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Effects of type of grass silage and level of concentrate on the flow of soluble non-ammonia nitrogen entering the omasum of dairy cows*

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ABSTRACT

Four ruminally cannulated Finnish-Ayrshire dairy cows were used to study effects of type of grass silage (primary vs secondary) and level of concentrate supplementation on concentration and the flow of soluble non-ammonia N (SNAN) in the liquid phase of digesta entering the omasum. The treatments in a 4 x 4 Latin square design consisted of either primary growth of grass silage (PG) or secondary growth of grass silage (SG) each with either 8.1 or 12.1 kg/d of concentrate (air dry basis). Digesta entering the omasum was collected, and SNAN fractions (free amino acids, peptides and soluble protein) in the digesta were assessed using ninhydrin. The microbial contribution to SNAN was estimated using ^{15}N as a microbial marker. Concentrations of free amino acids, peptides and soluble protein N averaged 22.3, 59.8 and 23.1 mg N/l, respectively. PG diets tended (P=0.09) to increase peptide N concentration in omasal digesta compared with SG diets, whereas level of barley concentrate did not affect concentrations of SNAN fractions. Peptide N constituted the largest proportion of SNAN in omasal digesta, supporting the previous observation that hydrolysis of peptides to AA is the most limiting step in rumen proteolysis. The microbial contribution to SNAN averaged 0.71, indicating that a substantial proportion of the SNAN was of microbial origin. Soluble dietary NAN flow averaged 9.1 g N/d, accounting for approximately 0.05 to 0.08 of total dietary NAN flow.

KEY WORDS: dairy cows, grass silage, soluble non-ammonia nitrogen, omasum, ^{15}N

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INTRODUCTION

Ruminal digesta contains large amounts of soluble non-ammonia, non-protein N that is not precipitated by trichloroacetic acid (Winters et al., 1964). This fraction, mainly peptide N, often accumulates in rumen fluid *in vitro* (Russell et al., 1983) and *in vivo* (Chen et al., 1987a; Williams and Cockburn, 1991). Our recent studies also showed that peptide N constituted the highest proportion in soluble non-ammonia N (SNAN) in omasal digesta and escaped ruminal degradation (Choi et al., 2002a,b).

The distribution of grass silage N fractions is influenced by stage of maturity (Rinne et al., 1997), type of additive (Nsereko et al., 1998) and harvest time of grass (McAllan et al., 1994). The changes in the distribution of N fractions may affect ruminal digestion and provision of nutrients from the rumen. McAllan et al. (1994) reported higher flow of non-ammonia N (NAN) at the duodenum of dairy cows fed early cut grass silage compared with late cut grass silage. Ahvenjärvi et al. (1999) observed that proportionately 0.22 of NAN from the rumen was in the form of non-protein N (NPN) and soluble protein when primary growth grass silage (PG) was fed with a supplement of barley concentrate. When secondary growth grass silage (SG) was fed to dairy cows, relatively high concentration of SNAN, accounting for 97 mg N/l of omasal digesta, flowed out from the rumen, whereas barley supplementation did not affect the SNAN escape from the rumen (Choi et al., 2002a).

The rate and extent of protein degradation in the rumen depend on several factors, e.g., proteolytic activity, retention time of protein in the rumen, microbial species, source of protein and rumen pH (Nolan, 1993). Usually proteins of cereal grain are completely degraded when given a prolonged period (Nolan, 1993). Increasing intake of barley grain in the diet was associated with decreased rumen pH (Jaakkola and Huhtanen, 1993), which subsequently could reduce proteolytic activity (Nolan, 1993). Thus, increasing intake of barley grain may affect the extent of protein-escape as SNAN from ruminal degradation. However, high intake of barley grain is associated with long rumen retention time and with low passage rates of both liquid and particle flow from the rumen (Jaakkola and Huhtanen, 1993).

The present experiment aimed to study the effects of harvest date of grass used for silage (PG vs SG) and level of concentrate supplementation on concentration and flow of SNAN in the liquid phase of omasal digesta escaping the rumen of dairy cows. We also determined microbial contribution to SNAN using $^{15}NH_4$-sulphate as a microbial marker. Data on nutrient flow and subsequent animal responses have been reported in companion paper (Korhonen M., Vanhatalo A. and Huhtanen P., unpublished).

MATERIAL AND METHODS

Experimental procedures

Four multiparous rumen cannulated Finnish-Ayrshire dairy cows averaging 71 days in milk (SD=12) and 607 kg body weight (SD=53) were used in a 4 x 4 Latin-square with a 2 x 2 factorial arrangement. The four treatments consisted of either PG or SG each with concentrate consisting on air dry basis of 2.1 kg of rapeseed meal and either 6.0 kg (L) or 10.0 kg (H) of coarsely milled barley daily. Grass silage was made of predominately timothy grass (*Phleum pratense*) and meadow fescue (*Festuca pratensis*) sward. The grass was cut with a disk mower and harvested after a short wilting period with a precision-chop forage harvester. The primary growth grass was harvested on 16th of June and the secondary growth grass from the same field plot 52 days later. At ensiling, a formic acid-based additive (AIV 2000; formic acid 55%, ammonium formate 24%, propionate 5%, ethyl benzoate 1%, benzoic acid 1%; Kemira Chemicals Ltd., Helsinki, Finland) was applied at a rate of 5.1 for PG or of 6.1 l/tonne for SG. More additive was used for the secondary growth grass relative to the primary growth grass because of the lower dry matter (DM) content of the secondary growth grass (200 vs 250 g/kg). A 300 g of mineral mixture (Suomen Rehu Ltd., Helsinki, Finland) was given daily. Cows were housed individually and had continuous access to drinking water and salt block. Each experimental period lasted for 28 days including 4 days for omasal sampling. Grass silage was fed *ad libitum* and concentrates were offered twice daily at 06.00 and 18.00 h. Refusals and DM intake of feeds were recorded daily. The cows were milked at 07.00 and 16.30 h.

