# Flow of Soluble Non-ammonia Nitrogen in the Liquid Phase of Digesta Entering the Omasum of Dairy Cows Given Grass Silage Based Diets

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**ABSTRACT :** An experiment was conducted to quantify the flow of soluble non-ammonia nitrogen (SNAN) in the liquid phase of ruminal (RD) and omasal digesta (OD), and to investigate diurnal pattern in SNAN flow in OD. Five ruminally cannulated Finnish-Ayrshire dairy cows in a 5×5 Latin square design consumed a basal diet of grass silage and barley grain, and that supplemented with four protein feeds (kg/d DM basis) as follows: skimmed milk powder (2.1), wet distiller's solubles (3.0), untreated rapeseed meal (2.1) and treated rapeseed meal (2.1). Ruminal digesta was sampled using a vacuum pump, whereas OD was collected using an omasal sampling system at 1.0 h interval during a 12 h feeding cycle. Both RD and OD were acidified, centrifuged to remove microbes and precipitated with trichloroacetic acid followed by centrifugation. The SNAN fractions (free amino acid (AA), peptide and soluble protein) in RD and OD were assessed using ninhydrin assay. Free AA, peptide and soluble protein averaged 60.0, 89.4 and 2.1 g/d, respectively, for RD, and 81.8, 121.5 and 2.5 g/d, respectively, for OD. Although free AA flow was relatively high, mean peptide flow was quantitatively the most important fraction of SNAN, indicating that degradation of peptide to AA rather than hydrolysis of soluble protein to peptide or deamination may be the most limiting step in rumen proteolysis. Diurnal pattern in flow of peptide including free AA in OD during a 12 h feeding cycle peaked 1 h post-feeding, decreased by 3 h post-feeding and was relatively constant thereafter. Protein supplementation showed higher flow of peptide including free AA immediately after feeding compared with no supplemented diet. There were no differences among protein supplements in diurnal pattern in flow of peptide including free AA in OD. ***(Asian-Aust. J. Anim. Sci. 2003. Vol 16, No. 10 : 1460-1468)***

**Key Words :** Soluble Non-ammonia Nitrogen, Omasal Digesta, Grass Silage, Diurnal Pattern, Peptide

## INTRODUCTION

Due to low concentration of free amino acid (AA) in ruminal digesta (RD), it was suggested an observation that hydrolysis of protein to peptide or deamination is the rate-limiting step in proteolysis in the rumen (Wright and Hungate, 1967; Tamminga, 1979; Van Straalen and Tamminga, 1990). Rumen bacteria, however, can take up peptide faster than AA (Pittman et al., 1967; Russell et al., 1983), indicating that ruminal AA may not be extracellular intermediate. Besides, peptide can accumulate in RD, and it may escape the rumen (Chen et al., 1987c). Thus, the previous observation in terms of the rate-limiting step in rumen proteolysis may be not valid.

Studies on responses of the flow of soluble non-ammonia nitrogen (SNAN) in the liquid phase of omasal digesta (OD) to various protein feeds are limited. Also, few data on diurnal pattern in SNAN flow escaping the rumen are available. Chen et al. (1987a) reported that an increase in CP of diets (14.5 to 17.5% DM basis) did not affect flow of peptide and soluble protein in RD. Broderick and Wallace (1988) showed a peak of ruminal peptide concentration immediately after feeding when casein was fed. In addition, similar responses of SNAN concentration in RD between no protein supplements and soybean meal have been reported (Robinson et al., 1998). However, most of previous SNAN data (Chen et al., 1987c; Broderick and Wallace, 1988; Robinson and McQueen, 1994) were obtained from RD, thus the extent of SNAN flow escaping the rumen was still unclear.

Recent development of omasal sampling technique (Huhtanen et al., 1997; Ahvenjärvi et al., 2000; Choi et al., 2002) provides an opportunity for the accurate quantification of proteolysis in the rumen. The potential advantages of the omasal sampling technique have been introduced as follows: (1) only rumen cannulated animals are required, (2) less endogenous nitrogen (N) is contaminated in OD compared with sampling of abomasal or duodenal digesta, and (3) samples of OD are devoid of exposure to acid pepsin hydrolysis occurring in the abomasum (Ahvenjärvi et al., 2000; Choi et al., 2002).

The present study using omasal sampling technique aimed to assess the flow of SNAN fractions and to investigate diurnal pattern in the flow of SNAN fractions escaping the rumen of cows fed grass silage based diets with various protein supplements containing different soluble N content.

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## MATERIALS AND METHODS

### Animals and their management

Five Finnish Ayrshire dairy cows weighing on an average 534 (SE±42.5) kg fitted with 100 mm i.d. ruminal cannulas were used in a 5×5 Latin square experiment. The cows were housed in individual stalls, and had free access to water and a salt block throughout the experiment. They were provided with two equal meals daily at 06:00 and 18:00 h and milked at 07:00 and 17:00 h.

