0.76
SMNAN								
Total	50.0	55.1	56.4	52.2	8.64	0.55	0.92	0.65
SDNAN								
Total	19.8	43.7	39.1	39.7	5.94	0.02	0.56	0.94

[a] Control = grass silage + barley; FM = grass silage + barley + fishmeal; SBM = grass silage + barley + soybean meal; MGM = grass silage + barley + maize gluten meal.
[b] SEM = standard error of the mean.
[c] C1 = control vs. other diets; C2 = FM vs. SBM + MGM; C3 = SBM vs. MGM.

Vol. 11 (2002): 79–91.

Table 5. Effect of protein supplements on flow (g N d^{-1}) of soluble non-ammonia N (SNAN), soluble microbial non-ammonia N (SMNAN) and soluble dietary non-ammonia N (SDNAN) in the liquid phase of diegeta entering the omasal canal.

	Diet[a]					Orthogonal contrasts[c]		
	Control	FM	SBM	MGM	SEM[b]	C1	C2	C3
SNAN								
Free amino acids	2.93	3.59	3.12	3.73	0.976	0.64	0.90	0.67
Peptide	11.8	16.1	13.5	14.3	1.17	0.06	0.15	0.62
Protein	0.05	0.15	0.06	0.06	0.048	0.45	0.15	0.96
Total	14.7	19.8	16.7	18.1	1.73	0.12	0.29	0.59
SMNAN								
Total	10.7	10.6	9.6	10.1	1.12	0.69	0.58	0.74
SDNAN								
Total	4.08	9.16	7.11	7.96	1.403	0.05	0.37	0.67

[a] Control = grass silage + barley; FM = grass silage + barley + fishmeal; SBM = grass silage + barley + soybean meal; MGM = grass silage + barley + maize gluten meal.
[b] SEM = standard error of the mean.
[c] C1 = control vs. other diets; C2 = FM vs. SBM + MGM; C3 = SBM vs. MGM.

there were not significant differences in SNAN fractions entering the omasal canal between the protein supplements. Approximately 0.64 of SNAN in the liquid phase of digesta was of microbial origin. Protein supplements did not affect microbial SNAN concentration whereas dietary SNAN concentration was significantly increased by the supplements ($P = 0.02$).

Flow

Protein supplements tended to increase total SNAN ($P = 0.12$) and peptide N flow ($P = 0.06$) (Table 5). Approximately 0.80 of SNAN flow into the omasum was in the form of peptides, while free AA and protein N accounted for proportionately of 0.19 and 0.02 SNAN, respectively. The contribution of dietary SNAN to the total SNAN was 0.28, 0.46, 0.43 and 0.44 for control, FM, SBM and MGM diets, respectively. The dietary SNAN flow significantly increased when protein supplements were given ($P = 0.05$).

Proportion of soluble N in total NAN flow

Examination of individual nitrogenous fractions in SNAN indicated that the proportion in the form of peptide present in total NAN was markedly higher than the other two fractions (Table 6). Mean proportions of free AA, peptide, protein and total SNAN were 8.6, 37.5, 0.23 and 46.3 g kg^{-1} total NAN, respectively.

Based on ^{15}N enrichments, the proportions of microbial SNAN and dietary SNAN were on average 28.0 and 18.4 g kg^{-1} total NAN, respectively. The proportion of microbial SNAN in total microbial NAN was on an average 46.4 g kg^{-1}, whereas the proportion of dietary SNAN in total dietary NAN was 46.8 g kg^{-1}. Protein supplements increased the proportion of dietary SNAN in total NAN flow ($P = 0.03$).

Diurnal variation in SNAN

Diurnal variations in SNAN and dietary SNAN only tended to be influenced by diet × time interaction ($P < 0.10$). Mean diurnal pattern of SNAN fractions for the experimental diets are shown in Fig. 1. Mean peptide N peaked at 1 – 3 h post-feeding and declined thereafter, while the peak of mean free AA was shown only at 1 h post-feeding. Soluble protein N concentration was very low and remained relatively constant throughout the feeding cycle. Diurnal pattern of free AA ($P = 0.02$) and peptide ($P = 0.005$) frac-

Table 6. Effect of protein supplements on the proportion (g kg^{-1}) of soluble non-ammonia N (SNAN), soluble microbial non-ammonia N (SMNAN) and soluble dietary non-ammonia N (SDNAN) in the liquid phase of omasal digesta in total non-ammonia N (NAN).