Sampling and chemical analyses

Samples of grass silage and concentrate feeds were collected over the last 4 days of each 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). Soluble N fractions (NPN and soluble true protein N) of feeds were analysed as described by Licitra et al. (1996). Free AA and peptide in feed N fractions were determined using the ninhydrin assay (NHA) (Choi et al., 2002a) while ammonia was analysed using a colorimetric method (McCullough, 1967).

Rumen fluid was sampled before the morning feeding and 1.5, 3.0, 4.5, 6.0, 7.5 and 9.0 h thereafter, and analysed for rumen pH and ammonia N concentration. To assess digesta flow into the omasum, indigestible neutral-detergent fibre, Yb-acetate and LiCo-EDTA were used as markers for large particle, small particle and liquid phase, respectively, using the triple-marker method described by France and Siddons (1986). Details of the procedure for the digesta markers have been described by Ahvenjärvi et al. (2002). Digesta samples were collected from the omasum and analysed for flows of

total NAN (TNAN), total microbial NAN (TMNAN) and total dietary NAN (TDNAN) into the omasum as described by Ahvenjärvi et al. (2000).

To estimate the flow of SNAN fraction in the liquid phase of digesta, digesta entering the omasum was sampled according to Choi et al. (2002b). In brief, omasal digesta was collected using the omasal sampling method described by Huhtanen et al. (1997), with three modifications as follows: 1. larger sampling tube (14 vs 9.5 mm i.d.), 2. solenoid valves replaced a three-way ball valve to control system vacuum and pressure phases and 3. a 0.5 kg-weight was inserted into the abomasum to secure the placement of the sampling device in the omasal canal. Approximately 30 ml of digesta was collected at 4 h intervals during a 12h feeding cycle on day 25. On subsequent sampling day, the time of sampling was advanced by 1h relative to the previous sampling day. Totally 12 digesta samples during 4 days were collected. Free AA, peptide and soluble protein N in the liquid phase of omasal digesta were assessed using the NHA. Details of digesta sample treatment and the NHA have been described (Choi et al., 2002a), with the additional step that supernatant after trichloroacetic acid-precipitation was treated using 10 N NaOH and heated to eliminate ammonia N prior to the NHA. Amino acid compositions of the experimental feeds and omasal free AA fraction were determined using the AA analyser (Biochrom 20, AA analyser, Autoloader version, Pharmacia Biotech (Biochrom) Ltd., Cambridge, UK).

Microbial contribution to SNAN in the liquid phase of omasal digesta was estimated using $^{15}NH_4$-sulphate as a microbial marker. The natural enrichment of ^{15}N in the omasal digesta averaged 0.3663 atom%. The microbial contribution to the SNAN in the liquid phase of omasal digesta was analysed as previously described by Choi et al. (2002a) with the modification that oven-drying (60ºC for 48 h) instead of freeze-drying was used. The ^{15}N from ammonia N in the omasal digesta was not considered in the calculation of $NA^{15}N$ since a preliminary analysis of alkaline-heating step eliminated over 99% residual ammonia N in the sample of omasal digesta. The ^{15}N-atom% in rumen microbes was analysed from mixed bacteria, instead of liquid-associated bacteria, collected from a centrifugation of digesta (Ahvenjärvi et al., 2000). The proportion of microbial contribution to the omasal SNAN was calculated as (^{15}N-atom% excess in the sample of SNAN - the natural ^{15}N-atom% excess) / (^{15}N-atom% excess in rumen bacteria - the natural ^{15}N-atom% excess).

Statistical analysis

Data on feed N intake and liquid, TNAN, TMNAN and TDNAN flow were analysed using the MIXED procedure of SAS (Littell et al., 1998) according to the following statistical model:

$$Y_{ijk} = \mu + A_i + P_j + G_k + C_l + (S \times C)_{kl} + e_{ijkl} \quad 1)$$

where A, P, G, C and S x C are animal, period, grass silage and concentrate effects and interaction between silage and concentrate, respectively.

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

$$Y_{ijkl} = \mu + A_i + P_j + G_k + C_l + (S \times C)_{kl} + e_{ijkl} + T_m + (A \times T)_{im} + (P \times T)_{jm} + (G \times T)_{km} + (C \times T)_{lm} + (G \times C \times T)_{klm} + e_{ijklm} \quad 2)$$

where T is time effect, and A x T, P x T, G x T, C x T and G x C x T are animal by time, period by time, silage by time, concentrate by time and silage by concentrate by time interactions, respectively. Animal effect, animal by time interaction and error terms (e_{ijkl} defined as between unit error and e_{ijklm} as within unit error) are multivariate normally distributed random effects with AR (1) covariance structure.

RESULTS

Feed composition and DM intake

The chemical composition and soluble N fractions of experimental feeds are shown in Table 1. Since the formic acid-based additive contained ammonium

TABLE 1

Chemical composition of experimental feeds

Indices	Primary grass silage	Secondary grass silage	Barley	Rapeseed meal
Dry matter (DM), g/kg	265	232	884	883
Component, g/kg DM				
organic matter	934	908	974	925
nitrogen	18.7[a]	17.7[a]	19.7	61.0
neutral detergent fibre	547	491	189	258
Feed soluble N fractions, g/kg total N				
NPN	658	482	157	117
ammonia	43.5	38.8	0.9	0.7
free amino acids	427	279	77	55
peptide	188	164	79	61
soluble true protein N	53	47	177	169
Silage fermentation quality				
pH	4.08	4.07		
lactic acid, g/kg DM	53.5	41.4		
acetic acid, g/kg DM	15.8	14.6		
water soluble carbohydrate, g/kg DM	66.8	97.6		
soluble N, g/kg total N	727	561		

[a] data were corrected for N derived from a formic acid-based additive

formate, total N, ammonia N and soluble N in silage were corrected for N present in the additive. Proportion of soluble N in feed total N was much higher for PG than for SG. Proportion of free AA in silage NPN was higher in PG than in SG. As for silage fermentation quality, pH did not differ between PG and SG. Both grass silages contained relatively low concentrations of fermentation acids and a low proportion of ammonia N in total N. Barley and RSM contained relatively high proportion of soluble true protein N but extremely low proportion of ammonia N. Proportions of free AA and peptide N in total N of barley and RSM were relatively similar. The NPN in all feeds was mainly in the forms of free AA and peptide N.