### Experimental diets and treatments

The cows were offered grass silage and rolled barley grain *ad libitum* during an adaptation period, and then the DMI was restricted to 90% of the *ad libitum* intake. A basal diet (% DM) consisted of grass silage (49.5), rolled barley grain (49.5), and a commercial mixture of mineral and vitamin (10) (control). Part of the basal diet was replaced (kg/d) by one of protein supplements as follows: skimmed milk powder (2.1) (diet SMP), wet distiller's solubles (3.0) (WDS), untreated rapeseed meal (2.1) (URSM), or treated rapeseed meal (2.1) (TRSM).

The silage was made from predominately timothy (*Phleum pratense*) and meadow fescue (*Festuca pratensis*) swards. Wilted herbage was harvested using a precision-chop forage harvester and ensiled with a formic acid-based additive (5 l/ton; AIV-2, Kemira-Agro, Helsinki, Finland) in a tower silo. Barley was coarsely milled using a roller mill prior to feeding, and all protein supplements were purchased from commercial sources (Suomen Rehu Ltd., Helsinki; Rehuraisio Ltd., Raisio, Finland). Wet distiller's solubles was mixed with the rolled barley grain just before feeding.

### Sampling procedures and chemical analysis

The whole experiment lasted 14 days. Feed intake was recorded daily. Representative samples of feeds were collected on day 10 to 14, pooled, subsampled and subsequently dried until analysed. Feed DM concentration was determined using a forced-air oven for 18 h at 105°C, whereas OM was determined by ashing for 18 h at 600°C. Crude protein content of feed was determined using a Dumas-type N analyser (Leco FP-428; Leco Corporation, St Joseph, MI, USA). Feed NDF was determined according to Van Soest et al. (1991). Concentrations of VFA in grass silage and RD were measured according to Huhtanen et al. (1998). Lactic acid and water soluble carbohydrate in grass silage were determined according to Barker and Summerson (1941) and Somogyi (1945), respectively. Soluble N content of grass silage was analysed by the Kjeldahl method, and ammonia N was determined according to McCullough (1967). Feed AA composition was analysed using an AA analyser (Biochrom 20, AA analyser, Autoloader version, Pharmacia Biotech (Biochrom) Ltd., Cambridge, UK).

To assess liquid outflow from the rumen, LiCoEDTA (12 g/d) was mixed with rumen contents at 0.5 h before feeding, and RD was collected at -1, 0, 2, 4, 6, 8, and 10 h post-feeding on day 13. The marker was prepared as previously described by Mwenya et al. (2003). Cobalt concentration was determined using a Perkin-Elmer 2100 atomic absorption spectrophotometer (Bodenseewerk Perkin-Elmer GmbH, Ueberlingen, Germany) based on a procedure described by Williams et al. (1962). Liquid outflow rate from the rumen was calculated as the slope of the regression plotted using natural logarithm of Co concentration against time. Concentration at 0 h was computed from anti-logarithm of the intercept of the equation. Rumen volume was computed as Co dose divided by Co concentration at 0 h, and liquid outflow from the rumen was calculated as rumen volume×liquid outflow rate from the rumen.

To estimate SNAN fractions in digesta, RD and OD were collected at 0, 2, 4, 6, 8, and 10 h post-feeding on day 13. On the subsequent day, sampling time was advanced by 1 h relative to the previous day. Sample of OD was collected from the omasum by means of a plastic tube (14 mm i.d.) connected to a pump according to procedures outlined by Huhtanen et al. (1997), with the exceptions as follows: 1) larger sampling tube (14 vs. 9.5 mm i.d.), 2) solenoid valves instead of a three way ball valve to control vacuum and pressure phases in a pump and 3) a 0.5 kg weight inserted into the abomasums for securing the sampling device in the omasum. Approximately 150 ml of samples of RD and OD was acidified with 6.25 ml of 10% sulphuric acid and filtered through cheesecloth. Filtrates of RD and OD were centrifuged at 1,000×g for 10 min at 4°C, and the supernatant was decanted into a fresh tube followed by high-speed centrifugation (10,000×g, 30 min, 4°C) to discard small particles and rumen microbes. The supernatant was precipitated with trichloroacetic acid (TCA) (final concentration 5% w/v), placed in ice overnight and high-speed centrifuged. Different N fractions (free AA, peptide and soluble protein) of SNAN in the liquid phase of RD and OD were assessed using ninhydrin assay (NHA). Details of sample preparation and NHA have previously been described by Choi et al. (2002). In brief, SNAN within RD and OD was fractionated as

free AA: estimated from the supernatant without acid-hydrolysis,

peptide: estimated from hydrolysed supernatant (6 M HCl, 24 h, 110ºC) minus free AA and

soluble protein: estimated from hydrolysis of TCA-precipitate.

