

## **Effect of concentrate supplementation to grass silage diets on rumen fermentation, diet digestion and microbial protein synthesis in growing heifers**

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A 4 x 4 latin square experiment was carried out with four growing heifers, each with a rumen cannula and a simple T-cannula inserted in the proximal duodenum. The purpose was to study the effects of the supplementation of concentrate to grass silage on rumen fermentation, microbial protein synthesis and digestion of organic matter (OM), fibre components and N. The diets were composed of grass silage alone (S); grass silage and barley (SBU, 50:50 % on dry matter (DM) basis); and grass silage, barley and protein concentrate based either on rapeseed meal (SBR), or meat and bone meal (SBM) (50:40:10). To make the diets isonitrogenous, 23 g of urea was given with the SBU diet.

The supplementation of concentrates, irrespective of their type, increased the average rumen ammonia-N and total concentration of volatile fatty acids (VFA) and decreased the molar proportion of acetate. Inclusion of concentrates in the diet had a negative effect on the digestibility of cell wall constituents. The production of microbial protein and the efficiency of microbial protein synthesis were not affected by the diet. It appears, therefore, that the supply of nitrogenous constituents for rumen microbes through ruminally degraded protein was adequate in silage feeding, and that no extra benefit, at the utilized level of application, was gained by the supplementation of any of the concentrates.

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Key words: rapeseed meal, meat and bone meal, fibre, digestion

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### **Introduction**

The rate of live weight gain (LWG) in growing cattle is usually improved by the inclusion of concentrates in grass silage diets owing to the increased energy intake (THOMAS et al. 1988, LAMPILA and MICORDIA 1990). In some experiments (HAKKOLA 1985, HUHTANEN et al. 1985, JOKI-TOKOLA 1989, 1991, ARONEN and VANHATALO 1992, ARONEN et al. unpubl.) a partial replacement of cereal grains by protein feed has had a positive

effect on LWG, while in others the effect has either been small (ARONEN 1991) or nonexistent (HUHTANEN 1989, ARONEN 1990).

The improved LWG may have been related either to an increased uptake of amino acids or to improved digestibility of the diet and thereby increased feed intake. In regard to grass silage intake, somewhat contradictory results have been obtained. HUHTANEN et al. (1985), STEEN (1988), ARONEN (1990) and MOLONEY (1991) did not find any changes in voluntary grass silage intake when pro-

tein supplements were included in the diet, while HAKKOLA (1985), ARONEN (1991), ARONEN and VANHATALO (1992) and ARONEN et al. (unpublished) recorded a significant increase in grass silage intake.

It has been suggested that the positive effects of protein supplements on grass silage intake may have been related to the supply of preformed amino acids or peptides to rumen microbes (OLDHAM 1984). On the other hand, protein supplementation may enhance silage intake not only through its effect on digestion in the rumen, but also through an increased amount or improved balance of amino acids flowing to the intestines (OLDHAM 1984, HUNT et al. 1989).

The aim of the present experiment was to study the amounts and proportions of the nutrients absorbed from the gastrointestinal tract as influenced by concentrates added to grass silage fed to growing heifers. Furthermore, the aim was to investigate whether the positive effects of protein supplements on grass silage intake are mediated by a stimulation of rumen microbes and by the thereby improved digestibility of dietary fibre and/or by an increase in the amount of nonammonia nitrogen (NAN) flowing to the intestines. Due to their central role in cattle feeding, rapeseed meal (RSM) was chosen as a source of vegetable protein and meat and bone meal (MBM) as a protein of animal origin.

## Material and methods

### Animals and their feeding

Four growing Finnish Ayrshire heifers (initial live weight (W) 197 kg), each with a rumen cannula and a simple T-cannula inserted in the proximal duodenum, were used for a digestibility trial designed as a 4 x 4 latin square. The heifers were fed 70 g DM kg<sup>-1</sup> W<sup>0.75</sup> in two equal meals at 12 h intervals.

The four diets were composed of grass silage alone (S); grass silage and barley (SBU, 50:50 on a DM basis); grass silage, barley and a RSM-based protein concentrate (SBR, 50:40:10); or grass

silage, barley and MBM -based protein concentrate (SBM, 50:40:10). To make the diets isonitrogenous, on average 23 g of urea was fed with the SBU-diet. In addition, a commercial mineral mixture was given (100 g d<sup>-1</sup>) to the animals and water was freely available.

Formulation of the diets and the average feed intake are presented in Table 1. In order to make the pelleted protein concentrates isonitrogenous, different amounts of ingredients were used. Due to the higher protein content in meat and bone meal, a larger amount of barley was included in the MBM-based concentrate than in the RSM-based type. However, the proportion of molasses was equal in both concentrates.

Table 1. Formulation of diets and mean quantities (kg DM/d) of dietary components given daily.

	Diet			
	S	SBU	SBR	SBM
Grass silage	3697	1944	1864	1942
Barley		1840	1482	1543
RSM <sup>1)</sup>			371	
MBM <sup>2)</sup>				389
Urea		23		
Minerals	150	150	150	150

RSM<sup>1)</sup> Composition of rapeseed meal-based concentrate in air dry basis; Rapeseed meal: Barley: Molasses (81:15:5).

MBM<sup>2)</sup> Composition of meat and bone meal-based concentrate in air dry basis; Meat and bone meal: Barley: Molasses (51:44:5).

The grass silage was prepared on 5-7 June 1989 from a first cut of cocksfoot-timothy (*Dactylis glomerata-Phleum pratense*) grass harvested using a flail harvester. It was ensiled in a bunker silo with a formic acid based additive (80 % (w/w) formic acid, 2 % orthophosphoric acid), applied at the rate of 5 l/t.

## Experimental procedures and analytical methods

Each experimental period lasted 21 days with a 12-day adaptation period. When changing the diets, the change in the rumen environment of each animal was accelerated by transferring 15 litres of the rumen contents from the animal previously fed that particular diet.