The AA composition of experimental feeds is shown in Table 2. The proportions of individual AA in total CP between PG and SG were similar. However, lysine and glutamic acid were higher for PG than for SG, whereas the opposite was true for arginine and tyrosine.

TABLE 2

Amino acid (AA) composition of experimental feeds

Amino acids	Feeds			
	primary grass silage	secondary grass silage	barley	rapeseed meal
AA, g/kg crude protein				
arginine	21.0	33.1	48.7	58.3
histidine	11.6	12.3	18.5	25.7
isoleucine	39.1	36.4	33.7	38.3
leucine	69.7	68.4	65.9	69.2
lysine	37.2	24.0	35.4	51.4
methionine	18.1	15.2	17.3	20.4
phenylalanine	42.2	43.8	46.5	38.4
threonine	38.1	36.7	34.8	43.3
valine	54.4	50.9	50.4	45.5
alanine	61.5	57.6	40.2	42.4
aspartic acid	77.0	76.0	58.8	74.4
cystine	11.3	8.8	28.1	19.4
glutamic acid	78.6	74.5	201.3	157.0
glycine	44.2	45.6	40.8	49.1
proline	46.5	45.0	93.5	56.9
serine	34.1	33.8	39.5	40.4
taurine	2.7	4.0	5.8	0.8
tyrosine	13.9	22.4	32.3	29.5
Total AA	702	689	891	860

Silage DM intake was significantly ($P = 0.002$) higher for PG compared with SG diets (Table 3). Increasing the amount of barley in the diet increased total DM ($P = 0.004$) and N intakes ($P = 0.007$) but reduced grass silage DM intake ($P = 0.04$).

TABLE 3

Effect of grass silage type and level of concentrate on intake of dietary ingredients

Indices	Diet[a]				SEM[b]	Statistical significance[c]		
	PG		SG					
	L	H	L	H		S	C	S x C
Dry matter (DM) intake, kg/d								
grass silage	10.8	9.9	9.0	8.1	0.51	0.002	0.04	0.95
concentrate[d]	7.2	9.8	7.3	10.4	0.28	0.10	<0.0001	0.18
total	18.0	19.7	16.2	18.5	0.55	0.01	0.004	0.49
Nitrogen intake, g/d	425	453	386	431	11.5	0.02	0.007	0.39

[a] PG = primary growth grass silage; SG = secondary growth grass silage; L = low level of concentrate; H = high level of concentrate

[b] SEM = standard error of the mean

[c] S = effect of grass silage; C = effect of concentrate; S x C = interaction between grass silage and concentrate

[d] concentrate consisted of barley, rapeseed meal and mixture of mineral and vitamin. The mixture of mineral and vitamin contained (per kg DM) Ca 160 g, P 64 g, Na 90 g, Mg 80 g, Cu 530 mg, Se 20 mg, Zn 4200 mg, Mo 20 mg, Co 15 mg, Mn 2250 mg, I 140 mg, vit. A 150,000 IU, vit. D 100,000 IU, vit. E 950 mg

Rumen pH, ammonia N and flow measurement

Mean rumen pH was higher (P = 0.03; data not shown) for L (6.63) than for H diets (6.47). Compared with SG diets, PG diets had significantly higher rumen ammonia N concentration (P<0.05; SEM = 1.40) during a feeding cycle (Figure 1). Ammonia N concentration peaked at 3.0 h and 10.5 h post feeding.

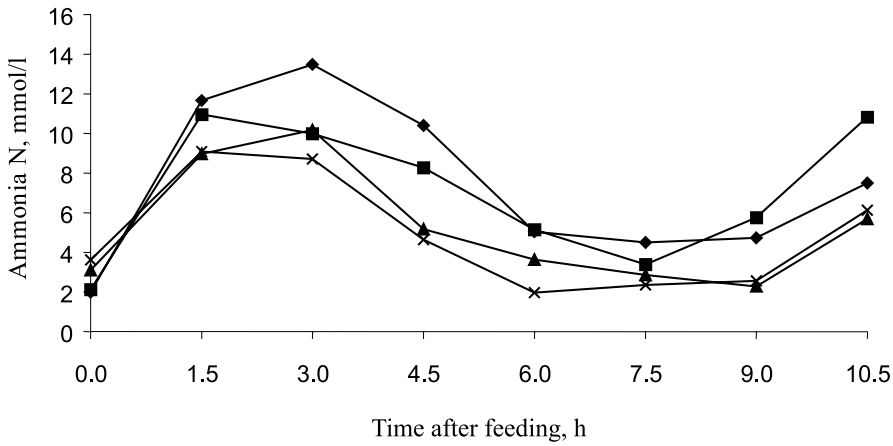

Figure 1. Diurnal variation in ammonia N concentration in rumen fluid of dairy cows fed primary or secondary growth grass silage with 8.1 kg (◆ or ▲) or with 12.1 kg concentrates (■ or x)

Data on liquid and N flow into the omasum are given in Table 4. The liquid flow was not significantly affected by dietary treatments, but it tended (P = 0.14) to be higher for SG than for PG diets (284 vs 269 l/d). Neither the silage type nor the level of concentrate significantly (P>0.05) affected TNAN (mean 445 g N/d) and TDNAN flow (mean 144 g N/d) into the omasum. Compared with L diets, H diets significantly (P = 0.01) increased TMNAN flow.