	Diet[a]					Orthogonal contrasts[c]		
	Control	FM	SBM	MGM	SEM[b]	C1	C2	C3
SNAN in total NAN								
Free amino acids	7.9	8.6	8.6	9.3	1.76	0.66	0.89	0.77
Peptide	33.8	40.7	39.6	35.8	5.30	0.15	0.37	0.33
Protein	0.1	0.5	0.2	0.1	0.16	0.52	0.16	0.93
Total	41.9	49.8	48.4	45.2	5.97	0.22	0.54	0.58
SMNAN[d]								
Proportion in total NAN	30.0	27.4	28.8	25.6	4.64	0.48	0.97	0.50
Proportion in TMNAN	43.5	46.8	46.3	48.8	7.64	0.55	0.90	0.74
SDNAN[d]								
Proportion in total NAN	11.9	22.4	19.6	19.6	2.89	0.03	0.41	1.00
Proportion in TDNAN	39.0	54.3	52.1	41.8	7.72	0.21	0.39	0.30

[a] Control = grass silage + barley; FM = grass silage + barley + fishmeal; SBM = grass silage + barley + soybean meal; MGM = grass silage + barley + maize gluten meal.

[b] SEM = standard error of the mean.

[c] C1 = control vs. other diets; C2 = FM vs. SBM + MGM; C3 = SBM vs. MGM.

[d] Proportions of SMNAN and SDNAN expressed as g kg^{-1} total microbial NAN (TMNAN) and g kg^{-1} total dietary NAN (TDNAN), respectively.

tions of SNAN were affected by the diet as indicated by significant diet × time interaction. At 1 h post-feeding diet MGM had the highest (74.8 mg N l^{-1}) concentration of free AA followed by diet SBM (Fig. 2). Free AA concentration for control and FM diets were higher at 0 h than in samples taken at 1 h post-feeding, and those for the other diets were also relatively high. Peptide N concentration in the liquid phase of digesta entering the omasum remained to be higher for protein supplemented-diets than that for control diet during the feeding cycle (Fig. 3).

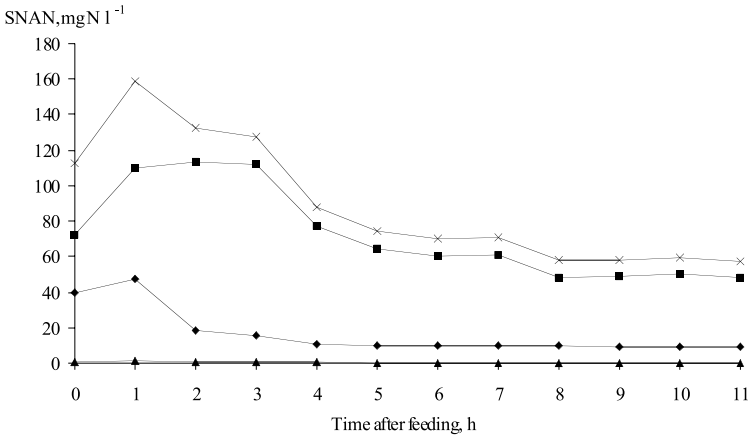

Fig. 1. Diurnal variations of nitrogenous fractions of soluble non-ammonia N (SNAN) in the liquid phase of digesta entering the omasum during a 12 h feeding cycle (◆ = free amino acid; ■ = peptide; ▲ = protein; × = total SNAN; standard error of the mean for the free amino acid, peptide, protein and total SNAN were 4.87, 9.36, 0.28 and 11.25, respectively).

Vol. 11 (2002): 79–91.

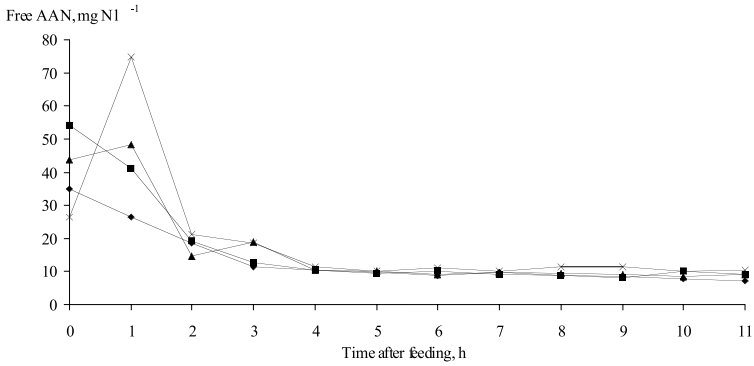

Fig. 2. Influence of dietary treatment on the extent of diurnal variation in free amino acid (AA) N of the liquid phase of digesta entering the omasum during a 12 h feeding cycle (◆ = grass silage + barley; ■ = grass silage + barley + fishmeal; ▲ = grass silage + barley + soybean meal; × = grass silage + barley + maize gluten meal; standard error of the mean for free AA N was 7.98).