Representative samples of the feeds were collected at regular intervals throughout each period and pooled for subsequent analysis. The flow of dietary constituents at the duodenum was determined by using the graphic alternative of FAICHNEY'S (1975) double-marker method (MCALLAN and SMITH 1983). Cr-mordanted straw and LiCo-EDTA, prepared as described by UDEN et al. (1980), were used as markers.

For the assessment of the overall digestibility of the diets, using acid-insoluble ash (VAN KEULEN and YOUNG 1977) as a natural marker, faecal grab samples were taken from day 13 to day 17 when feeding the animals. Calculations of duodenal nutrient flows were based on the amounts of Co and Cr excreted in faeces. Microbial N flow at the duodenum was estimated using purine bases of nucleic acids as markers. To prepare a microbial sample, three samples were taken from the rumen content on day 20 just before feeding, and 4 h and 8 h after feeding.

Administration of markers, sampling and handling of duodenal digesta, preparation of microbial samples and rumen fermentation measurements were carried out as described by VANHATALO et al. (1992), but for the assessment of the liquid outflow rate from the rumen, only rumen liquid samples were used.

Ruminal degradation of grass silage was determined as described by VANHATALO et al. (1992) and that of barley and RSM-based and MBM-based concentrates as described by ARONEN et al. (1991) with the exception that the incubation periods for grass silage were 3, 6, 12, 24, 48, 72 and 96 hours and for concentrates 3, 6, 12, 24 and 48 hours. Degradability of crude protein (DEG) was

calculated according to ØRSKOV and McDONALD (1979) using a rumen outflow rate ( $k$ ) of 0.08 as suggested by HVELPLUND and MADSEN (1990) and making a correction for microbial contamination in grass silage samples in accordance with MICHALET-DOREAU and OULD-BAH (1989).

The methods used in all the chemical analyses are presented by VANHATALO et al. (1992).

## Calculations and statistical analyses

The liquid dilution rate from the rumen was calculated as the slope of regression of the natural logarithm of the Co concentration against time after a single dose of LiCo-EDTA into the rumen.

An analysis of variance, appropriate to the latin square design, was carried out on the digestibility and dilution rate data. Rumen fluid data were studied by analyses of variance using the following model:

$$Y_{ijklm} = \mu_{\dots} + A_i + P_j + T_k + e_{ijk} + H_l + AH_{il} + PH_{jl} + TH_{kl} + e_{ijklm}$$

where A, P, T and H are the effects of animal, period, treatment and sampling hour, respectively, and  $e_{ijklm}$  is the residual error term.  $e_{ijk}$  was used as an error term for testing the main effects A, P and T.

The differences between the treatments were tested by using orthogonal contrasts. The treatment comparisons were S vs SBU, SBR, SBM; SBU vs SBR, SBM; SBR vs SBM.

## Results

### Feeds and feed intake

The palatability of the feeds was found to be good and only some refusals were recorded for one of the animals on the SBR-diet and for another on the S-diet. Grass silage was of good quality with a rather high protein content (Table 2). Also the protein content in barley meal was high. The rumen degradability of protein was similar in RSM-based and in MBM-based concentrates (Table 2), whereas both in silage and barley it was higher than in RSM-based and MBM-based concentrates.

Table 2. Chemical composition and degradability of crude protein (DEG) of the experimental feeds.

	Silage	Barley	RSM	MBM
Dry matter (g/kg)	215	886	901	916
In dry matter (g/kg)				
Ash	71	26	70	177
Crude protein	172	147	325	332
Ether Extract	61	22	51	90
Crude fibre	296	47	124	27
Nitrogen free extracts	400	759	430	374
NDF <sup>1)</sup>	575	262	292	141
ADF <sup>1)</sup>	320	58	191	30
DEG, %	85	80	69	67

In silage: pH 4.07; in dry matter (g/kg): sugars 29, lactic acid 58, acetic acid 17; in total nitrogen (g/kg): ammonia N 47, soluble N 543.

<sup>1)</sup> NDF, neutral detergent fibre; ADF, acid detergent fibre.

## Rumen fermentation

Rumen fermentation parameters are given in Table 3 and the diurnal fluctuation of rumen fermentation is illustrated in Figures 1 to 3. Inclusion of concentrates in the diet was followed by a decrease in

Table 3. The effect of different supplements on rumen pH, NH<sub>3</sub>-N and volatile fatty acids (VFA) in growing heifers given grass silage.

Diet <sup>1)</sup>	S (1)	SBU (2)	SBR (3)	SBM (4)	SEM <sup>2)</sup>	Statistical significance of effect <sup>3)</sup>		
						1 vs 2,3,4	2 vs 3,4	3 vs 4
pH	6.56	6.33	6.30	6.35	0.11	*	NS	NS
NH <sub>3</sub> -N (mmol/l)	8.78	10.82	10.75	9.85	1.25	*	NS	NS
Total VFA (mmol/l)	111.5	119.4	116.1	119.1	6.37	*	NS	NS
Molar proportion of VFAs <sup>4)</sup> (mmol/mol)								
Ac	668	646	642	651	9.46	o	NS	NS
Pr	192	196	198	192	6.95	NS	NS	NS
Bu	108	121	121	119	4.11	**	NS	NS
Ival	18.3	22.3	22.8	22.3	1.97	o	NS	NS
Val	12.9	14.8	16.0	15.4	1.54	*	NS	NS
Ratio (Ac + Bu)/Pr	4.07	4.03	3.93	4.08	0.19	NS	NS	NS
Ratio Pr/Bu	1.81	1.63	1.66	1.62	0.09	*	NS	NS

<sup>1)</sup> S, silage; SBU, silage, barley and urea; silage, barley and protein concentrate based on rapeseed meal (SBR) or meat and bone meal (SBM).

<sup>2)</sup> Standard error of the treatment effect means. Mean treatment effects were deduced from the fermentation curves.