All N fractions were corrected for ammonia N except for soluble protein because TCA-precipitate was assumed to

**Table 1.** Chemical composition of experimental feeds

| | Feed[1] | | | | | |
|---|---|---|---|---|---|---|
| | Silage | Barley | SMP | WDS | URSM | TRSM |
| Composition, % DM | | | | | | |
| DM, % | 28.5 | 88.3 | 96.0 | 33.7 | 89.5 | 89.0 |
| OM | 92.2 | 97.5 | 91.9 | 87.6 | 92.3 | 92.5 |
| CP | 16.9 | 12.9 | 36.4 | 25.2 | 36.6 | 35.3 |
| NDF | 54.5 | 21.8 | 0.0 | 0.6 | 27.3 | 23.1 |
| Silage fermentation quality, % DM excluding pH | | | | | | |
| pH | 4.09 | | | | | |
| Acetic acid | 1.70 | | | | | |
| Propionic acid | 0.02 | | | | | |
| Butyric acid | 0.08 | | | | | |
| Lactic acid | 5.76 | | | | | |
| Water soluble carbohydrate | 27.6 | | | | | |
| Soluble N | 1.78 | | | | | |
| Ammonia N | 0.16 | | | | | |

[1] SMP=skimmed milk powder, WDS=wet distiller's solubles, URSM=untreated rapeseed meal, TRSM=treated rapeseed meal.

contain negligible amounts of ammonia N.

**Statistical analysis**

Data on feed DMI, CP intake and rumen VFA concentration were fitted using the MIXED procedure of SAS (Littell et al., 1998) according to the following statistical model:

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

where A is a random effect of animal, and P and D are the fixed effects of period and diet, respectively.

Data on rumen pH, ammonia N, flow measurement and SNAN fractions determined at each sampling interval were fitted using the MIXED procedure of SAS (Littell et al., 1998) for repeated measures according to the following statistical model:

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

where T is a fixed effect of time after feeding, and A×T, P×T and D×T are animal by time, period by time and diet by time interactions, respectively. In the repeated measures models, animal, animal by time interaction and error terms ($e_{ijk}$ defined as between unit error and $e_{ijkl}$ as within unit error) are assumed to be multivariate normally distributed random effects with AR (1) covariance structure.

**Animal care**

All cows involved in the present experiment were managed according to legislation documented within the Finnish Animal Welfare Act (247/96), the order of using vertebrate animals for scientific purposes (1,076/85), and

**Table 2.** Amino acid (AA) composition of experimental feeds

| | Feed | | | | | |
|---|---|---|---|---|---|---|
| | Silage | Barley | SMP | WDS | URSM | TRSM |
| AA, g/kg crude protein | | | | | | |
| Arginine | 37.5 | 51.2 | 32.1 | 34.7 | 59.9 | 61.9 |
| Histidine | 16.5 | 24.0 | 28.7 | 23.2 | 27.1 | 26.5 |
| Isoleucine | 40.1 | 35.7 | 49.8 | 21.8 | 39.5 | 40.0 |
| Leucine | 73.3 | 65.7 | 99.8 | 42.0 | 70.7 | 70.9 |
| Lysine | 47.8 | 35.9 | 75.8 | 29.3 | 56.0 | 53.3 |
| Methionine | 17.2 | 17.6 | 24.0 | 11.8 | 20.1 | 21.1 |
| Phenylalanine | 46.2 | 48.2 | 47.3 | 28.2 | 39.3 | 40.5 |
| Threonine | 39.4 | 34.7 | 41.5 | 33.5 | 43.7 | 43.3 |
| Valine | 52.4 | 61.1 | 67.6 | 45.3 | 51.2 | 50.1 |
| Alanine | 64.8 | 38.9 | 30.7 | 49.9 | 43.0 | 42.3 |
| Aspartic acid | 87.2 | 59.8 | 73.8 | 54.2 | 73.2 | 76.4 |
| Cystine | 7.8 | 22.7 | 6.7 | 22.4 | 18.8 | 18.7 |
| Glutamic acid | 88.3 | 217.9 | 210.4 | 193.4 | 145.8 | 155.1 |
| Glycine | 44.7 | 39.9 | 18.4 | 52.8 | 49.2 | 49.4 |
| Proline | 56.0 | 100.3 | 102.8 | 97.5 | 57.0 | 56.9 |
| Serine | 35.3 | 41.5 | 53.0 | 39.6 | 42.0 | 41.9 |
| Tyrosine | 26.5 | 31.5 | 40.7 | 20.7 | 30.7 | 30.4 |
| EAA[1] | 370 | 374 | 467 | 270 | 408 | 408 |
| NEAA[2] | 411 | 553 | 537 | 531 | 460 | 471 |
| Hydrophobic AA[3] | 254 | 307 | 358 | 234 | 249 | 249 |
| Hydrophilic AA[3] | 261 | 365 | 392 | 312 | 335 | 347 |
| TAA | 781 | 927 | 1,003 | 800 | 867 | 879 |

[1] Essential AA=sum of arginine, histidine, isoleucine, leucine, lysine, methionine, phenylalanine, threonine and valine.
[2] Nonessential AA=sum of alanine, aspartic acid, cystine, glutamic acid, glycine, proline, serine and tyrosine.
[3] Calculated according to Chen et al. (1987b); Hydrophobic AA=sum of leucine, phenylalanine, valine, proline and tyrosine; Hydrophilic AA=sum of arginine, lysine, aspartic acid and glutamic acid. For abbreviations of feeds, see Table 1.