TABLE 4

Effect of grass silage type and level of concentrate on liquid and nitrogen flow entering the omasum

Indices	Diet[a]				SEM[b]	Statistical significance[c]		
	PG		SG					
	L	H	L	H		S	C	S x C
Flow into the omasal canal								
liquid, l/d	262	276	280	287	9.8	0.14	0.27	0.66
total NAN[d], g N/d	420	438	444	477	22.5	0.14	0.21	0.71
TMNAN[d]	279	302	289	335	11.3	0.07	0.01	0.29
TDNAN[d]	141	136	155	142	12.8	0.43	0.47	0.73

[a] PG = primary growth grass silage; SG = secondary growth grass silage; L = low level of concentrate; H = high level of concentrate

[b] SEM = standard error of the mean

[c] S = effect of grass silage; C = effect of concentrate; S × C = interaction between grass silage and concentrate

[d] total NAN = total non-ammonia N; TMNAN = total microbial NAN; TDNAN = total dietary NAN

Soluble NAN entering the omasum

Quantification of SNAN. Concentration and flow of SNAN fractions in the liquid phase of omasal digesta are given in Table 5. There were not significant interactions between grass silage type and level of concentrate in the concentration or flow of SNAN fractions in the liquid phase of omasal digesta.

Although there were not significant differences in the SNAN fractions between dietary treatments, PG diets resulted in numerically higher concentration of SNAN fractions than SG diets. Except for free AA for PG diets, concentrations of SNAN fractions were slightly higher for L than for H diets, but the differences were not statistically significant. Peptide N concentration in omasal digesta tended (P = 0.09) to be higher for PG than for SG diets (65.4 vs 54.3 mg N/l).

There were not significant differences in the flow of SNAN fractions between dietary treatments (P>0.05). However, peptide N flow for PG diets (17.6 g N/d) was numerically higher than that for SG diets (15.5 g N/d). Proportionately 0.57 of SNAN flow was in the form of peptides, whereas free AA and protein N accounted for 0.21 and 0.22 of SNAN, respectively. Little differences (P>0.05) in the flow of SNAN fractions between L and H diets were observed.

TABLE 5

Effect of grass silage type and level of concentrate on concentration and estimated flow of 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 omasum

	Diet[a]				SEM[b]	Statistical significance[c]		
	PG		SG					
	L	H	L	H		S	C	S x C
Concentration	mg N/l							
SNAN								
free amino acids	22.3	26.1	21.3	19.3	2.84	0.21	0.76	0.34
peptide	68.5	62.2	54.9	53.6	7.45	0.09	0.51	0.65
protein	24.3	22.3	24.5	21.3	2.47	0.88	0.31	0.81
total	115.1	110.5	100.7	94.3	9.92	0.17	0.59	0.93
SMNAN								
total	76.9	73.3	71.2	68.1	6.64	0.43	0.63	0.97
SDNAN								
total	38.3	37.3	29.6	26.2	4.93	0.09	0.67	0.82
Flow	g N/d							
SNAN								
free amino acids	5.8	7.2	5.9	5.5	0.70	0.31	0.50	0.24
peptide	17.9	17.2	15.5	15.5	2.27	0.16	0.78	0.80
protein	6.4	6.1	6.8	6.1	0.65	0.70	0.43	0.74
total	30.2	30.5	28.3	27.1	2.86	0.28	0.86	0.75
SMNAN								
total	20.2	20.2	19.8	19.6	1.95	0.79	0.97	0.95
SDNAN								
total	10.0	10.3	8.5	7.5	1.38	0.13	0.80	0.62

[a] PG = primary growth grass silage; SG = secondary growth grass silage; L = low level of concentrate; H = high level of concentrate

[b] SEM = standard error of the mean

[c] S = effect of grass silage; C = effect of concentrate; S x C = interaction between grass silage and concentrate

The microbial contribution to SNAN in the liquid phase of digesta entering the omasum tended ($P = 0.06$; data not shown) to be lower for PG than SG diets (mean 0.69 vs 0.73). The concentration of dietary SNAN tended ($P= 0.09$) to be higher for PG compared with SG diets. Neither the silage type nor the level of concentrate affected ($P>0.05$) microbial and dietary SNAN flow into the omasum.

Proportion of N fractions in SNAN. The proportion of free AA, peptide, protein and total SNAN in TNAN averaged 13.9, 37.2, 14.5 and 65.6 g/kg, respectively, and was not different (P>0.05) among dietary treatments (Table 6). The proportion of peptide N in TNAN of omasal digesta was substantially higher than the other two fractions (free AA and soluble protein), and it tended (P = 0.09) to be higher for PG than for SG diets. Except for free AA, the proportions of all SNAN fractions in TNAN flow were slightly higher for L than H diets, but the differences were not statistically significant. Based on the ^{15}N-enrichment in the liquid phase of omasal digesta, the proportions of microbial and dietary SNAN averaged 45.1 and 20.5 g/kg TNAN, respectively. The proportion of microbial SNAN in TMNAN averaged 66.4 g/kg. The proportion of dietary SNAN in TDNAN tended (P = 0.09) to be higher for PG than SG diets (mean 75.7 vs 53.7 g/kg). There were not significant (P>0.05) interactions between grass silage type and level of concentrate in the proportion of microbial and dietary SNAN in TNAN flow into the omasum.