<sup>3)</sup> Statistical significance: NS, not significant; o, P<0.10; \*, P<0.05; \*\*, P<0.01; \*\*\*, P<0.001.

<sup>4)</sup> Ac, acetic acid; Pr, propionic acid; Bu, butyric acid; Val, valeric acid; Ival, isovaleric acid.

rumen pH. Supplementation with concentrates, irrespective of their type, increased the average rumen ammonia-N concentration. Also the curve pattern of ammonia-N concentration tended to differ (P<0.10) between the treatments; the highest values after feeding were recorded for the SBU-diet, but at the end of the feeding interval the highest values were found with the SBR-diet. Inclusion of concentrates in the diet increased (P<0.05) the total VFA concentration. However, the differences in total VFA concentration between the SBU-diet and the other two supplemented diets were insignificant. This was also the case in the molar proportions of individual VFAs; differences were observed only between the supplemented and unsupplemented diets.

## Digestion of organic matter and fibre

There were some dissimilarities in the OM intake between the treatments (Table 4). Neither these nor the differences in the amounts of OM entering the duodenum were significant. On an average 0.560 of

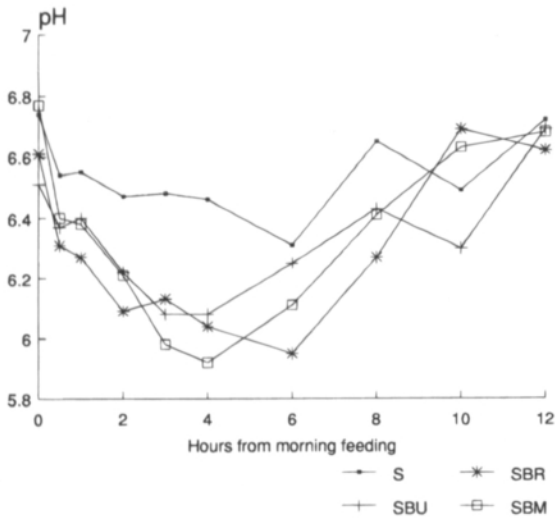


Fig. 1. The effect of type of supplement on rumen pH. (S, silage; SBU, silage, barley and urea; silage, barley and protein concentrate based on rapeseed meal (SBR) or meat and bone meal (SBM)).

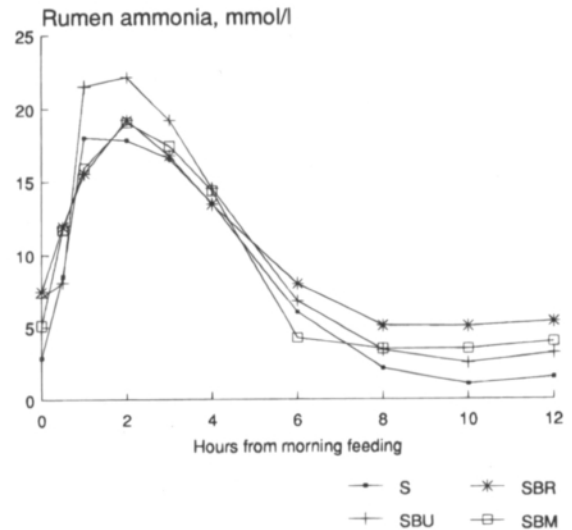


Fig. 3. The effect of type of supplement on ammonia concentration in the rumen fluid. (S, silage; SBU, silage, barley and urea; silage, barley and protein concentrate based on rapeseed meal (SBR) or meat and bone meal (SBM)).

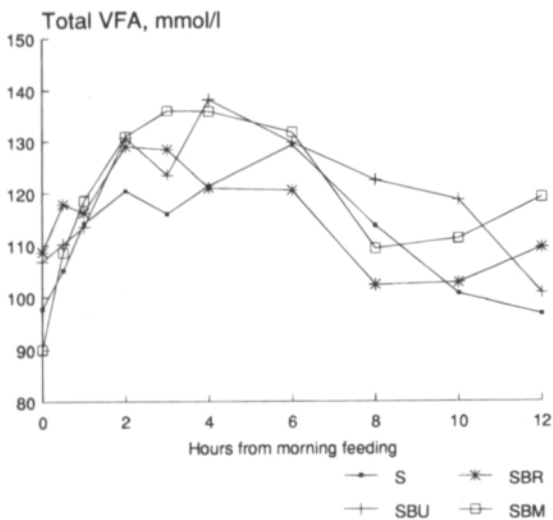


Fig. 2. The effect of type of supplement on volatile fatty acid (VFA) concentration in the rumen. (S, silage; SBU, silage, barley and urea; silage, barley and protein concentrate based on rapeseed meal (SBR) or meat and bone meal (SBM)).

OM was apparently and 0.704 truly digested in the rumen. An average of 0.905 of digestible OM had been truly digested before the small intestine.

The content of fibre components, in terms of NDF and ADF, was highest in grass silage, which was reflected in a higher NDF and ADF intake in the S-diet than in the other diets (Table 5). The inclusion of concentrates in the diet, irrespective of their type, impaired the total tract digestibility and ruminal digestion of diet NDF and ADF. The difference in digestibility of diet NDF and ADF between the total tract and the rumen was of no practical importance.

#### Disappearance of grass silage DM from nylon bags

Disappearance of grass silage DM from the nylon bags was not significantly influenced by the diet. However, the highest values were recorded for the S-diet (Figure 4).

Table 4. The effect of different supplements on organic matter digestion in growing heifers given grass silage.

Diet <sup>1)</sup>	S	SBU	SBR	SBM	SEM <sup>2)</sup>
OM (g 24 h <sup>-1</sup> )					
In feed	3433	3598	3519	3627	92.3
At duodenum	1600	1490	1535	1575	155.4
Microbial OM	558	481	507	500	76.4
In faeces	819	736	785	759	57.5
Digestibility in the rumen					
Apparent	0.531	0.574	0.572	0.562	0.0430
True	0.692	0.711	0.714	0.700	0.0286
Disappearance of digestible OM before small intestine					
Apparent	0.698	0.721	0.738	0.711	0.0437
True	0.911	0.898	0.921	0.888	0.0282
Apparent digestibility	0.759	0.790	0.775	0.790	0.0131

<sup>1)</sup> S, silage; SBU, silage, barley and urea; silage, barley and protein concentrate based on rapeseed meal (SBR) or meat and bone meal (SBM).