**Table 3.** Intake of dietary ingredients

| | Diet[1] | | | | | SEM[2] | Contrast[3] | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | Control | SMP | WDS | URSM | TRSM | | C1 | C2 | C3 | C4 |
| DM intake, kg/d | | | | | | | | | | |
| Grass silage | 9.3 | 8.4 | 7.9 | 8.5 | 8.5 | | | | | |
| Barley grain | 9.5 | 8.7 | 7.4 | 8.7 | 8.6 | | | | | |
| Skimmed milk powder | | 2.1 | | | | | | | | |
| Wet distiller's solubles | | | 2.8 | | | | | | | |
| Untreated rapeseed meal | | | | 2.1 | | | | | | |
| Treated rapeseed meal | | | | | 1.8 | | | | | |
| Mineral and vitamin mixture | 0.25 | 0.25 | 0.25 | 0.25 | 0.25 | | | | | |
| Total | 19.0 | 19.5 | 18.3 | 19.5 | 19.1 | 0.31 | NS | NS | * | NS |
| CP | 2.90 | 3.41 | 3.01 | 3.43 | 3.26 | 0.064 | *** | † | ** | NS |

[1] Control=grass silage+barley grain, SMP=grass silage+barley grain+skimmed milk powder, WDS=grass silage+barley grain+wet distiller's solubles, URSM=grass silage+barley grain+untreated rapeseed meal, TRSM=grass silage+barley grain+treated rapeseed meal.
[2] SEM=standard error of the mean. [3] C1=control vs. other diets; C2=SMP+WDS vs. URSM+TRSM; C3=SMP vs. WDS; C4=URSM vs. TRSM; NS; $p>0.10$, †; $p<0.10$, * $p<0.05$, ** $p<0.01$ and *** $p<0.001$, respectively.

the European convention for the protection of vertebrate animals used for experimental and other scientific purposes implemented under the auspices of the local Animal Use and Care Committee.

## RESULTS AND DISCUSSION

### Feed

The chemical composition of experimental feeds is given in Table 1. Grass silage contained relatively low proportions of total acids and ammonia N in DM, indicating that the silage used was restrictively fermented and of good fermentation quality (Choi et al., 2002). It is widely accepted that application of formic acid-based additive during ensiling reduces protein breakdown and ammonia N production in silage, and increases silage peptide concentration compared with untreated silage (Nsereko et al., 1998).

The AA composition of feeds is shown in Table 2. The AAs of leucine, alanine, aspartic acid, glutamic acid and proline were quantitatively the most important in CP of grass silage. The proportion of AA in CP was highest for skimmed milk powder followed by barley. In the present study, hydrophobic and hydrophilic AAs were calculated as sum of leucine, phenylalanine, valine, proline and tyrosine, and sum of arginine, lysine, aspartic acid and glutamic acid, respectively, according to Chen et al. (1987b). Protein feeds contained more hydrophilic AA in total CP than hydrophobic AA. Intake of hydrophilic and hydrophobic AA was calculated to be 865 and 781 (control), 1,085 and 984 (diet SMP), 920 and 801 (diet WDS), 1,051 and 908 (diet URSM) and 1,008 and 871 (diet TRSM), respectively (based on Tables 2 and 3).

Experimental animals consumed grass silage and concentrates as planned, except for diet WDS (Table 3) presumably due to poor palatability. Protein supplements had no effect ($p>0.10$) on total DMI, but increased CP intake ($p<0.001$). However, DMI ($p<0.05$) and CP intake ($p<0.01$) were lower for diet WDS than diet SMP.

### Rumen flow measurement and fermentation characteristics

Data on rumen liquid flow measurement and fermentation are given in Table 4. Protein supplements did not affect ($p>0.10$) rumen outflow rate, rumen volume or liquid outflow from the rumen. Absence of effect of protein supplements on rumen outflow rate, rumen volume or rumen liquid outflow is consistent with our previous results (Choi et al., 2002) where barley or rapeseed meal supplements did not affect rumen flow measurements of cows fed grass silage based diet. Both rumen volume and liquid outflow tended ($p<0.10$) to be higher for diet URSM than for diet TRSM.