TABLE 6

Effect of grass silage type and level of concentrate on the proportion 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) flow entering the omasum

Indices	Diet[a]				SEM[b]	Statistical significance[c]		
	PG		SG					
	L	H	L	H		S	C	S x C
SNAN in total NAN	g/kg							
free amino acids	13.9	16.5	13.4	11.7	1.92	0.22	0.80	0.30
peptide	42.6	39.2	34.8	32.0	4.46	0.09	0.44	0.93
protein	15.2	14.4	15.6	12.9	1.68	0.73	0.29	0.55
total	71.7	70.1	63.8	56.7	6.09	0.11	0.49	0.66
SMNAN								
proportion in total NAN	47.8	46.6	45.0	41.0	3.95	0.31	0.52	0.73
proportion in TMNAN[d]	71.8	67.1	68.5	58.2	4.95	0.25	0.16	0.57
SDNAN								
proportion in total NAN	23.9	23.6	18.8	15.7	3.07	0.07	0.59	0.66
proportion in TDNAN[d]	72.7	78.6	54.3	53.0	11.56	0.09	0.85	0.76

[a] PG = primary growth grass silage; SG = secondary growth grass silage; L = low level of concentrate; H = high level of concentrate

[b] SEM = standard error of the mean

[c] S = effect of grass silage; C = effect of concentrate; S x C = interaction between grass silage and concentrate

[d] proportions of SMNAN and SDNAN expressed as g/kg total microbial NAN (TMNAN) and g/kg total dietary NAN (TDNAN), respectively

Composition of omasal free AA fraction. The proportions of individual AA in free AA fraction in the liquid phase of omasal digesta are shown in Table 7.

The proportion of lysine was significantly (P = 0.01) higher whereas tyrosine was lower (P = 0.009) when PG was fed. Quantitatively glutamic acid and alanine were the most important AA in the free AA fraction entering the omasum, followed by lysine, valine and alanine. Concentration of glutamic acid was higher (P = 0.24) for SG than for PG diets. Concentration of omasal free AA determined using the AA analyser averaged 12.9 mg N/l.

TABLE 7

Effect of grass silage type and level of concentrate on amino acid (AA) composition of free AA fraction in the liquid phase of omasal digesta

Amino acids	Diet[a]				SEM[b]	Statistical significance[c]		
	PG		SG					
	L	H	L	H		S	C	S x C
Free AA, g/kg of total free AA								
arginine	44.2	32.9	45.1	36.7	8.26	0.77	0.24	0.86
histidine	23.3	20.0	18.5	21.7	1.84	0.44	0.99	0.13
isoleucine	28.1	34.7	22.9	25.3	7.87	0.39	0.59	0.80
leucine	51.4	64.1	40.7	49.2	17.05	0.48	0.56	0.91
lysine	84.4	89.8	59.9	68.1	6.82	0.01	0.36	0.84
methionine	2.5	5.6	1.8	2.1	1.39	0.19	0.26	0.36
phenylalanine	31.0	39.2	29.7	34.8	7.73	0.71	0.40	0.84
valine	73.1	85.5	69.7	69.4	8.36	0.29	0.49	0.48
alanine	155.3	167.0	173.0	168.2	10.78	0.41	0.76	0.47
cystine	64.5	72.1	46.4	56.2	9.49	0.10	0.36	0.90
glutamic acid	319	266	375	345	52.4	0.24	0.46	0.83
glycine	50.3	53.3	48.2	50.4	6.03	0.69	0.67	0.95
ornithine	22.6	27.3	12.4	21.1	6.54	0.26	0.34	0.77
proline	48.3	40.7	53.4	48.4	6.99	0.40	0.40	0.86
tyrosine	2.1	2.0	3.1	3.3	0.31	0.009	0.92	0.76
Total free AA[d]	79.9	93.5	68.8	79.3	17.07	0.49	0.50	0.93

[a] PG = primary growth grass silage; SG = secondary growth grass silage; L = low level of concentrate; H = high level of concentrate

[b] SEM = standard error of the mean

[c] S = effect of grass silage; C = effect of concentrate; S × C = interaction between grass silage and concentrate

[d] expressed as mg/l of omasal digesta

Diurnal variation. Mean diurnal change in concentration of each SNAN fraction over the experimental diets is shown in Figure 2. Concentration of each SNAN fraction was influenced by sampling time (at least P<0.01), but the interactions between diets × sampling time were not significant (P>0.05). Mean concentration of peptide N peaked at 2 h post-feeding and approached pre-feeding level at 5 h post-feeding, while soluble protein N remained relatively constant throughout the

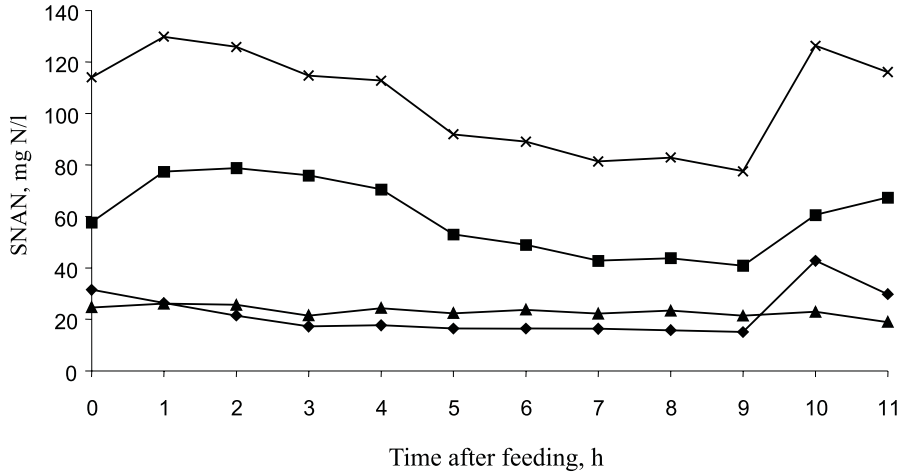

Figure 2. Diurnal variation in concentrations of soluble non-ammonia N (SNAN) fractions in the liquid phase of omasal digesta during a 12 h feeding cycle (◆ = free amino acid; ■ = peptide; ▲ = protein; x = total SNAN. (Standard error of the mean for free amino acid, peptide, protein and total SNAN were 3.37, 7.30, 1.69 and 8.26, respectively)