<sup>2)</sup> Differences between treatment comparisons were not significant.

Table 5. The effect of different supplements on digestion of cell wall fractions in growing heifers on grass silage feeding.

Diet <sup>1)</sup>	S (1)	SBU (2)	SBR (3)	SBM (4)	SEM	Statistical significance of effect <sup>2)</sup>		
						1 vs 2,3,4	2 vs 3,4	3 vs 4
NDF (g 24 h <sup>-1</sup> )								
In feed	2123	1591	1562	1569	43.7	***	NS	NS
At duodenum	408	385	382	351	39.4	NS	NS	NS
In faeces	431	383	413	393	33.3	NS	NS	NS
Digestibility								
Rumen	0.806	0.751	0.762	0.776	0.0238	NS	NS	NS
Total	0.795	0.755	0.735	0.748	0.0170	*	NS	NS
ADF (g 24 h <sup>-1</sup> )								
In feed	1184	728	752	722	25.6	***	NS	NS
At duodenum	206	166	197	173	18.5	NS	NS	NS
In faeces	211	162	203	171	15.8	NS	NS	NS
Digestibility								
Rumen	0.824	0.767	0.741	0.761	0.0192	*	NS	NS
Total	0.820	0.774	0.729	0.762	0.0156	*	NS	NS

<sup>1)</sup>S, silage; SBU, silage, barley and urea; silage, barley and protein concentrate based on rapeseed meal (SBR) or meat and bone meal (SBM).

<sup>2)</sup>Statistical significance: see Table 3.

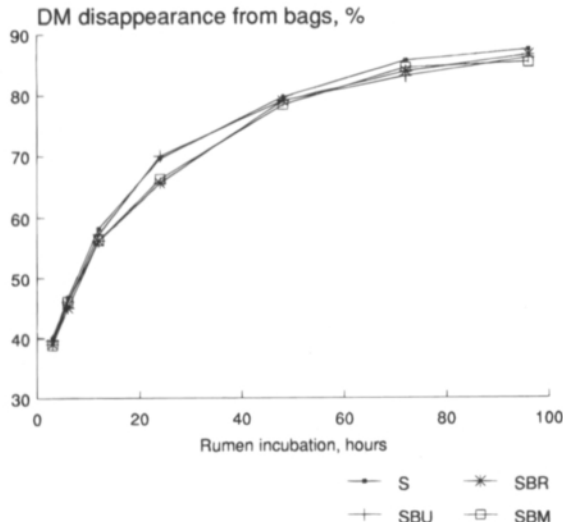


Fig. 4. The effect of type of supplement on dry matter (DM) disappearance of grass silage from nylon bags incubated in the rumen of cattle. (S, silage; SBU, silage, barley and urea; silage, barley and protein concentrate based on rapeseed meal (SBR) or meat and bone meal (SBM).

### Digestion of nitrogen and liquid dilution rate

The flow of N in the digestive tract is presented in Table 6. The amount of total N entering the duodenum tended to be higher in the S-diet than in the other diets. Also the amount of ammonia-N entering the duodenum tended to be higher ( $P < 0.10$ ) in the S-diet. There was no significant difference in the amount of microbial N at the duodenum between the treatments. In the S-diet the flow of feed N into the duodenum was greater than in the other diets ( $P < 0.05$ ). There were no differences in the amount of N voided in the faeces, or in the apparent digestibility of N. The efficiency of microbial protein synthesis was not significantly affected by the diet.

The liquid dilution rate was highest in the S-diet, while the differences between the other diets were not significant (Table 6).

Table 6. Intake and flow of nitrogen through the digestive tract and digestion of nitrogen and liquid dilution rate in growing heifers given grass silage and different supplements.

Diet	S (1)	SBU (2)	SBR (3)	SBM (4)	SEM	Statistical significance of effect <sup>1)</sup>		
						1 vs 2,3,4	2 vs 3,4	3 vs 4
Nitrogen (g 24 h <sup>-1</sup> )								
In feed	101.7	105.2	105.2	110.4	3.05	NS	NS	NS
At duodenum								
Total N	105.2	81.4	89.3	90.2	8.41	NS	NS	NS
Ammonia N	3.6	2.4	2.9	2.3	0.39	o	NS	NS
NAN	101.6	79.0	86.4	87.9	8.30	NS	NS	NS
Microbial N	50.2	43.3	45.6	45.0	6.88	NS	NS	NS
Feed N <sup>2)</sup>	44.1	28.3	33.4	35.6	4.06	*	NS	NS
In faeces	27.4	25.6	29.4	29.2	1.66	NS	NS	NS
Apparent digestibility	0.729	0.750	0.716	0.732	0.0130	NS	NS	NS
Degradability of feed N	56.1	72.3	68.9	67.2	0.039	*	NS	NS
Microbial N kg <sup>-1</sup> OMADR <sup>3)</sup>	27.9	23.2	23.1	22.4	4.55	NS	NS	NS
Microbial N kg <sup>-1</sup> OMTDR <sup>3)</sup>	21.2	17.9	18.1	17.8	2.96	NS	NS	NS
Microbial N kg <sup>-1</sup> DCHO <sup>4)</sup>	25.3	20.2	21.1	20.5	3.28	NS	NS	NS
Liquid dilution rate (l/h)	0.114	0.055	0.071	0.063	0.0086	**	NS	NS

<sup>1)</sup> Statistical significance and abbreviations: see Table 3.

<sup>2)</sup> Assuming endogenous N 130 mg (kg W<sup>0.75</sup>)<sup>-1</sup> (ØRSKOV and MACLEOD 1983).