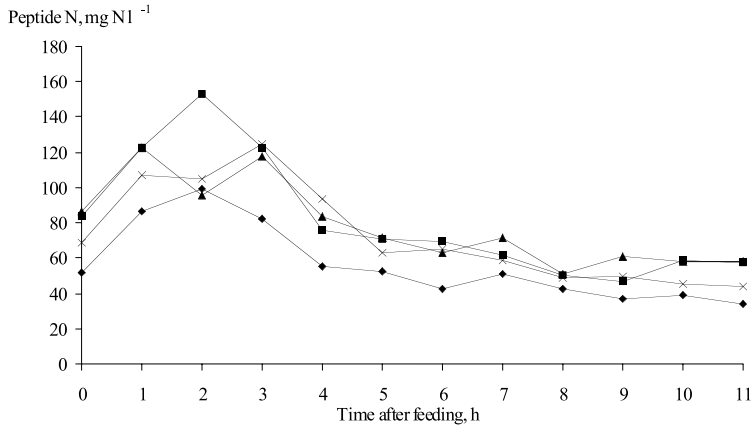

Fig. 3. Influence of dietary treatment on the extent of diurnal variation in peptide N of the liquid phase of digesta entering the omasum during a 12 h feeding cycle (◆ = grass silage + barley; ■ = grass silage + barley + fishmeal; ▲ = grass silage + barley + soybean meal; × = grass silage + barley + maize gluten meal; standard error of the mean for peptide N was 11.7).

Peptide N concentration in omasal digesta peaked at 1 h (diet SBM), 2 h (control and FM diets) and 3 h (diet MGM) post-feeding and declined to the pre-feeding level.

Discussion

Contribution of microbial N to total SNAN

Previous studies have neglected the potential microbial contamination of SNAN (Chen et al. 1987a, b, Broderick and Wallace 1988). However, extracellular AAs are probably excreted by rumen bacteria and protozoa cells or released during the cell lysis (Nolan 1993). Our recent study using ^{15}N as a microbial marker suggested that a high proportion of SNAN in the liquid phase of digesta entering the omasal canal was of microbial origin (Choi et al. 2002a). It may also be partly explained by high concentration of glutamic acid, as an intermediate of bacterial AA metabolism, in free AA fraction of SNAN in omasal digesta (Choi et al. 2002a).

In the present study, ^{15}N was also used to estimate microbial contamination of the liquid phase of digesta. The $NA^{15}N$ was assumed to be entirely derived from liquid associated bacteria because engulfed LAB-NAN is spilled outside of protozoa cells (Jouany et al. 1988). The

present microbial contribution to SNAN in the liquid phase of digesta (mean 0.64) is consistent with that of 0.63 – 0.86 (Hristov and Broderick 1996) and of 0.61 (Choi et al. 2002a). The proportion of microbial contribution to the SNAN could, however, be overestimated as microbial lysis could have occurred during acid treatment of digesta and freezing after sampling.

Here, the microbial contribution to SNAN was similar to the microbial contribution to total NAN flow (mean 0.61). Besides, neither microbial NAN nor microbial SNAN were significantly affected by the supplements, supporting the previous result that rapeseed meal supplementation had no influence on microbial N flow in dairy cows (Ahvenjärvi et al. 1999). An increase of dietary SNAN by protein supplements may indicate that release of dietary N in the form of peptide and/or protein exceeds the proteolysis in the rumen. Consequently the peptide N could have an opportunity to escape the rumen and be absorbed in the small intestine as AA source by the host animal.

Soluble NAN

Protein supplements

Robinson et al. (1998) using dairy cows fed timothy silage, whole-crop barley silage and a mixed concentrate showed that protein supplements gave no significant increase in peptide N concentration in the liquid phase of ruminal digesta (mean 83.6 and 83.0 mg N l^{-1} for without and with protein supplements, respectively). In the present study, peptide N concentration was, however, increased by protein supplements (mean 56.0 and 77.4 mg N l^{-1}). This is consistent with a considerable increase in peptide N flow from the rumen when the cows were fed grass silage based diets received protein supplements (Choi et al. 2002a, b).

