목초 사일리지 급여 시 보리와 채종박 보충급여에 의한 제 3위 소화액내 Soluble Non-ammonia Nitrogen Fraction의 Flow 패턴 변화

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Diurnal Patterns in the Flow of Escapable Soluble Non-Ammonia Nitrogen Fractions in Omasal Digesta as Influenced by Barley and Rapeseed Meal Supplementation in Cows Fed Grass Silage Based Diet

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ABSTRACT

The present study was conducted to measure diurnal patterns in the flow of soluble non-ammonia nitrogen (SNAN) fractions in the liquid phase of digesta entering the omasum of cows fed grass-red clover silage supplemented with barley and rapeseed meal. Four ruminally cannulated cows were fed, in a 4 × 4 Latin square design, grass-red clover silage alone (GS) or supplemented with (on a DM basis) 6.0 kg/d of barley grain, 2.1 kg/d of rapeseed meal or 6.0 kg/d of barley and 2.1 kg/d rapeseed meal. Omasal digesta was taken using an omasal sampling system at 1.5 h intervals during a 12 h feeding cycle, and SNAN fractions (free AA, peptide and soluble protein) in the omasal digesta were assessed using ninhydrin assay. Dietary supplementation numerically increased the mean flow of SNAN fractions relative to GS diet despite the lack of statistical significance. Diurnal patterns in the flow of peptide entering the omasum during a 12 h feeding cycle appeared to be highest immediately after feeding, declined by 10.0 h post-feeding and slightly increased thereafter. In SNAN fractions, the flow of peptide was higher for supplemented diets than for GS diet throughout the feeding cycle. Based on the microbial contribution to total SNAN using ^{15}N, diurnal patterns in the flow of dietary SNAN for dietary supplemented diets appeared to be higher compared with GS diets. Present results may conclude that peptide flow is quantitatively the most important N in SNAN fractions and that dietary supplementation can increase peptide flow entering the omasal canal.

(**Key Words** : Supplementation, Soluble Non-Ammonia Nitrogen, Omasal Digesta)

Ⅰ. INTRODUCTION

Ruminal protein degradability has generally been estimated using a nylon bag method (Ørskov and McDonald, 1979; Volden and Harstad 1995) because of its simplicity, directness and cost effectiveness (Van Straalen and Tamminga, 1990; Michalet-Doreau and Ould-Bah, 1992). However,

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the validity of estimates obtained using the nylon bag method has been an area of concern due to numerous sources of variation (Nocek, 1988; Michalet-Doreau and Ould-Bah, 1992; Huhtanen et al., 1998). In addition, calculating effective protein degradability in studies obtained using the nylon bag method requires an assumption that the rate of a-fraction occurs at an infinite rate and only insoluble dietary nitrogen (N) escapes ruminal degradation (Madsen et al., 1995). However, this assumption has been shown to be seriously flawed (Dhanoa et al., 1999; Udén, 2000; Volden et al., 2002). For example, when skimmed milk powder was used as a protein supplement for dairy cows, the soluble non-ammonia N (SNAN) concentration in omasal digesta was 110 mg N/l (Choi et al., 2002b). Assuming a rumen volume of 80.1 and a liquid passage rate of 0.15/h, approximately 32 g N/d of SNAN could potentially escape the rumen. It was calculated that 116 g/kg of skimmed milk powder N escaped ruminal degradation as SNAN in dairy cows (Choi et al., 2002b).

Inconsistent with the assumption of the nylon bag method, many *in vivo* studies showed that substantial concentration of soluble N compounds, particularly peptide fraction may escape the rumen (Chen et al., 1987a, c; Williams and Cockburn, 1991; Robinson and McQueen, 1994). Some studies also showed responses of SNAN fractions in the liquid phase of rumen digesta to dietary CP levels (Chen et al., 1987a; Broderick and Wallace, 1988; Robinson et al., 1998). For instance, flow of peptide and soluble protein in rumen digesta was not clearly associated with an increase in CP of diets (Chen et al., 1987a), whereas casein supplementation increased peptide concentration in rumen digesta (Broderick and Wallace, 1988). However, the SNAN data in rumen digesta described above may not be same to actual amounts of rumen-escapable N because SNAN flow in rumen digesta was considerably lower than that in omasal digesta (Choi et al., 2002b). Also, determining SNAN flow from omasal digesta can minimize the contribution of endogenous N to SNAN flow (Ørskov et al., 1986). Thus, responses of SNAN flow to dietary supplementation should be investigated from omasal digesta.