<sup>3)</sup> Organic matter apparently (OMADR) and truly (OMTDR) digested in the rumen.

<sup>4)</sup> Digestible carbohydrates in the diet (DCHO).

## Discussion

The decreased ruminal pH resulting from concentrate supplementation was found in the present study to be related to the higher total VFA concentrations in the rumen. The lowest pH values were recorded for diets containing RSM and MBM, 4 and 6 hours after feeding, respectively. However, these values, being slightly below pH 6, were higher than the critical value of 5.5 for normal rumen functioning (KAUFMANN et al. 1980).

In agreement with VANHATALO (1991), the proportion of acetate in total VFA decreased when the grass silage diet was supplemented with concentrates. Analogous to observations by HUHTANEN (1987), the proportion of butyrate in total VFA increased when barley-based concentrates were included in the diet.

The molar proportions of valerate and isovalerate increased as a result of concentrate supplementation. Isobutyrate and isovalerate are derived from the deamination of the branched-chain amino acids, valine and leucine. In the experiment by HUSSEIN et al. (1991), the replacement of fish meal with soyabean meal was followed by an increase in the proportion of isobutyrate and isovalerate, which reflected the higher rumen degradability of soyabean meal. Therefore, the results obtained in the present experiment may indicate that a smaller amount of amino acids was degraded in the rumen in the S-diet compared to the supplemented diets, or that the incorporation of amino acids in the microbial protein synthesis in it was larger.

The average ruminal  $\text{NH}_3\text{-N}$  concentration was lower for the S-diet than for the supplemented diets, which is inconsistent with the high rumen degradability of grass silage N measured by the nylon bag technique. On the other hand, the degradability of feed N *in vivo* was lower for the S-diet than for the other diets.

The lower rumen ammonia concentration in the S-diet may have been due, in addition to the lower N intake, to the more efficient capture of rumen degradable nitrogen. It is also possible that the higher rumen pH with the S-diet had encouraged

extensive absorption of  $\text{NH}_3\text{-N}$  from the rumen (ROOKE and ARMSTRONG 1989). As suggested by HUHTANEN (1988), a larger number of protozoa in the rumen with the concentrate-supplemented diets, and the resulting increased recycling of N in the rumen, may have caused an elevated concentration of rumen ammonia. A higher  $\text{NH}_3\text{-N}$  peak after feeding with the SBU-diet compared to the other diets reflects the high degradability of urea N.

For optimal microbial growth, minimum rumen  $\text{NH}_3\text{-N}$  concentrations of 3.6 - 5.7 mmol  $\text{l}^{-1}$  should be maintained (SATTER and SLYTER 1974). According to ROOKE et al. (1985), the synthesis may be impaired if the level of  $\text{NH}_3\text{-N}$  remains lower than 3.6 mmol  $\text{l}^{-1}$  for longer periods. In the present experiment the  $\text{NH}_3\text{-N}$  concentration remained below 2.2 mmol  $\text{l}^{-1}$  for three to four hours at the end of the feeding interval in the S-diet. Nevertheless, the production of microbial protein and the efficiency of synthesis was highest in the S-diet. This finding is supported by BOWMAN and ASPLUND (1988), who state that the low levels of ruminal  $\text{NH}_3\text{-N}$  (2.2-4.3 mmol  $\text{l}^{-1}$ ) in sheep did not limit the microbial protein synthesis.

Another aim of the present experiment was to investigate whether the positive effects of protein supplements on feed intake are mediated by the increased microbial activity and the thereby improved digestibility of dietary fibre (NOCEK and RUSSELL 1988) and/or by an increased amount or improved balance of amino acids flowing to the intestines (OLDHAM 1984, HUNT et al. 1989).

No significant differences in the apparent digestibility of OM in the rumen or in the total tract were noticed between the diets. However, the inclusion of concentrates in the diet had a negative effect on the digestibility of cell wall constituents. The lowest pH values during the feeding interval in the present experiment were higher than the critical values proposed for normal rumen functioning by KAUFMANN et al. (1980). They were, however, lower than the critical pH values (6.0 - 6.1) for fibre digestion proposed by MOULD et al. (1983). Therefore, the decreased digestion of fibre components in the supplemented diets may have reflected



impaired cellulolytic activity in the rumen.

The differences in fibre digestion between the diets may have originated from the differences in diet fibre composition and potential degradability, too. This possibility is supported by the finding that the disappearance of grass silage DM from the nylon bags incubated in the rumen was not significantly affected by the diet.

The central role of the rumen in fibre digestion was reflected by the observation that practically all NDF and ADF digestion took place in the rumen.

No differences in the digestibility of fibre components between the various concentrate-supplemented diets were found in the present experiment. There was no significant difference in grass silage DM disappearance from the nylon bags, either. Most of the experiments in which protein supplementation or replacing urea with protein has resulted in positive effects on fibre digestion, have been conducted with medium- or poor-quality roughages (MCALLAN and GRIFFITH 1987, MCALLAN et al. 1988, OLSSON et al. 1991). The positive response to protein supplementation has been related to low rumen degradability of feed protein (e.g., fish meal, blood meal, rumen protected meals) and a more gradual release of  $\text{NH}_3\text{-N}$ , peptides and branched-chain VFA, allowing the essential growth factors to remain available in the rumen for a longer period of time after feeding (HUSSEIN et al. 1991).

On the other hand, MCALLAN et al. (1988) did not relate the positive effect of rapeseed meal and maize-gluten meal supplementation on rumen fibre digestion in a straw diet to differences in  $\text{NH}_3\text{-N}$  release or differences in degradability alone. In dairy cows which were fed a grass silage-based diet, the ruminal protein degradability of rapeseed meal-based concentrate had no significant effect on diet OM or on NDF digestibility, either (BERTILSSON et al. 1991).

ROOKE and ARMSTRONG (1989) concluded that the extent of stimulation on rumen microbial N synthesis achieved by rumen degradable protein in silage-based diets is dependent both on silage composition and on the synchronization of protein and energy supply.