Despite lack of significance, ruminal pH was numerically lower for control than for protein-supplemented diets. Cows fed the control diet had significantly ($p<0.01$) lower molar concentration of ruminal ammonia N than cows fed protein-supplemented diets. Consistent with the present results, Ahvenjärvi et al. (1999) reported that increase in ruminal ammonia concentration when cows fed grass silage based diet were supplemented with protein feed (rapeseed meal). In addition, Chen et al. (1987b) reported an increase of ruminal ammonia N concentration (51 mg N $l^{-1}$ to 105 mg N $l^{-1}$) as CP increased (145 to 171 g $kg^{-1}$) in the diets contained corn silage, hay crop silage, barley and corn meal supplemented with soybean meal. The increases in ammonia concentration were probably associated with increased intake of rumen degradable protein (Ahvenjärvi et al., 1999). Protein supplements did not affect ($p>0.10$) total VFA concentration. However, the supplementation increased ($p<0.05$) molar proportion of acetate, whereas cows fed rapidly degradable protein diets had lower ($p<0.01$) molar proportion of acetate than slowly degradable protein diets. Treatment of rapeseed meal tended

**Table 4.** Effect of protein supplements on rumen outflow rate, volume and liquid flow measurements, and rumen fermentation parameters

| | Diet[1] | | | | | SEM[2] | Contrast[3] | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | Control | SMP | WDS | URSM | TRSM | | C1 | C2 | C3 | C4 |
| Rumen outflow rate, /h | 0.162 | 0.168 | 0.166 | 0.178 | 0.175 | 0.0093 | NS | NS | NS | NS |
| Rumen volume, litre | 87.2 | 81.5 | 76.1 | 90.5 | 79.1 | 4.15 | NS | NS | NS | † |
| Liquid outflow, l/h | 14.3 | 14.1 | 12.6 | 16.1 | 13.7 | 0.85 | NS | NS | NS | † |
| Rumen fermentation | | | | | | | | | | |
| pH | 6.14 | 6.29 | 6.25 | 6.25 | 6.21 | 0.124 | NS | NS | NS | NS |
| $NH_3$-N, mmol/l | 6.41 | 8.54 | 7.58 | 8.24 | 8.84 | 0.745 | ** | NS | NS | NS |
| Total VFA, mmol/l | 120 | 118 | 113 | 118 | 117 | 2.6 | NS | N | NS | NS |
| Molar proportion of VFA, mmol/mol | | | | | | | | | | |
| Acetate | 651 | 622 | 624 | 640 | 655 | 5.5 | * | ** | NS | † |
| Propionate | 167 | 168 | 200 | 185 | 176 | 9.2 | NS | NS | * | NS |
| Isobutyrate | 9.1 | 11.8 | 7.2 | 9.3 | 8.6 | 0.33 | NS | NS | *** | NS |
| Butyrate | 133 | 145 | 135 | 130 | 125 | 7.7 | NS | NS | NS | NS |
| Isovalerate | 15.5 | 21.7 | 10.1 | 14.1 | 14 | 2.16 | NS | NS | ** | NS |
| Valerate | 16.3 | 21.4 | 19.0 | 16.7 | 16.1 | 0.53 | ** | *** | * | NS |

[1] Control=grass silage+barley grain, SMP=grass silage+barley grain+skimmed milk powder, WDS=grass silage+barley grain+wet distiller's solubles, URSM=grass silage+barley grain+untreated rapeseed meal, TRSM=grass silage+barley grain+treated rapeseed meal.
[2] SEM=standard error of the mean. [3] C1=control vs. other diets; C2=SMP+WDS vs. URSM+TRSM; C3=SMP vs. WDS; C4=URSM vs. TRSM; NS; p>0.10, †: p<0.10, * p<0.05, ** p<0.01 and *** <0.001, respectively.

**Table 5.** Effect of protein supplements on the flow (g/d) of soluble non-ammonia nitrogen fractions in ruminal and omasal digesta

| | Diet[1] | | | | | SEM[2] | Contrast[3] | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | Control | SMP | WDS | URSM | TRSM | | C1 | C2 | C3 | C4 |
| Free amino acid | | | | | | | | | | |
| Ruminal | 39.0 | 63.1 | 56.4 | 68.8 | 72.8 | 10.39 | * | NS | NS | NS |
| Omasal | 47.3 | 98.5 | 71.4 | 102.9 | 88.6 | 18.90 | ** | NS | NS | NS |
| Peptide | | | | | | | | | | |
| Ruminal | 82.4 | 94.3 | 86.2 | 101.6 | 82.7 | 11.02 | NS | NS | NS | † |
| Omasal | 105.2 | 129.2 | 124.5 | 140.5 | 108.2 | 18.05 | NS | NS | NS | NS |
| Soluble protein | | | | | | | | | | |
| Ruminal | 1.5 | 2.2 | 1.2 | 3.4 | 2.3 | 0.42 | NS | * | NS | † |
| Omasal | 1.6 | 2.8 | 2.1 | 3.0 | 2.9 | 0.57 | † | NS | NS | NS |
| Total | | | | | | | | | | |
| Ruminal | 122.9 | 159.6 | 143.7 | 173.8 | 157.8 | 18.18 | * | NS | NS | NS |
| Omasal | 154.0 | 230.6 | 198.1 | 246.4 | 199.7 | 28.36 | * | NS | NS | NS |

[1] Control=grass silage+barley grain, SMP=grass silage+barley grain+skimmed milk powder, WDS=grass silage+barley grain+wet distiller's solubles, URSM=grass silage+barley grain+untreated rapeseed meal, TRSM=grass silage+barley grain+treated rapeseed meal.
[2] SEM = standard error of the mean. [3] C1=control vs. other diets; C2=SMP+WDS vs. URSM+TRSM; C3=SMP vs. WDS; C4=URSM vs. TRSM; NS; p>0.10, †; p<0.10, * p<0.05 and ** p <0.01, respectively.