Present study aimed to assess the effects of dietary supplements (energy and protein sources) on diurnal pattern in the flow of SNAN fractions (free amino acid (AA), peptide and soluble protein) in omasal digesta of cows fed grass silage diets. Data on SNAN quantification and its analytical methodology have been reported elsewhere (Choi et al., 2002a).

Ⅱ. MATERIALS AND METHODS

1. Animals and management

Four dairy cows, mean BW 571 (SE ± 33.0) kg, previously fitted with 100 mm i.d. ruminal cannulas were used in a 4 × 4 Latin square design experiment with four periods of 21 days each. All animals 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.

2. Diets and treatments

Animals were allowed grass silage (GS) *ad libitum* as a basal diet, and fed 6.0 kg (per day on a DM basis) of barley grain (GB), 2.1 kg of rapeseed meal (GR) or 6.0 kg of barley grain and 2.1 kg of rapeseed meal (GBR). Grass silage was prepared from secondary growths of swards containing predominately timothy grass (*Phleum pratense*) and red clover (*Trifolium pratense*) swards. Once cut, herbage was wilted and

harvested using a precision-chop forage harvester and ensiled with a formic acid-based additive applied at a rate of 5 l/t. Prior to feeding, barley grain was coarsely milled using a roller mill. Solvent-extracted rapeseed meal was obtained from a commercial source (Rehuraisio Ltd., Raisio, Finland).

3. Sampling procedures and chemical analysis

Dry matter intake and chemical analyses of experimental feeds have previously been reported (Choi et al., 2002a). Feed AA composition of feed ingredients was analysed using an AA analyser (Biochrom 20, AA analyser, Autoloader version, Pharmacia Biotech (Biochrom) Ltd., Cambridge, UK).

To assess digesta outflow from the rumen, a triple-marker method (France and Siddons, 1986) was used using indigestible NDF, Yb-acetate and LiCoEDTA as markers for large particle, small particle and liquid phases, respectively. Microbial contamination of omasal SNAN flow was estimated using $^{15}NH_4$-sulphate. Details of marker methods and sampling procedures and results of liquid (mean 227 l/d) and total N flow (348 g N/d) have previously been reported (Choi et al., 2002a). To estimate SNAN fractions in digesta, omasal digesta were collected from the omasal canal using an omasal sampling system including a plastic tube (14 mm i.d.) connected to a vacuum/compressor pump according to procedures described by Choi and Choi (2003). Details of omasal digesta sampling have previously been reported (see Choi et al., 2002a). In brief, eight omasal digesta samples were taken every 1.5 h after morning feeding during a 12 h feeding cycle (i.e. 1.0, 2.5, 4.0, 5.5, 7.0, 8.5, 10.0 and 11.5 h). Immediately after sampling, omasal digesta was frozen in a liquid nitrogen tank for 30 sec to stop microbial activity, and stored at −20℃ prior to analysis. Diurnal patterns of free AA, peptide and soluble protein fractions in the liquid phase of omasal digesta was assessed using ninhydrin assay (NHA).

4. Statistical analysis

Experimental data 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 Y_{ijkl} and are the dependent variable and the overall mean, A_i is a random effect of animal, P_j, D_k and T_l are the fixed effects of period, diet and time after feeding, and $(A \times T)_{il}$, $(P \times T)_{jl}$ and $(D \times T)_{kl}$ 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.

Ⅲ. RESULTS AND DISCUSSION

1. AA composition of feed

Chemical composition of feedstuffs, silage fermentation quality and DMI have been reported in Choi et al. (2002a). The AA composition of experimental feeds is shown in Table 1. The AAs of leucine, phenylalanine, alanine, aspartic acid and glutamic acid were quantitatively the most important in AA composition of grass silage. Barley grain and rapeseed meal contained relatively high proportion of glutamic acid in CP (23.3 and 15.3%, respectively).