In spite of the high level of readily available carbohydrates in the supplemented diets there was a greater N loss from the rumen with the supplemented diets than with the S-diet. These losses are consistent with the higher concentrations of rumen ammonia in heifers fed the supplemented diets. Therefore, it is likely that the supply of protein and energy to rumen microbes was better synchronized in the S-diet than in the concentrate supplemented diets. The good quality of the grass silage may have contributed to this.

Rumen degradability of feed N, measured by the nylon bag technique, was highest for the grass silage, but when the comparisons were made with the *in vivo* data, the rumen degradability of N was lowest for the S-diet. This discrepancy remains unsolved, but it must be noted, that the degradability of diet N *in vivo* was calculated by difference. Therefore, the possible inaccuracy in measuring the microbial flow and in the assumptions for the endogenous N would be accumulated in the feed N. On the other hand, there are shortcomings in the nylon bag method, too (VARVIKKO and LINDBERG 1985).

Slightly higher values of NAN entering the duodenum were recorded for diets SBR and SBM than for diet SBU. The difference was not significant, however. Therefore, the hypothesis that the positive effects of protein supplements on feed intake are mediated by an increase in the amount of amino acids flowing to the intestines (OLDHAM 1984, HUNT et al. 1989) could not be supported either. The possibility that the increased feed intake caused by protein feeds is mediated by an improved balance of amino acids flowing to the intestines cannot be excluded, however.

In disagreement with the figures given by ARC (1984), the efficiency of microbial protein synthesis in this experiment was found to be higher, though not significantly, for the S-diet than for the concentrate supplemented diets (27.9 vs. 22.9 g microbial N  $\text{kg}^{-1}$  OMADR). According to ARC (1984), grass silage alone appears to support lower microbial yields, averaging 23 g N  $\text{kg}^{-1}$  OMADR, whereas grass silage supplemented with concentrates may result in higher efficiency (30 g N  $\text{kg}^{-1}$

OMADR). Similarly in a review by McALLAN et al. (1987) reported lower values of microbial protein synthesis for diets of grass silage alone than for diets of grass silage supplemented with concentrates (27 vs. 33 g microbial N kg<sup>-1</sup> OM digested in the rumen).

On the other hand, HARSTAD and VIK-MO (1985) and JAAKKOLA and HUHTANEN (1990) noticed that a small addition of barley to silage improved the rate of bacterial nitrogen synthesis, whereas further substitution of silage by barley gradually reduced the efficiency. One reason for the high efficiency of microbial protein production in silage feeding in the present experiment may have been the good digestibility and fermentation quality of the grass silage.

In agreement with studies on rapeseed meals with different degradabilities (LINDBERG 1984), the probable explanation for the limited sensitivity of microbial protein synthesis to a change in protein quality found in the present study may have been the high nitrogen content and degradability of the basal diet and the relatively small addition of nitrogen from the supplements; only 0.08, 0.183 and 0.188 of the total diet N originated from urea, RSM and MBM, respectively. Additionally, the differences in rumen degradability between barley and RSM or MBM were quite small.

The efficiency of microbial protein synthesis in the Nordic system is given in relation to digestible carbohydrates (DCHO, HVELPLUND and MADSEN 1990). The efficiency of microbial N synthesis was 25.3 g kg<sup>-1</sup> DCHO for diet S and, on an average, 20.6 for the other three diets. With an average amino acid content of 70 % in microbial protein (HVELPLUND and MOLLER 1980), this is equivalent

to a synthesis of 17.7 g microbial amino acid N kg<sup>-1</sup> DCHO in diet S and 14.4 in the other diets. These figures are lower than the efficiency adopted for the AAT-PBV system (20 g microbial amino acid N kg<sup>-1</sup> DCHO, HVELPLUND and MADSEN 1990). One reason for this discrepancy may be the difference in the methods used in determining the microbial protein.

The rumen liquid outflow rate and the efficiency of microbial protein synthesis have been found to be positively related (HARRISON and McALLAN 1980; LINDBERG 1984). This was also the case in this study.

To conclude, the supplementation of a grass silage diet with concentrates, irrespective of their type, increased the average rumen ammonia-N concentration. Supplementation was also followed by an increase in the total VFA concentration with a lower proportion of acetate. Inclusion of concentrates in the diet had a negative effect on the digestibility of cell wall constituents. Microbial protein production and the efficiency of microbial protein synthesis were not significantly affected by the diet. Therefore, it appears that the supply of nitrogenous constituents for rumen microbes through ruminally degraded protein was adequate in silage feeding, and no extra benefit was gained by supplementation with barley and urea, RSM or MBM at the applied levels.

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## References

- ARC 1984. The nutrient requirements of ruminant livestock. Supplement No 1. 45 p. Commonwealth Agricultural Bureaux, Slough, England.
- ARONEN, I. 1990. Barley Protein and Rapeseed Meal as Protein Supplements for Growing Cattle. *Acta Agric. Scand.* 40: 297-307.
- 1991. Influence of frequency and accuracy of supplement feeding on rumen fermentation, feed intake, diet digestion and performance of growing cattle. 1. Studies with growing bulls fed grass silage ad libitum. *Anim. Feed Sci. Technol.* 34: 49-65.
- & VANHATALO, A. 1992. Heat-Moisture Treatment of