(p<0.10) to increase molar proportion of acetate in RD. As compared to diet SMP, diet WDS had lower isobutyrate (p<0.001), isovalerate (p<0.01) and valerate (p<0.05), whereas the opposite was true for propionate (p<0.05). Protein supplements increased (p<0.01) valerate because of mainly the increase by SMP and WDS diets. No responses of rumen fermentation to chemical treatment to rapeseed meal are consistent with Khorasani et al. (1993) in which the acetate-treated rapeseed meal did not affect rumen pH, ammonia N and VFA concentration compared with untreated RSM.

**Quantification of SNAN flow**

Flow of SNAN fractions in the liquid phase of RD and OD is given in Table 5. Protein supplements significantly increased free AA flow in RD (p<0.05) and OD (p<0.01). Relatively high flow of free AA in both digesta is inconsistent with Chen et al. (1987c) and Nolan (1993) where free AA concentration in RD was low even immediately post-feeding. In addition, a recent study (Choi et al., 2002) also showed relatively low flow of free AA (mean 22.2 g/d) in OD. However, these values of free AA flow analysed using NHA are partly incorrect because of the terminal amino groups of peptides reacting on ninhydrin. Assuming that peptides in digesta in the present study were mainly in the form of short peptides, N materials as free AA analysed using NHA may have been somewhat overestimated. Alternatively, peptide N may be

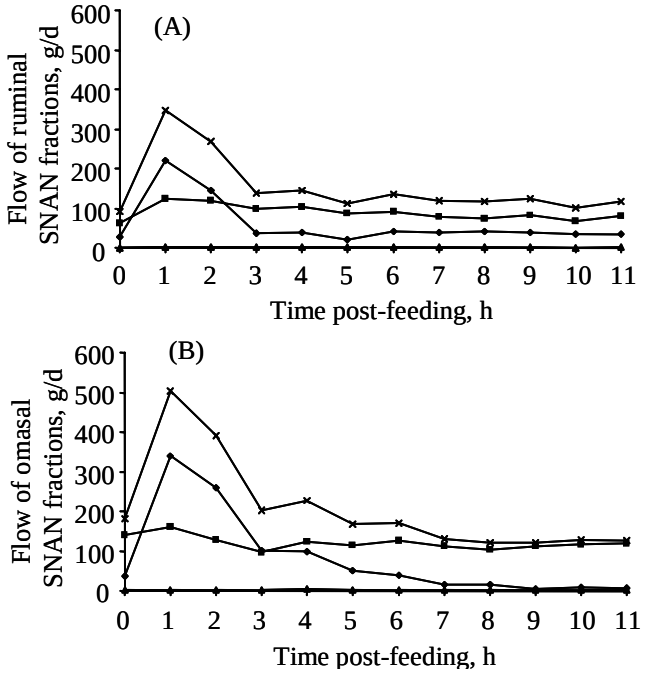


**Figure 1.** Diurnal pattern in flow of soluble non-ammonia nitrogen (SNAN) fractions in the liquid phase of ruminal (A) or omasal digesta (B) during a 12 h feeding cycle (Markers indicate free amino acid (♦), peptide (■), soluble protein (▲) and total (×), respectively).

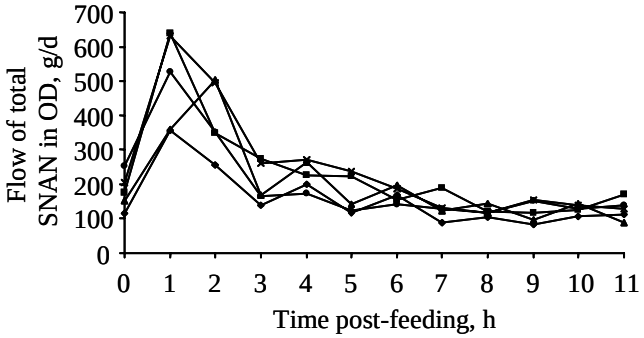


**Figure 2.** Diurnal pattern in flow of total soluble non-ammonia nitrogen (SNAN) in the liquid phase of omasal digesta (OD) during a 12 h feeding cycle as influenced by various protein supplements (Markers indicate no supplement (♦), skimmed milk powder (■), wet distiller's solubles (▲), untreated rapeseed meal (×) and treated rapeseed meal (●), respectively).

underestimated to the same extent.