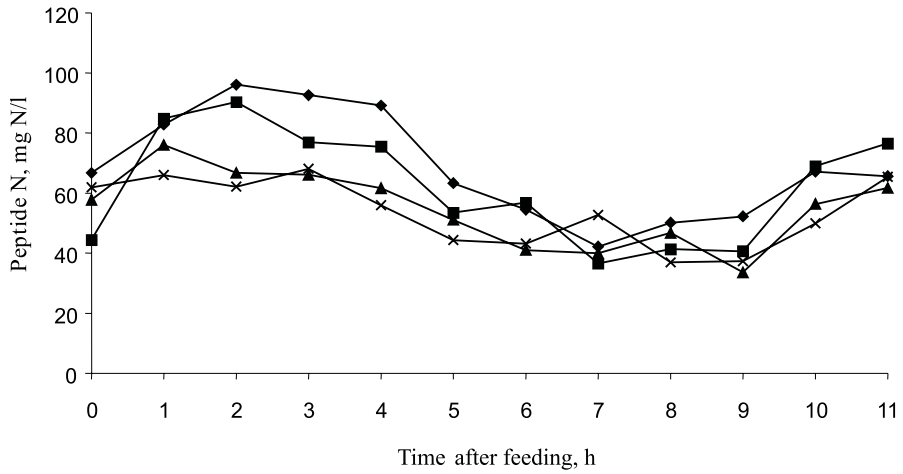

Figure 3. Diurnal variation in peptide N concentration in liquid phase of omasal digesta of dairy cows fed primary or secondary growth grass silage with 8.1 kg (◆ or ▲) or with 12.1 kg concentrates (■ or x) (Standard error of the mean for peptide N was 10.9)

feeding cycle. Another peak in peptide and free AA N appeared at 10-11 h post-feeding. Peptide N was substantially higher than free AA and protein N during the entire period post feeding (maximum 4 times at 1 - 3 h post-feeding).

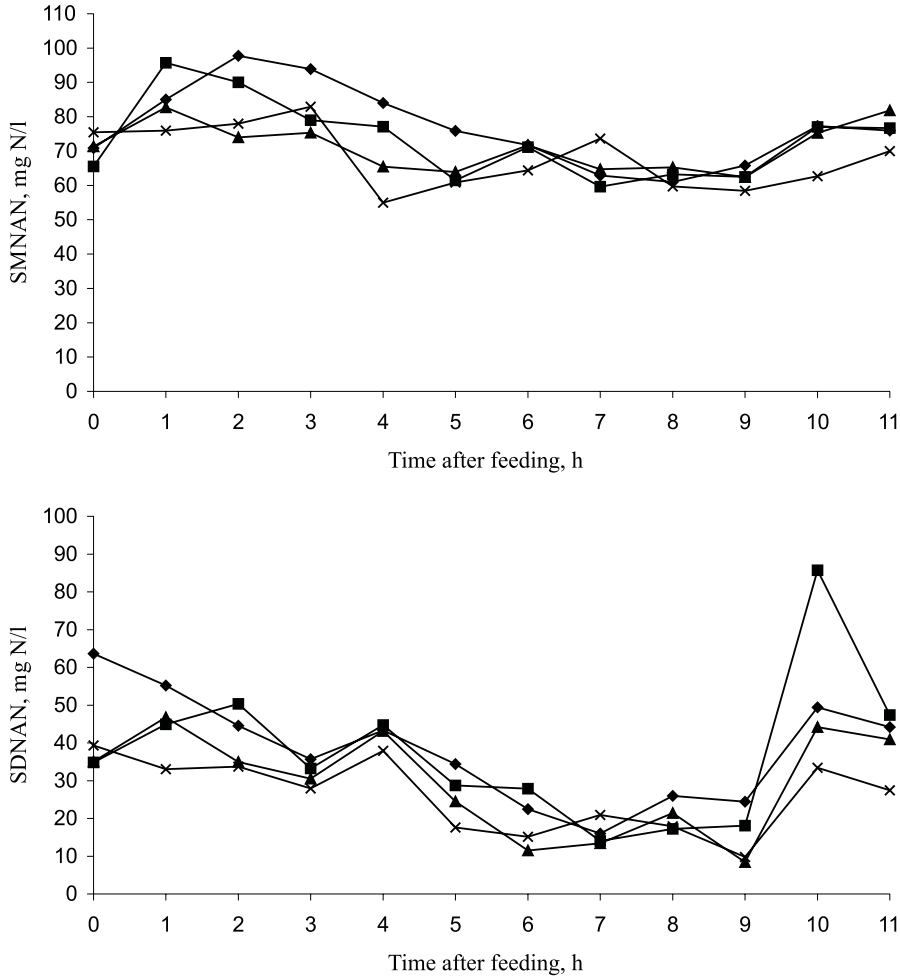

Figure 4. Diurnal variation in soluble non-ammonia N concentration from either microbial (SMNAN) or dietary origin (SDNAN) in the liquid phase of omasal digesta of dairy cows fed primary or secondary growth grass silage with 8.1 kg (◆ or ▲) or with 12.1 kg concentrates (■ or x) (Standard errors of the mean for SMNAN and SDNAN were 4.73 and 5.40, respectively)

Effect of dietary treatments on diurnal variation in peptide N concentration is presented in Figure 3. Diurnal changes in peptide N concentration appeared to be higher for PG than for SG diets. Level of concentrate did not affect diurnal variation in peptide N concentration, but the diurnal variation seemed to be higher for L than for H diets within same type of grass silage fed. Diurnal changes in concentrations of microbial and dietary SNAN during the feeding cycle are shown in Figure 4. The diurnal change in the microbial SNAN concentration was higher (silage x time, P

= 0.04) for PG than for SG diets. The highest dietary SNAN concentrations were observed during and immediately after the morning feeding and another peak at 10-11 h (time effect, P<0.001).