- Rapeseed Meal: Effect on Digestibility of the Diet, Voluntary Grass Silage Intake and Growth Rate of Ayrshire Bulls. Acta Agric. Scand., Sect. A, Animal Sci. 42. In press.
- BERTILSSON, J., LINDBERG, J.E. & GONDA, H. 1991. Different protein levels to dairy cows - effect of reduced protein degradability and lower nitrogen supply on animal performance. 6th Int. Symp. Prot. Metab. and Nutr. Herning, Denmark. p. 309-311.
- BOWMAN, J.G.P. & ASPLUND, J.M. 1988. Nitrogen Utilization, Ruminal Fermentation and Abomasal Nitrogen Flow in Sheep Fed Caucasian Bluestem Hay Supplemented with Lucerne or Urea. Anim. Feed Sci. Technol. 20: 33-44.
- FAICHNEY, G.H. 1975. The use of markers in partitioning digestion within gastrointestinal tract of ruminants. In: McDonald & Warner (eds.). Digestion and metabolism in ruminant. The University of New England Pub. Unit, Sydney. p. 277-291.
- HAKKOLA, H.S. 1985. Barley and protected protein concentrate as supplements to timothy silage for growing bulls. The International Grassland Congress, XV. Kyoto, Japan.
- HARRISON, D.G. & McALLAN, A.B. 1980. Factors affecting microbial growth yields in the reticulo-rumen. In: Ruckebusch & Thivend (eds.). Digestive physiology and metabolism in ruminants. CT: AUI Publishing Co, Westport. p. 205-226.
- HARSTAD, O.M. & VIK-MO, L. 1985. Estimation of microbial and undegraded protein in sheep on grass silage based diets. Acta Agric. Scand. Suppl. 25: 37-48.
- HUHTANEN, P. 1987. The effect of carbohydrate supplements on the utilization of grass silage diets. Department of Animal Husbandry. University of Helsinki. Diss. 45 p.
- 1988. The Effects of Barley, Unmolassed Sugar-beet Pulp and Molasses Supplements on Organic Matter, Nitrogen and Fibre Digestion in the Rumen of Cattle Given a Silage Diet. Anim. Feed Sci. Technol. 20: 259-278.
- , POUTAINEN, E. & MIKKOLA, T. 1985. The effect of supplementation of grass silage with rapeseed meal or Gasol-treated barley on the performance of growing cattle. J. Agric. Sci. Finl. 57: 75-84.
- , NÄSI, M. & KHALILI, H. 1989. By-products from integrated starch-ethanol production from barley in the diets of growing cattle. J. Agric. Sci. Finl. 61: 451-462.
- HUNT, C.W., PARKINSON, J.F., ROEDER, R.A. & FALK, D.G. 1989. The delivery of cottonseed meal at three different time intervals to steers fed low-quality grass hay: effects on digestion and performance. J. Anim. Sci. 67: 1360-1366.
- HUSSEIN, H.S., JORDAN, R.M. & STERN, M.D. 1991. Ruminal protein metabolism and intestinal amino acid utilization as affected by dietary protein and carbohydrate sources in sheep. J. Anim. Sci. 69: 2134-2146.
- HVELPLUND, T. & MADSEN, J. 1990. A study of the quantitative nitrogen metabolism in the gastro-intestinal tract, and the resultant new protein evaluation system for ruminants. The AAT-PBV system. Institute of Animal Science. The Royal Veterinary and Agricultural University, Copenhagen. Diss. 62 p.
- & MØLLER, P.D. 1980. Fodringens indflydelse på vom-bakteriernes kemiske sammensætning. Medd. nr. 310, Statens Husdyrbrugsforsøg, Copenhagen.
- JAAKKOLA, S. & HUHTANEN, P. 1990. Nitrogen digestion and rumen fermentation in cattle given silage or dried grass with three levels of concentrate. Proc. of the 9th Silage Conference. Paper No 61: 116-117. Univ. of Newcastle upon Tyne.
- JOKI-TOKOLA, E. 1989. Valkuaislisän tarve sonnien säilö-rehu-ruokinnassa. Koetoin. ja Käyt. 46: 46.
- 1991. Lihaluurehujauho ja rypsirouhe sonnien valkuaishuna. Koetoin. ja Käyt. 48: 64.
- KAUFMANN, W., HAGEMEISTER, H. & DIRKSEN, G. 1980. Adaptation to changes in dietary composition, level and frequency of feeding. In: Ruckebusch & Thivend (eds.). Digestive Physiology and Metabolism in Ruminants, MTP Press Limited, Lancaster. p. 587-602.
- LAMPILA, M. & MICORDIA, A. 1990. Lihanautojen tarvenormien vertailu eläinkokeiden perusteella. Rehuarvojärjestelmät ja tuotantovaikutus. Suom. Maatal.tiet. Seur. tiedote nro 13, p. 15-29.
- LINDBERG, J.E. 1984. Nitrogen Metabolism in Sheep. Swedish J. agric. Res. 14: 29-36.
- McALLAN, A.B. & SMITH, R.H. 1973. Factors affecting the digestion of dietary carbohydrates between the mouth and duodenum. Br. J. Nutr. 50: 445-454.
- & GRIFFITH, E.S. 1987. The Effects of Different Sources of Nitrogen Supplementation on the Digestion of Fibre Components in the Rumen of Steers. Anim. Feed Sci. Technol. 17: 65-73.
- , SIDDON, R.C. & BEEVER, D.E. 1987. The efficiency of conversion of degraded nitrogen to microbial nitrogen in the rumen of sheep and cattle. In: Jarrige & Alderman (eds.). Feed Evaluation and Protein Requirement Systems for Ruminants. Luxembourg: CEC. p. 111-128.
- , COCKBURN, J.E., WILLIAMS, A.P. & SMITH, R.H. 1988. The degradation of different protein supplements in the rumen of steers and the effects of these supplements on carbohydrate digestion. Br. J. Nutr. 60: 669-692.
- MICHALET-DOREAU, B. & OULD-BAH, M.Y. 1989. Estimation of the extent of bacterial contamination in bag residues and its influence on *in sacco* measurements of forage nitrogen degradation in rumen. XVI Int. Grassl. Congr. Nice, France. p. 909-910.
- MOLONEY, A.P. 1991. Growth, digestibility and nitrogen retention in young friesland steers offered grass silage and concentrates which differed in protein concentration and degradability. 6th Int. Symp. Prot. Metab. and Nutr. Herning. p. 342-344.
- MOULD, F.L., ØRSKOV, E.R. & MANN, S.O. 1983. Associative effects of mixed feeds. 1. Effects of type and level of supplementation and the influence of the rumen fluid pH