Although feeding protein supplements did not affect flow of peptide in both digesta, the peptide flow was numerically increased except for diet TRSM. Treatment of rapeseed meal tended ($p<0.10$) to decrease ruminal peptide flow. Peptide fraction averaging 89.4 g/d for RD and 121.5 g/d for OD, respectively, was quantitatively the greatest in SNAN fractions. The present result is consistent with Chen et al. (1987c) and Robinson and McQueen (1994) for RD and with Choi et al. (2002) for OD. Therefore, this clearly indicates that the previous observation that hydrolysis of protein to peptide or deamination is the rate-limiting step in rumen proteolysis (Wright and Hungate, 1967; Tamminga, 1979; Van Straalen and Tamminga, 1990) is not valid. Instead, the current result confirms our suggestion (Choi et al., 2002) that degradation of peptide to AA may be the rate-limiting step in rumen proteolysis.

According to Chen et al. (1987b), hydrophobicity of peptide in RD may influence SNAN flow escaping the rumen. Peptides including hydrophobic AA may have more opportunities to accumulate in and flow out from the rumen while rumen microbes primarily degrade peptides containing hydrophilic AA (Chen et al., 1987b; Russell et al., 1991). In the present study, hydrophobic AA intake for diet SMP was calculated to be higher than other diets, but the flow of peptides in OD for diet SMP was similar to that for diet WDS and even lower than that for diet URSM. This discrepancy may be explained by ratio of intake of hydrophobic to hydrophilic AA. The ratio of hydrophobic to hydrophilic AA intake for diet SMP was similar to that for control diet (1.10 vs. 1.11). The ratio of other diets was 1.15, 1.16 and 1.16 for WDS, URSM and TRSM diets, respectively. Therefore, it is concluded that high ratio of hydrophobic to hydrophilic AA intakes may be closely associated with high flow of peptides (or SNAN) in digesta even though high intake of hydrophobic AA in diets is necessarily required. However, more *in vivo* experiments should be directed toward investigation of factors including hydrophobic and hydrophilic AA affecting SNAN flow in digesta, because kinetic of peptides (or SNAN) in the rumen could be more related to peptide size than the hydrophobicity (Chen et al., 1987c).

Flow of soluble protein was extremely low in RD (mean 2.1 g/d) and OD (2.5 g/d). Proportion of soluble protein flow in total SNAN flow was 1.4% for RD and 1.2% for OD. This is inconsistent with previous results of relatively high proportion of soluble protein in total SNAN flow in the rumen (8.6%; Robinson et al., 1998) and entering into the omasum (12.1%; Choi et al., 2000). The relatively low flow of soluble protein has probably resulted from soluble protein being rapidly degraded to peptides that are not precipitated.

**Diurnal pattern in SNAN flow**

Diurnal patterns in mean flow of SNAN fractions were similar between RD and OD (Figure 1), and the flow of SNAN fractions was higher for OD than RD (Table 5). Therefore, effects of protein supplements on diurnal patterns in the flow of SNAN fractions are shown based on OD (Figures 2 to 5). Protein supplementation produced relatively higher patterns in total SNAN flow than control diet (Figure 2). The diurnal patterns in total SNAN flow peaked at 1 h, declined by 3 h after feeding and remained

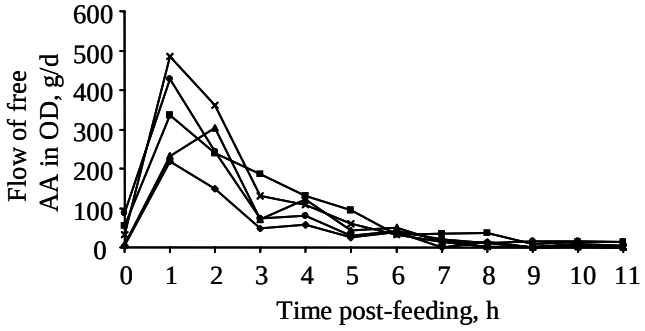


**Figure 3.** Diurnal pattern in flow of free amino acid (AA) in the liquid phase of omasal digesta (OD) during a 12 h feeding cycle as influenced by various protein supplements (Markers indicate no supplement (♦), skimmed milk powder (■), wet distiller's solubles (▲), untreated rapeseed meal (×) and treated rapeseed meal (●), respectively).

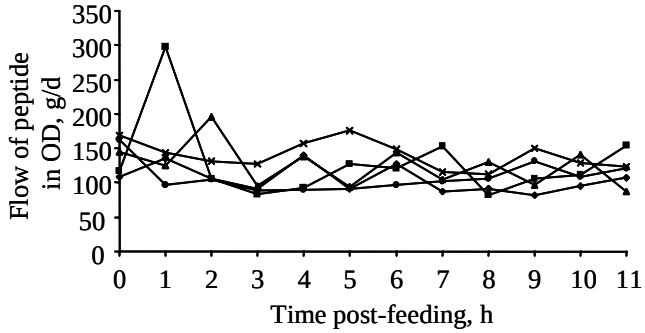


**Figure 4.** Diurnal pattern in flow of peptide in the liquid phase of omasal digesta (OD) during a 12 h feeding cycle as influenced by various protein supplements (Markers indicate no supplement (♦), skimmed milk powder (■), wet distiller's solubles (▲), untreated rapeseed meal (×) and treated rapeseed meal (●), respectively).

relatively constant thereafter. When cows consumed diet WDS, a peak of total SNAN flow appeared at 2 h after feeding.