Soybean meal has produced a higher SNAN concentration than other protein supplements e.g. maize gluten meal and/or blood meal (Robinson 1997) and fishmeal (Chen et al. 1987b) but not always (Williams and Cockburn 1991). Peptide N concentration in ruminal digesta at 1 h post-feeding was much higher for maize gluten meal than for fishmeal supplement (Williams and Cockburn 1991). However, the present concentration of peptide N and/or total SNAN was rather similar between protein supplemented-diets, even though the soluble N composition differed in protein feeds. Different peptide N concentrations on similar protein feeds between the studies could be influenced by variation in the quality of protein feeds, e.g. it is well known that the quality and composition of fishmeal can be different depending on a fish-drying and adding antioxidants (Mehrez et al. 1980). In addition, a rate of slowly degradable N fraction (*b-fraction*) is very different for experimental supplements, e.g. a rate (% h^{-1}) of *b-fraction* of soybean meal (9.4) is the fastest rate and that of maize gluten meal (2.3) is the slowest (NRC 2001). The similar peptide N concentration for the protein supplemented-diets supports the claim that peptide N concentration is poorly correlated with degradability and solubility of protein feeds (Williams and Cockburn 1991). Many studies have also shown that there was no difference in peptide N and SNAN concentration between diets containing different type of protein supplements (Chen et al. 1987b, Robinson and McQueen 1994, Choi et al. 2002b).

Here, protein supplements were replaced not by barley concentrate but by a part of control diet in order to keep grass silage to barley ratio (55:45) constant. However, the actual ratio of DM intake failed to keep the ratio planned (see Table 3), but it was still similar between the diets (57:43). The purpose of this was to allow to estimate SNAN flow in the liquid phase of omasal digesta per kg DM intake from the basal diet, and then calculate eSNAN from each protein supplement. Based on the equations 1–3, eSNAN was 49, 22 and 37 g kg^{-1} of fishmeal, soybean meal and maize gluten meal, respectively.

Daily intake of soluble NAN in feed N of each protein supplement, calculated as soluble NAN in total N of protein feed × daily N intake of protein feed, appeared to be approx. 23, 28

Vol. 11 (2002): 79–91.

and 8 g N d^{-1} for fishmeal, soybean meal and maize gluten meal, respectively.

Overall, the eSNAN increased by each protein feed does not seem to be subjected to the intake of soluble NAN in protein feeds. The present results support our previous observation that the eSNAN is not related to *a-fraction* of in situ determination, and effective protein degradability can not necessarily be estimated as a sum of the escape calculated from *a-* and *b-fractions* of in situ determination (Choi et al. 2002b).

Metabolism of SNAN

Wallace and McKain (1990) reported that a colourimetric method using ninhydrin does not give estimate of peptide concentration reliably because of extremely high ammonia concentration in acid-hydrolysates after alkaline-heating. We have also observed markedly high ammonia in samples after the acid-hydrolysis (Choi et al. 2002a). However, our preliminary analysis showed that proportionately 0.99 of ammonia in the pre-hydrolysis samples was eliminated by the alkaline-heating method. Therefore, in the present study, SNAN obtained in the acid-hydrolysates was corrected for ammonia N concentration determined in the pre-hydrolysis.

In the present study, markedly higher peptide N in total SNAN than the other two fractions is consistent with the previous observation of high peptide N in ruminal fluid (Chen et al. 1987a, Choi et al. 2002b) and in omasal fluid (Choi et al. 2002a, b). Free AA concentration in ruminal digesta is relatively low (Williams and Cockburn 1991) even during the period immediately post-feeding (see Nolan 1993). However, in the present study, free AA concentration in omasal digesta was relatively high immediately post-feeding. On an average, the present free AA concentration is consistent with our previous results (mean 15.3 mg N^{-1}) (Choi et al. 2002a). In the diurnal pattern of free AA in the present study, the reason for the lacks of the peaks for control and FM diets was unclear (Fig. 2). However, although many studies reported clear peaks in free AA concentration (Broderick and Wallace 1988, Choi et al. 2002b), some protein supplements produced the constant diurnal pattern without peaks in free AA concentration (Choi et al. 2002a). Extremely low soluble protein fraction of SNAN in the present study is consistent with other studies, in which different protein supplements were given (Williams and Cockburn 1991, Choi et al. 2002b). The low soluble protein N could be explicated by that soluble protein in feed N is rapidly degraded to non-precipitable peptides (Choi et al. 2002b). Partitioning of SNAN fractions may be less important as regards to determination of the supply of AA from soluble N fraction, since all N fractions are assumed to be completely digested in the small intestine.