- on cellulolysis *in vivo* and dry matter digestion of various roughages. *Anim. Feed Sci. Technol.* 10: 15-30.
- NOCEK, J.E. & RUSSELL, J.B. 1988. Protein and Energy as an Integrated System. Relationship of Ruminal Protein and Carbohydrate Availability to Microbial Synthesis and Milk Production. *J. Dairy Sci.* 71: 2070-2106.
- OLDHAM, J.D. 1984. Protein-Energy Interrelationships in Dairy Cows. *J. Dairy Sci.* 67: 1090-1114.
- OLSSON, I., LINDBERG, J.E. & WEJDEMAR, K. 1991. The protein value of peas for growing bull calves. 6th Int. Symp. Prot. Metab. and Nutr. Herning, Denmark. p. 321-323.
- ØRSKOV, E.R. & McDONALD, I. 1979. The estimation of protein degradability in the rumen from incubation measurements weighted according to rate of passage. *J. Agric. Sci., Camb.* 92: 499-503.
- ROOKE, J.A., BRETT, P.A., OVEREND, M.A. & ARMSTRONG, D.G. 1985. The energetic efficiency of rumen microbial protein synthesis in cattle given grass silage-based diets. *Anim. Feed Sci. Technol.* 13: 255-267.
- & ARMSTRONG, D.G. 1989. The importance of the form of nitrogen on microbial protein synthesis in the rumen of cattle receiving grass silage and continuous intrarumen infusions of sucrose. *Br. J. Nutr.* 61: 113-121.
- SATTER, L.D. & SLYTER, L.L. 1974. Effect of ammonia concentration on rumen microbial protein production *in vitro*. *Br. J. Nutr.* 32: 194-208.
- STEEN, R.W.J. 1988. The effect of supplementing silage-based diets with soya bean and fish meals for finishing beef cattle. *Anim. Prod.* 46: 43-51.
- THOMAS, C., GIBBS, B.G., BEEVER, D.E. & THURNHAM, B.R. 1988. The effect of date of cut and barley substitution on gain and on the efficiency of utilization of grass silage by growing cattle. *Br. J. Nutr.* 60: 297-306.
- UDEN, P., COLUCCI, P.E. & VAN SOEST, P.J. 1980. Investigation of chromium, cerium and cobalt as markers in digesta. Rate of passage studies. *J. Sci. Fd Agric.* 31: 625-632.
- VANHATALO, A. 1991. Kotimaisten rehujen ja niihin perustuvien dieettien sulaminen naudan ruoansulatuskanavassa. Lisensiaattityö. Helsingin yliopisto, kotieläintieteen laitos, 153 p.
- , VARVIKKO, T. & ARONEN, I. 1992. The effect of enzyme or acid based additives on digestion of grass silage by cattle. *Agric. Sci. Finl.* 1: 000-000.
- VAN KEULEN, K. & YOUNG, B.A. 1977. Evaluation of insoluble ash as a natural marker in ruminant digestibility studies. *J. Anim. Sci.* 44: 282-287.
- VARVIKKO, T. & LINDBERG, J.E. 1985. Estimation of microbial nitrogen in nylon-bag residues by feed <sup>15</sup>N dilution. *Br. J. Nutr.* 54: 473-481.

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## SELOSTUS

### Valkuaisrehun vaikutus säilörehulla ruokitun naudan pötsifermentaatioon, rehuannoksen sulavuuteen ja mikrobisynteesiin

ILMO ARONEN ja AILA VANHATALO  
Maatalouden tutkimuskeskus

Aikaisemmissa tutkimuksissa valkuaisrehujen on todettu lisäävän lihanautojen vapaaehtoista karkearehun syöntiä. On esitetty, että valkuaisrehujen karkearehun syöntiä lisäävä vaikutus perustuu pötsimikrobien lisääntyneeseen aminohappojen ja peptidien saantiin ja tehostuneeseen kuidun sulatukseen pötsissä.

Tämän tutkimuksen tarkoituksena oli selvittää, miten säilörehun lisäksi annettava valkuaisrehu vaikuttaa pötsifermentaatioon, rehuannoksen sulavuuteen ruoansulatuskanavan eri osissa ja mikrobivalkuaisen tuotantoon. Koemallitaan Latinalaisen nelion mukaisessa tutkimuksessa koeruo- kinnat olivat pelkkä säilörehu; säilörehu ja ohra (50:50); säilörehu, ohra ja rypsirouheeseen (RSM) tai lihaluurehujauhoon (MBM) perustuva valkuaiistiiviste (50:40:10). Säilörehu-ohra-ruokinnalla eläimet saivat lisäksi 23 g ureaa

d<sup>-1</sup>. Koe-eläiminä olivat neljä pötsi- ja ohutsuolifistelöityä, kasvavaa Ayrshire-hiehoa ruokintatasolla 70 g dieetin kuiva-ainetta kg<sup>-1</sup> W<sup>0.75</sup>.

Väkirehun sisällyttäminen rehuannokseen nosti pötsinesteen keskimääräistä ammoniakkipitoisuutta ja haihtuvien rasvahappojen (VFA) yhteismäärää mutta laski etikkahapon osuutta VFA:sta. Väkirehutyypistä riippumatta väkirehun sisällyttäminen rehuannokseen laski dieetin kuidun sulavuutta. Dieetillä ei ollut vaikutusta muodostuneen mikrobivalkuaisen määrään tai mikrobisynteesin tehokkuuteen. Tämän perusteella on ilmeistä, että pötsissä vapautuvien tyrellisten aineiden määrä ja laatu oli riittävä säilörehu-ruokinnalla eikä ohran ja urean, rypsirouheen tai lihaluurehujauhon lisäyksellä tässä tutkimuksessa käytetyillä annostus- tasoilla ollut vaikutusta.