Table 1. Amino acid (AA) composition of experimental feeds

	Feed		
	Grass silage	Barley grain	Rapeseed meal
AA, g/kg crude protein			
Arginine	37.9	50.8	59.7
Histidine	17.3	23.7	26.6
Isoleucine	38.7	34.0	38.2
Leucine	71.0	71.7	68.2
Lysine	43.5	35.1	53.2
Methionine	18.5	19.6	19.8
Phenylalanine	55.2	52.4	43.3
Threonine	38.2	32.5	43.3
Valine	48.5	54.4	50.3
Alanine	60.9	41.4	42.4
Aspartic acid	82.2	57.1	73.5
Cystine	7.8	23.6	18.6
Glutamic acid	86.9	233.0	152.7
Glycine	45.0	40.7	47.5
Proline	43.2	107.5	56.4
Serine	36.0	39.2	41.7
Tyrosine	29.5	32.9	31.6
EAA[1)]	369	374	403
NEAA[2)]	392	575	464
Hydrophobic AA[3)]	247	319	250
Hydrophilic AA[4)]	251	376	339
TAA	760	950	867

[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)] Hydrophobic AA = sum of leucine, phenylalanine, valine, proline and tyrosine.
[4)] Hydrophilic AA = sum of arginine, lysine, aspartic acid and glutamic acid.

2. Omasal SNAN flow measurement

Liquid flow into the omasum was 225, 257, 221 and 204 l/d for GS, GB, GR and GBR, respectively (Choi et al., 2002a). Based on the liquid flow, omasal flow of SNAN fractions in the liquid phase of omasal digesta was on average 21.7, 127.8, 29.0 and 178.5 g/d for free AA, peptide, soluble protein and total SNAN, respectively (Choi et al., 2002a). Relatively low free AA flow in the liquid phase of omasal digesta is consistent that in ruminal digesta (Williams and Cockburn, 1991) even though the present values were higher than those of the previous study. In contrast, Choi and Choi (2003) showed approximately 60.0 g/d of free AA flow escaping the rumen of cows fed grass silage based diets supplemented with different protein feeds. Based on the microbial contribution to SNAN obtained using ^{15}N-enrichments (mean 61%; Choi et al., 2002a), dietary SNAN flow entering the omasum averaged 67.4 g/d while microbial SNAN was 111.0 g/d (data not shown). The present microbial contribution to SNAN clearly indicates that a considerable proportion of the SNAN flow entering the omasum is of microbial origin. This is consistent with the previous observation that some AAs incorporated into bacterial and protozoal cells which can excrete free AA and peptides into ruminal fluid (Coleman, 1967).

3. Hydrophobicity in ruminal N metabolism

Peptides containing hydrophobic AA may have more opportunities to accumulate in the rumen as rumen microbes primarily degrade hydrophilic peptides (Chen et al., 1987b; Russell et al., 1991). Chen et al. (1987b) reported that hydrophobic AAs contained leucine, phenylalanine, valine, proline and tyrosine, and hydrophilic AAs involved arginine, lysine,

aspartic acid and glutamic acid, respectively. Consistent with the previous observation of the hydrophobicity in ruminal N metabolism, Choi et al. (2003) reported that dietary intake of hydrophobic AA may be associated with SNAN concentration in omasal digesta of dairy cows fed grass silage and barley and rapeseed meal concentrates. In the present study, the proportions of hydrophobic and hydrophilic AAs in crude protein of grass silage were similar, whereas barley grain and rapeseed meal contained numerically higher hydrophobic than hydrophilic AAs (see Table 1). Based on the proportions of hydrophobic and hydrophilic AAs in crude protein of the present experimental feeds and N intake data (Choi et al., 2002a), hydrophobic and hydrophilic AA intakes (g/d) corresponded to 512 and 520 (GS), 666 and 710 (GB), 708 and 780 (GR) and 794 and 902 (GBR), respectively. Concerned the previous observation of the hydrophobicity on ruminal N metabolism (Chen et al., 1987b; Russell et al., 1991), in the present study, the highest value of SNAN flow should have appeared for GBR followed by GR. However, all SNAN flow fractions for GBR were obviously lower than those for GB or GR (see Choi et al., 2002a). This discrepancy may mean that further *in vivo* studies concerning the hydrophobicity on SNAN flow should be conducted because SNAN or N metabolism could be more related to peptide size than the hydrophobicity (Chen et al., 1987c).