Concentrations of peptide N varied between 82 and 111 mg N l^{-1} (Chen et al. 1987a, b, Robinson and McQueen 1994, Robinson et al. 1998). The present peptide N concentration in omasal fluid (mean 72 mg N l^{-1}) was marginally lower than the values reported previously. However, the previous peptide concentrations included free AA (Robinson and McQueen 1994, Robinson et al. 1998) since free AA fraction was not determined before hydrolysis. Taking this into account, the sum of peptide and free AA concentration for control, FM, SBM and MGM was 70, 98, 95 and 92 mg N l^{-1}, respectively, estimates that are in good agreement with the reported values. Our most recent study showed that peptide N concentration excluding free AA in omasal digesta was 54 – 64 mg N l^{-1} when protein supplements were given to cows (Choi et al. 2002b). Diurnal pattern in ruminal or omasal peptide concentration (and/or total SNAN) that peaked immediately post-feeding and declined thereafter has been observed in many studies (Chen et al. 1987a, Williams and Cockburn 1991, Robinson et al. 1998, Choi et al. 2002a, b). When ryegrass hay and maize based concentrate were fed with urea or ovalbumin diurnal pattern in peptide concentration in the rumen was rather constant (Broderick and Wallace 1988). In their study, however, ruminal peptide N reached a maximum immediately post-feeding, when the diet was supplemented with casein.

Conclusions

Present data confirm the previous observation (Choi et al. 2002a) that a substantial proportion of SNAN in the liquid phase of omasal digesta can escape ruminal degradation. Protein supplements increased peptide N and total SNAN fractions in the liquid phase of omasal digesta, whereas there were not differences in the SNAN concentration between different protein supplements. The omasal SNAN provided by protein feeds was not equated with soluble N the protein feeds. Quantitatively peptides rather than free AA or soluble protein were the most important N fraction of SNAN in the liquid phase of digesta. The potential microbial contribution to SNAN in omasal digesta using ^{15}N as a microbial marker suggested that the SNAN was substantially contaminated by microbes.

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SELOSTUS

Valkuaistäydennyksen vaikutus lypsylehmän pötsistä virtaavan liukoisen rehuperäisen typen pitoisuuteen ja määrään säilörehuruokinnalla

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Valkuaistäydennyksen vaikutusta lypsylehmän pötsistä virtaavan liukoisen rehuperäisen typen (N) pitoisuuteen ja määrään tutkittiin 4 × 4 latinalaisen neliön koemallin mukaisessa kokeessa. Koe-eläiminä oli neljä pötsifistelöityä lypsylehmää, jotka saivat kontrolliruokinnalla nurmisäilörehua ja ohraa siten, että syönti oli 95 % vapaasta syönnistä ja säilörehun ja ohran suhde oli 55:45. Muilla ruokinnoilla perusrehuja korvattiin kalajauholla (6 %), soijarouheella (9 %) ja maissigluteenilla (6 %) siten, että ohran ja säilörehun suhteelliset osuudet pysyivät vakioina ja valkuaistäydennyksenä tulevan typen määrä oli sama kaikilla valkuaisrehuilla.

Valkuaistäydennys lisäsi pötsistä virtaavan peptidi-N:n ja liukoisen rehuperäisen N:n pitoisuuksia satakerran ruokasulassa, mutta erot valkuaisrehujen välillä olivat pieniä. Peptidi-N muodosti suurimman osan liukoisesta rehuperäisestä N:stä osoittaen, että peptidien hydrolyysi aminohapoiksi on rajoittavin vaihe pötsin proteolyysissä. Mikrobi-N:n osuus liukoisesta rehuperäisestä N:stä oli 0,64 osoittaen, että suuri osa satakertaan virtaavasta rehuperäisestä liukoisesta N:stä oli mikrobista alkuperää. Laskelma liukoisen rehuperäisen N:n virtauksesta syötyä kuiva-ainekiloa kohti osoitti, että kalajauhosta n. 49 g kg^{-1}, soijarouheesta n. 22 g kg^{-1} ja maissigluteenista n. 37 g kg^{-1} virtasi ulos pötsistä hajoamatta liukoisessa muodossa.