Diurnal patterns in free AA flow were relatively similar to those in total SNAN flow (Figure 3). Free AA flow was lower for control than protein-supplemented diets at 1 to 3 h after feeding. Consistent with the present result, Broderick and Wallace (1988) showed free AA concentration reaching a maximum immediately after feeding and declined rapidly in RD of sheep fed ryegrass hay and concentrate supplemented with casein or urea. In contrast, a recent study showed no peaks of free AA flow throughout a 12 h feeding period even when cows were fed grass silage supplemented with rapeseed meal (CP 16.6%; C. W. Choi, unpublished data). The highest peak appeared 1 h post-feeding when cows consumed diet URSM followed by diet TRSM. Free AA flow for control and WDS diets at 1 h were similar, but that for diet WDS appeared to be higher than control at 2 h after feeding.

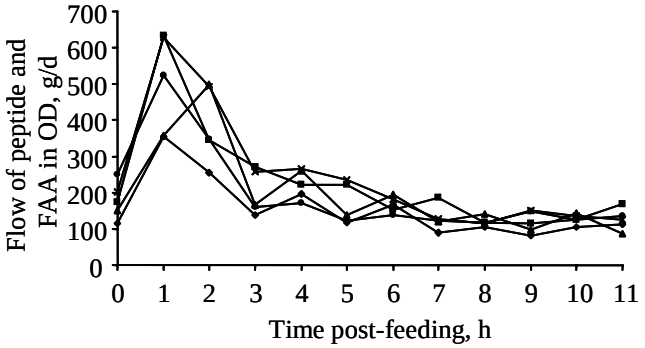


**Figure 5.** Diurnal pattern in flow of peptide and free amino acid (FAA) in the liquid phase of omasal digesta (OD) during a 12 h feeding cycle as influenced by various protein supplements (Markers indicate no supplement (♦), skimmed milk powder (■), wet distiller's solubles (▲), untreated rapeseed meal (×) and treated rapeseed meal (●), respectively).

Peptide flow did not show a clear peak during a 12 h feeding period except for diet SMP having a peak 1 h post-feeding (Figure 4), however the mean flow of peptide was highest 1 h post-feeding (Figure 1). Consistent with diurnal pattern in free AA flow, peptide flow for diet WDS was highest at 2 h after feeding. Diurnal pattern in peptide flow in the present study is inconsistent with previous studies (Chen et al., 1987c; Robinson et al., 1998; Choi et al., 2002) in which the diurnal pattern peaked at 1 to 2 h, and declined thereafter or declined by 3 to 5 h after feeding and remained constant thereafter. However, when ryegrass hay and maize based concentrate were fed with urea or ovalbumin, diurnal pattern in peptide concentration in RD was relatively constant (Broderick and Wallace, 1988). Chen et al. (1987c) discussed that they analysed peptide concentration in RD in the absence of separation between peptide and free AA because the peptide fraction may contain low concentration of free AA. In the present study, we analysed net peptide flow excluding free AA. As discussed earlier, free AA flow in the present study may have been overestimated due to the terminal amino group of peptide reacting on ninhydrin, in particular if the peptide was in the form of di- and tri-peptides. In addition, mean peptide flow was the highest proportion of SNAN fractions in OD. Therefore, in the present study, diurnal patterns in flow of peptide including free AA fraction replaced those in net peptide flow (Figure 5). As expected, clear peaks appeared at 1 h after feeding, and the flow declined thereafter except for diet WDS showing a peak at 2 h after feeding. The diurnal pattern in the flow of peptide including free AA was consistent with previous results (Robinson and McQueen, 1994; Robinson et al., 1998).

Protein supplements did not affect diurnal pattern in soluble protein flow entering into the omasum (Data not shown). Soluble protein flow was extremely low, and

relatively constant throughout the feeding period. Inconsistent with the present results, some studies showed relatively clear peaks of soluble protein concentration at 1 to 3 h after feeding (Robinson and McQueen, 1994; Robinson et al., 1998), but not all (Robinson et al., 1998).

## IMPLICATIONS

Protein supplements increased the flow of soluble non-ammonia nitrogen in the liquid phase of digesta entering into the omasum of dairy cows fed grass silage based diets. Peptide was quantitatively the greatest nitrogen fraction in soluble non-ammonia nitrogen flow, suggesting that hydrolysis of peptide to amino acid is the rate-limiting step in rumen proteolysis. Diurnal pattern in omasal flow of peptide including free AA peaked at 1 h and declined thereafter. Methodologically, the present omasal sampling system can provide an opportunity for the accurate quantification of 'actual' rumen-escapable nitrogen flow in the liquid phase of digesta.

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