4. Diurnal pattern in omasal SNAN flow fractions

Influences of dietary supplementation on diurnal patterns in mean flow of SNAN fractions in the liquid phase of omasal digesta were investigated in the present study (Figs. 1 to 5). Since digesta flow was not determined at each digesta sampling time (Choi et al., 2002a), only overall changes in omasal SNAN flow fractions

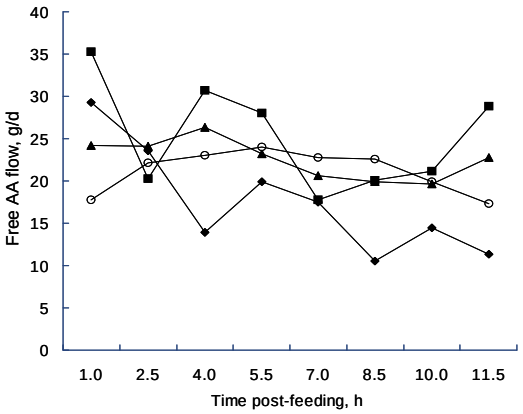

Fig. 1. Diurnal pattern in flow of free amino acid (AA) in the liquid phase of omasal digesta during a 12 h feeding cycle as influenced by dietary supplementation (Markers indicate no supplement(◆), barley(■), rapeseed meal(▲) and barley and rapeseed meal(○), respectively).

during an entire feeding cycle was studied. Diurnal patterns in free AA flow were relatively constant throughout the entire feeding cycle (time effect, p=0.16; Fig. 1). Dietary supplementation did not affect (p>0.05) diurnal pattern in free AA flow in the present study. Although free AA flow for GS and GB appeared to be highest at 1.0 h post-feeding, there were no clear peaks in the feeding cycle. The no clear peaks in free AA flow pattern by the treatments may have resulted from relatively low free AA flow (21.7 g/d). This observation may be true because ruminal free AA concentration was relatively low even immediately after feeding (Nolan, 1993) and diurnal pattern in free AA concentration was relatively constant during a 8 h feeding cycle when sheep were fed a diet of 67% ryegrass hay and 33% concentrate supplemented with ovalbumin (Broderick and Wallace, 1988). In contrast, diurnal pattern in omasal free AA flow appeared to be peaks at 1.0 h post-feeding when dairy cows consumed grass silage and barley supplemented with untreated rapeseed meal,

chemically-treated rapeseed meal or skimmed milk powder (Choi and Choi, 2003). In addition, a peak of free AA concentration in rumen digesta clearly appeared immediately after feeding in sheep was supplemented with casein supplementation increased ruminal free AA concentration to be a peak immediately after feeding (Broderick and Wallace, 1988).

Diurnal patterns in peptide flow appeared to be highest at 1.0 h, declined by 10.0 h and slightly increased at 11.5 h post-feeding (time effect, p=0.02; Fig. 2). Present peptide diurnal patterns in omasal digesta were relatively consistent with previous studies (Chen et al., 1987c; Robinson et al., 1998) where peptide flow in rumen digesta was highest immediately post-feeding and declined thereafter. Since peptide flow as well as SNAN flow in omasal digesta were significantly higher than that in rumen digesta (Choi et al, 2002b), actual rumen-escapable peptide flow in the previous studies should be higher than the ruminal peptide flow despite similar diurnal patterns in peptide flow between rumen and omasal digesta. Dietary supplementation did not show clear differences in peptide flow between treatments, but omasal peptide flow obtained from dairy cows fed supplemented diets was obviously higher ($p<0.05$) compared with GS diet during the entire feeding cycle. This is consistent with Choi and Choi (2003) in which omasal peptide outflow including free AA for dietary protein supplementation was clearly higher than non-supplemented diet during a 12 h feeding cycle. In addition, several studies showed that peptide flow in cattle fed dietary protein supplements was higher than that fed no protein supplements (Broderick and Wallace, 1988; Robinson et al., 1998). In the present study, it is surprising that increases in peptide flow were found at 11.5 h post-feeding. Similarly, Choi et al. (2003) showed peptide peaks appearing at 11.0 h after a morning feeding which was

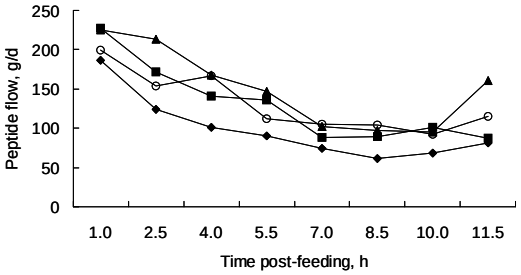

Fig. 2. Diurnal pattern in flow of peptide in the liquid phase of omasal digesta during a 12 h feeding cycle as influenced by dietary supplementation (Markers indicate no supplement(◆), barley(■), rapeseed meal(▲) and barley and rapeseed meal(○), respectively).

around afternoon milking time (16:30 h). The cows in this study were milked at 07:00 and 17:00 h. Therefore, it may be assumed that the increase in peptide flow was due to free access to grass silage and afternoon milking time, i.e. the milking time have presumably stimulated the appetite of dairy cows, and subsequently the cows have started eating grass silage.