DISCUSSION

Soluble N distribution in feed and DM intake

Both grass silages ensiled with formic acid-based additive were restrictively fermented and of good quality, as indicated by low proportion of ammonia N in total N and the low concentration of total acids. Nsereko et al. (1998) reported that peptides in grass silage N treated with formic acid was 210 g/kg total N and was higher compared with untreated grass silage. Consistent with their study (Nsereko et al., 1998), the proportion of silage peptide N in the present study was relatively high (188 and 164 g/kg total N for PG and SG, respectively). Compared with PG, the lower concentration of NPN of SG in the present study was partially a result of slightly lower soluble N content in the secondary growth grass than the primary growth grass (328 vs 379 g/kg of total N; data not shown). Soluble N fractions in barley and RSM were similar to results obtained in previous studies (Vanhatalo et al., 1995; Ahvenjärvi et al., 1999; NRC, 2001; Choi et al., 2002b).

Higher grass silage intake resulted in higher total DM intake for PG compared to SG diets. Based on neutral detergent fibre content of silages in the present study, the DM intake would have been expected to be higher for SG diets. The differences in concentrations of fermentation acids and water soluble carbohydrate between silages are neither consistent with the higher DM intake for PG than SG diets, because DM intake of silage was negatively correlated with total fermentation acids and positively correlated with concentration of water soluble carbohydrate (Huhtanen et al., 2002).

Microbial N contribution to SNAN

The present microbial contribution to SNAN (mean 0.71) is consistent with the values of 0.61 to 0.86 reported earlier (Hristov and Broderick, 1996; Choi et al., 2002a, b). As expected, the microbial contribution to total NAN flow increased as the intake of barley increased (Table 4) because increased supply of fermentable organic matter provides substrate for an increased microbial growth (Jaakkola and Huhtanen, 1993). However, the present results indicated only minor differences between L and H diets in microbial NAN flow. This is probably because of microbial N recycling in the rumen due to a large number of protozoa in the rumen with increasing amount of starch concentrate in the diet (Jaakkola and Huhtanen, 1993).

The level of barley did not affect the microbial SNAN in the liquid phase of omasal digesta (Table 5). Instead, PG diets produced more dietary SNAN compared with SG diets. This may have resulted from a much higher intake of feed soluble NAN from PG (mean 130 g/d) compared to that from SG (74 g/d) (Tables 1 and 3).

It has been suggested that alanine and glutamic acid in free AA of ruminal digesta are mainly the intermediates from microbial metabolism (Wright and Hungate, 1967; Volden et al., 2001). In the present study, the free AA in omasal digesta consisted mainly of alanine and glutamic acid (Table 7). Furthermore, the proportion of glutamic acid in free AA in omasal digesta was numerically lower for H than for L diets, despite high intake of this AA (see Tables 2 and 3). Provided that the present free AA fraction was mainly from microbial metabolism, the present microbial contamination in the other N fractions, i.e. peptide and soluble protein, would be lower than the mean microbial contribution to SNAN (0.71). The SNAN concentration from microbial origin was much higher than the concentration of free AA N fraction (Table 5), thus a substantial proportion of peptide N must be of microbial origin. However, the current ^{15}N-enrichment in rumen microbes was analysed from mixed bacteria not from liquid-associated bacteria. Ahvenjärvi et al. (2002) showed slightly higher ^{15}N-enrichment in liquid-associated bacteria than that in particle-associated bacteria in rumen digesta of cows fed grass silage based diets. Thus, microbial contribution to omasal SNAN in the present study could be slightly overestimated (for calculations, see Material and Methods), assuming that microbial N in the liquid phase of omasal digesta has solely been derived from liquid-associated bacteria. The microbial lysis from acid treatment of digesta and the freezing after sampling could also have led to an overestimation of the microbial contribution.

Responses of dietary treatments to SNAN

Free AA N and soluble protein N. Concentration of free AA N determined by the AA analyser was proportionately only 0.58 of that determined using the NHA (see Tables 5 and 7). However, it does not mean errors in estimation of free AA N since the NHA reacts also with the terminal amino groups of peptides as well as those of free AA (Rosen, 1957). Further, close relationship ($R^2 = 0.91$; data not shown) between the NHA and the AA analyser in estimating free AA N clearly indicates that the NHA is a reliable method for estimating AA in digesta.

In the present study, neither silage type nor level of concentrate affected the free AA N fraction in omasal digesta. The high concentration of free AA N in omasal digesta was partially associated with a high proportion of free AA in silage soluble NAN. Inconsistent with the previous observation that level and type of concentrate could affect ruminal digestion (Dewhurst et al., 2000), the free AA N concentration in the present study did not differ between L and H diets (Table 5). It may be a result

of recycling of microbial N in the rumen caused by an increase in the number of protozoa as amount of barley in the diet increases (Jaakkola and Huhtanen, 1993). The barley supplementation in a previous study (Choi et al., 2002a) did not affect free AA N fraction in omasal digesta.

Consistent with previous studies (Robinson et al., 1998; Choi et al., 2002a), soluble protein N concentration was relatively low in the present study, and suggests that soluble proteins are rapidly degraded to peptides during protein degradation in the rumen. In addition, the low concentration of soluble protein N in the rumen may partly have resulted from low concentration of soluble true protein in silage total N ensiled with formic acid additive (Rinne et al., 1997). Relatively constant diurnal pattern of soluble protein N concentration during the entire feeding cycle is in good agreement with several other studies (Chen et al., 1987a; Robinson et al., 1998) as well as our recent study (Choi et al., 2002a).