Diurnal patterns in soluble protein flow entering into the omasum were relatively constant throughout the feeding cycle (time effect, p=0.45; Fig. 3). When cows were fed GB or GBR diurnal patterns in soluble protein flow in omasal digesta were higher ($P<0.05$) compared with dairy cows fed GR or GS, and absolute values and patterns in soluble protein flow were rather similar between GR and GS. Present study did not show clear peaks of omasal soluble protein flow even immediately post-feeding. This result was maybe because soluble N contents in barley and rapeseed meal has little affected diurnal pattern in soluble protein flow in omasal digesta as soluble N contents in grass silage rather than the supplementary feeds was quantitatively the most important sources (see Choi et al., 2002a).

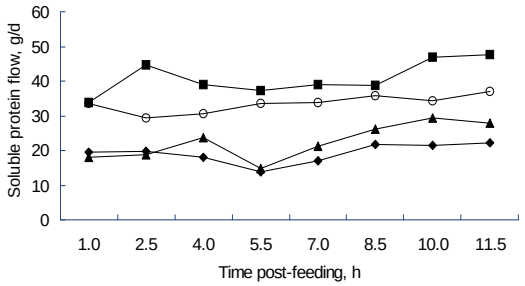

Fig. 3. Diurnal pattern in flow of soluble protein in the liquid phase of omasal digesta during a 12 h feeding cycle as influenced by dietary supplementation (Markers indicate no supplement (◆), barley(■), rapeseed meal (▲) and barley and rapeseed meal(○), respectively).

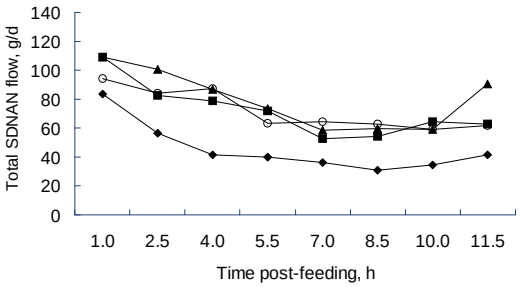

Fig. 4. Diurnal pattern in total soluble dietary non-ammonia nitrogen (SDNAN) flow in the liquid phase of omasal digesta during a 12 h feeding cycle as influenced by dietary supplementation (Markers indicate no supplement (◆), barley (■), rapeseed meal (▲) and barley and rapeseed meal (○), respectively).

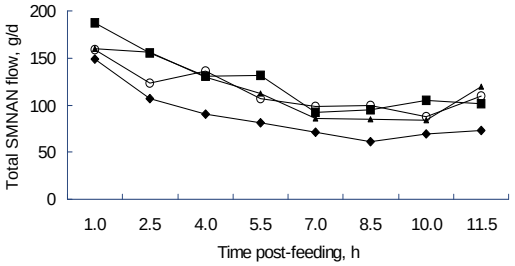

Fig. 5. Diurnal pattern in total soluble microbial non-ammonia nitrogen (SMNAN) flow in the liquid phase of omasal digesta during a 12 h feeding cycle as influenced by dietary supplementation (Markers indicate no supplement (◆), barley(■), rapeseed meal(▲) and barley and rapeseed meal(○), respectively).

This is consistent with the previous study (Robinson et al., 1998) where ruminal soluble protein flow appeared with no clear peaks in dairy cows fed timothy silage, whole crop barley silage and grain-based concentrate. In contrast, Robinson and McQueen (1994) showed clear peaks of soluble protein flow at 1 to 3 h after feeding in dairy cows fed alfalfa and timothy silage and barley and corn concentrate based diets. Since peptide flow was quantitatively the most important fraction in the SNAN fractions, diurnal patterns in total SNAN flow in omasal digesta as influenced by dietary supplementation were similar to those in peptide flow (data not shown).

5. Microbial contribution to diurnal patterns in omasal SNAN flow

Effects of dietary supplementation on diurnal changes in the flow of microbial and dietary SNAN during the feeding cycle are presented in Figs. 4 and 5, respectively. Although diurnal patterns in dietary SNAN flow for all diets were relatively similar to be highest at 1.0 h post-feeding and declined by 7 h post-feeding (time effect, p=0.002) the dietary SNAN flow in cows fed GS was higher than that for supplemented diets during the entire feeding cycle (Fig. 4) because of lower CP intake for GS (2.1 kg/d) than GB, GR and GBR (2.5, 2.9 and 3.0 kg/d; for details, see Choi et al., 2002a). Level of dietary SNAN flow for GB during the feeding cycle, to the same extent, would be expected to be lower than those for GR and GBR. However,

no clear differences between GB and other supplemented diets in dietary SNAN flow appeared in the present study. This may reflect microbial N recycling in the rumen because of a number of ruminal protozoa with increasing amount of starch concentrate in the GB diet (Jaakkola and Huhtanen, 1993). When cows consumed supplemented diets, microbial SNAN flow appeared to be higher than that obtained from cows fed GS (Fig. 5). Microbial SNAN flow appeared to be similar patterns (time effect, p=0.0004), but the absolute levels were much higher compared with dietary SNAN flow during the 12 h feeding cycle (see Figs. 4 and 5). The higher SNAN from microbial origin than dietary origin in the present study is consistent with a recent study reported by Choi et al. (2003). Also, the microbial SNAN flow was much higher than free AA or soluble protein flow, indicating that the present microbial contribution to peptide flow would be lower than the mean microbial contribution to total SNAN (61%; for details, see Choi et al., 2002a). This assumption may be probable because, in the present study, the diurnal patterns in peptide flow were clearly affected whereas those in free AA and soluble protein flow were not affected by the feeding time (see Figs. 1 to 3).

Ⅳ. IMPLICATIONS

Effect of dietary supplementation on diurnal patterns in the flow of SNAN fractions in the liquid phase of digesta entering the omasum of dairy cows fed grass silage based diets was investigated in the present study. Peptide flow peaked at 1 h and declined thereafter despite another peak at 11.5 h post-feeding. Diurnal patterns in peptide flow were higher for dietary supplemented diets than GS diet. No differences between the supplemented diets in diurnal patterns in peptide flow appeared in the present study. Microbial SNAN flow obtained using ^{15}N was higher than free AA or soluble protein flow during the entire feeding cycle, indicating peptide flow would be lower than the mean microbial contribution to total SNAN.

Ⅴ. 요 약

본 연구는 목초 사일리지를 기초사료로 급여한 젖소에 있어서 보리와 유채박 급여가 제 3위 소화액 내 soluble non-ammonia nitrogen fractions(SNAN fractions; 아미노산, 펩티드, 용해성 단백질 및 total SNAN)의 flow 패턴변화를 측정하기 위하여 실시하였다. 반추위 캐뉼라가 장착된 4마리 젖소는 4×4 라틴방각법에 의해 기초사료로 grass-red clover silage를 자유급여하여 사일리지 단독급여구(GS), 기초사료 + 보리 6.0 kg/d 급여구, 기초사료 + 유채박2.1 kg/d 급여구 및 기초사료 + 보리6.0 kg/d + 유채박2.1 kg/d 급여구로 배치하였다. 제 3위 소화액은 제 3위 소화액 채취기구를 이용하여 사료급여 후 1.5 h 간격으로 채취하였고, 그 소화액 내 SNAN fractions 정량은 ninhydrin assay를 이용하여 분석하였다. 보충사료 급여는 GS구에 비해 비록 통계학적 유의성은 없었지만 total SNAN flow를 증가시켰다. 제 3위 소화액 내 펩티드 flow 패턴은 12시간 feeding cycle 내내 SNAN fractions 중 양적으로 가장 높았으며, GS구에 비해 보충사료급여구에서 높은 펩티드 flow 패턴을 보였다. GS구를 제외한 보충사료 처리구간에는 SNAN fractions flow 패턴 차이가 없었다. ^{15}N을 이용하여 분석된 사료유래 SNAN fractions flow 패턴에서는 보충사료급여구가 GS구에 비해 높게 나타났다. 이상의 결과 제 3위로 유입되는 펩티드 flow는 전체 SNAN flow 중 양적으로 가장 중요한 N 이며, 보충사료 급여 시 그 펩티드 flow의 증가 가능성을 시사한다.

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