Peptide N. In the present study, peptide N concentration (59.8 mg N/l) in omasal digesta was lower than that reported in previous studies (82-111 mg N/l; Chen et al., 1987a; Robinson and McQueen, 1994; Robinson et al., 1998). However, the peptide N concentration in the previous studies has been obtained in the absence of free AA and peptide separation. Taking this into account, the sum (82.1 mg N/l) of concentrations of free AA N and peptide N in omasal digesta in the present study is good agreement with the published values.

The highest proportion of peptide N fraction in SNAN entering the omasum in the present study is consistent with our recent results (Choi et al., 2002a,b). This finding supports the previous observation that peptide degradation can be the rate-limiting step in ruminal proteolysis (Chen et al., 1987a; Choi et al., 2002a).

Peptide N concentration tended to be higher with the PG diets compared with the SG diets (64.4 vs 54.3 mg N/l). The differences may be partially because of the higher content of peptide N in PG than that in SG (Table 1). The relatively large differences in soluble N intake between the PG and the SG diets (210 vs 156 g N/d) also partially explain the differences in peptide N concentration in omasal digesta.

Diurnal changes in the concentrations of total SNAN and peptide N in omasal digesta during the entire feeding cycle were similar due to the largest proportion of peptide in SNAN. The peaks in peptide N concentration in omasal digesta appearing 1-3 h post feeding (Figure 3) were similar to those in previous studies (Chen et al., 1987a; Robinson and McQueen, 1994). However, in the present study, another peak in peptide N concentration occurred at 10 - 11 h after the morning feeding (i.e. 1-2 h before the afternoon feeding). The increase in peptide N concentration may result from free access to grass silage and afternoon milking time (16.30 h), i.e. the milking may have stimulated the appetite of cows, and subsequently the cows have started eating grass silage. This explanation may be probable because the diurnal changes

in dietary and microbial SNAN (Figure 4) showed that the peaks were mainly of dietary origin.

Approximately two times higher concentration of free AA obtained with the NHA than with the AA analyser and three times higher concentration of peptide than free AA assessed with the NHA were observed. The findings suggest that the average size of peptide escaping the rumen may be about 7 to 8 AA units per peptide. This may be true because *Bacteroides ruminicola*, which is a major proteolytic species on most diets (Nolan, 1993), degraded mainly oligo-peptides (Pittman et al., 1967). Our recent study also demonstrated that peptide escaping the rumen seemed to be mainly oligo- or polypeptides (Choi et al., 2002a).

Hydrophobic peptides may have more opportunities to accumulate in the rumen while rumen microbes primarily degrade hydrophilic peptides (Chen et al., 1987a; Russell et al., 1991). According to Chen et al. (1987b), hydrophobic peptides contained high level of leucine, phenylalanine, proline, tryptophan, tyrosine and valine, whereas hydrophilic peptides contained more arginine, aspartic acid, glutamic acid and lysine. In the present study, the intake of hydrophilic AA was calculated to be much higher for PG diets than that for SG diets (mean 259 vs 197 g/d) as well as the intake of hydrophobic AA (274 vs 218 g/d). However, further *in vivo* studies concerning the hydrophobicity on SNAN flow should be conducted because the metabolism of the SNAN from *in vivo* study (Chen et al., 1987a) is related rather to peptide size than the hydrophobicity. Besides, if hydrophilic AA is linked to hydrophobic AA, the hydrophilic AA might escape from ruminal degradation (Russell et al., 1991).

CONCLUSIONS

Compared to secondary growth of grass silage (SG) diets, primary growth of grass silage (PG) diets tended to increase the concentration of peptide N in omasal digesta. Increasing supply of barley concentrate did not affect SNAN concentration. The high microbial contribution to SNAN flow, using ^{15}N as a microbial marker, confirmed the previous observation that a substantial proportion of SNAN is of microbial origin.

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STRESZCZENIE

Wpływ rodzaju kiszonki z traw i udziału paszy treściwej w dawce dla krów mlecznych na przepływ rozpuszczalnego nieamonowego azotu dochodzącego do ksiąg

W doświadczeniu przeprowadzonym na 4 krowach mlecznych fińskich ayrshirach z przetokami żwacza, badano wpływ rodzaju kiszonki z traw (z pierwszego lub drugiego odrostu) oraz udziału paszy treściwej w dawce na stężenie i przepływ rozpuszczalnego nieamonowego azotu (SNAN) w płynnej treści dochodzącej do ksiąg. W doświadczeniu, o układzie kwadratu łacińskiego, zwierzętom podawano kiszonkę przygotowaną z pierwszego (PG) lub drugiego odrostu traw (SG), z dodatkiem 8,1 lub 12,1 kg paszy treściwej/dzień (w s.m.). Zbierano treść wpływającą do ksiąg i oznaczano w niej frakcje SNAN (wolne aminokwasy, peptydy i białko rozpuszczalne). Udział azotu drobnoustrojów w SNAN oznaczano przy pomocy ^{15}N, jako wskaźnika. Stężenie wolnych aminokwasów, peptydów i rozpuszczalnego białka w treści wynosiło 22,3; 59,8 i 23,1 mg N/l, odpowiednio. Przy podawaniu dawek PG wystąpiła tendencja ($P = 0,09$) zwiększenie koncentracji azotu peptydowego w treści ksiąg w porównaniu z dietami SG, natomiast nie stwierdzono wpływu paszy treściwej na stężenie frakcji SNAN. Udział N peptydowego, w SNAN w treści ksiąg był największy, co potwierdza wcześniejsze obserwacje, że hydroliza peptydów do aminokwasów jest najbardziej ograniczającym etapem przebiegu proteolizy w żwaczu. Udział N drobnoustrojów w SNAN wynosił średnio 0,71, co wskazuje na znaczący jego udział w SNAN. Przepływ rozpuszczalnego NAN diety wynosił średnio 9,1 N/d, co odpowiada około 0,05 do 0,08 całkowitego przepływu NAN pochodzącego z paszy.