The effect of type of additive on rumen fermentation and digestion of grass silage in cattle

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Four grass silages made from a second cut cocksfoot-timothy grass were ensiled with the application of water, i. e., without additive (NA), formic acid (FA), lignosulfonate + formic acid + acetic acid (LFA) and cellulase + glucose oxidase enzymes (E). The silages were fed at maintenance level to four dry cows, which had been equipped with a rumen cannula and a simple T-shaped duodenal cannula, in a digestibility experiment designed as a 4x4 latin square. The silages and a mixture of barley and oats (1:1) were given at a ratio of 70:30 on a dry matter basis.

All the silages were well preserved, but fermentation in the silo was more restricted in silages ensiled with acid-based additives. The enzyme treatment resulted in reduced levels of cell wall contents compared to the other silages. The apparent digestibilities of organic matter (OM) and neutral detergent fibre with E silage were higher ($P<0.05$) than with the other silages. The microbial N flow at the duodenum was significantly higher ($P<0.001$) with the LFA diet compared to the other diets (NA 52; FA 53; LFA 66 and E 47 g N/d) and the efficiency of microbial protein synthesis tended to be lower with the E diet compared to the other diets (NA 31; FA 31; LFA 38 and E 20 g N/OM apparently digested in the rumen).

The molar proportion of acetate in the rumen was significantly higher ($P<0.001$) and the proportion of propionate significantly lower ($P<0.001$) with acid silages than with E and NA silages. The proportion of butyrate was significantly higher with E silage compared to the others.

Key words: enzymes, acids, microbial protein, rumen degradation, mobile bag

Introduction

Ensiling grass into silage aims at maintaining the high nutritional value of original grass. This can be done using acids to create an environment with a sufficiently low pH within a short period of time to stagnate the biological activities in the grass. Alternatives to acid additives include so-called biological additives, e.g. lactic acid bacteria or fibre degrading enzymes, which develop an acidic environment through fermentation during the ensiling.

In Finland acid-based additives have been predominantly used during the past few decades in silage making to ensure sufficiently good grass silage for high-quality milk products, even for cheese making. However, as acid additives are corrosive to machinery and not user-safe, there is an increasing tendency to reduce the use of acids in silage making by replacing them with biological additives.

Thus, a number of trials have been carried out to study the response of cattle to grass silages preserv-

ed using biological or acid-based additives (e.g. GORDON 1989, HEIKKILÄ et al. 1989, JAAKKOLA and HUHTANEN 1990, JAAKKOLA et al. 1990, KENNEDY 1990, HEIKKILÄ et al. 1991). However, there are only few reports (JAAKKOLA et al. 1991, JACOBS and MCALLAN 1991, van VUUREN et al. 1991) comparing the digestibility and rumen fermentation patterns of grass silage preserved with acid and biological additives. The purpose of the present experiment was to compare digestibility, rumen fermentation, and ruminal and intestinal degradability of grass silages preserved with acid or biological additives.

Material and methods

Experimental silages

The experimental silages were prepared from a second cut cocksfoot (*Dactylis glomerata*)-timothy (*Phleum pratense*) grass by a flail harvester using:

— no additive (NA)

— AIV-2 (Valio Finnish Co-operative Dairies' Association) 5.5 l/t, containing 80 % (w/w) formic acid and 2 % orthophosphoric acid (FA)

— Farmi-solution (Farmos-Group Ltd.) 5,6 l/t, containing 50 % lignosulphonate, 25 % formic acid and 25 % acetic acid (LFA)

— Clampzyme (Finnish Sugar Ltd.) 0.2 l/t, containing cellulase and glucose oxidase enzymes (E)

All the additives were applied by a pressure pump at cutting using the application rates recommended by the manufacturers. The silages were ensiled in fibreglass silos of 3 m^3 for seven months.

Animals and their feeding

The four non-pregnant and non-lactating cows (live weight 550 kg) of Finnish Ayrshire breed used in the experiment were equipped with a rumen cannula and a simple T-piece duodenal cannula. The animals were fed in a balanced 4 x 4 latin square close to maintenance level (SALO et al. 1982), the diets consisting of experimental grass silages supplemented with a mixture of barley and oats (1:1). The ratio of forage to concentrate was 70:30 on a dry matter (DM) basis. The animals were fed twice daily in equal amounts at 12 h intervals. A commercial mineral supplement was included in the diet and water was freely available.

Experimental procedures

The length of each experimental period was 28 days with a 10-day adaptation period. Representative samples of the grass silages and concentrate mix were obtained from each experimental period.

The flow of nutrients to the small intestine was estimated using the graphic alternative (MCALLAN and SMITH 1983) of the double-marker method (FAICHNEY 1975). Cr-mordanted straw and LiCo-EDTA prepared as described by UDÉN et al. (1980) were used as a marker for the solid and liquid phase of digesta. Cr-mordanted straw was administered to the rumen from day 8 twice a day (2 x 7.5 g) and LiCo-EDTA (5g/d) was infused continuously into the rumen from day 9 onwards.

Duodenal digesta was spot-sampled on days 17, 18 and 19. Samples (150 ml) were collected at three hours' intervals, four times a day, starting at 8, 9 and 10 o'clock on consecutive collection days. The spot samples of each animal were pooled to provide one composite unrepresentative digesta sample. One half of this sample was centrifuged (700x *g*/10 min) to separate the particulate and liquid phases. The particulate phase and the other half of the digesta sample were dried at 60 °C and milled to pass a 1 mm screen. The liquid phase was stored at -20 °C before analysis.

In order to determine the overall digestibility of nutrients using acid-insoluble ash as a natural marker, faecal grab samples were taken twice a day from day 14 to day 18 when feeding the animals.

To estimate the rumen liquid outflow rate, a single dose of LiCo-EDTA (8 g) was infused into the rumen during one feeding interval. The infusion was started at evening feeding on day 19 and continued till the next morning feeding. On day 20, the ruminal and duodenal digesta samples were collected before morning feeding and at 1.5 hours'

intervals thereafter during the daytime feeding cycle. The liquid phase of the samples was separated and stored as described above.

Nitrogen of microbial origin in duodenal N was measured using purine bases of nucleic acids as markers (ZINN and OWENS 1986). The contents of the purine bases in the microbial mass were analyzed from the rumen samples collected on day 21 immediately before morning feeding and four hours thereafter. The microbial mass was isolated by separating the rumen liquid as described earlier and by further centrifuging (26000x *g*/20 min) the supernatant.

To measure rumen fermentation, samples of rumen fluid were collected on day 21 right before feeding and 0.5, 1, 2, 3, 4, 6, 8 and 10 h after feeding. The ammonia-N and pH of the digesta were measured immediately, and samples for the measurement of volatile fatty acids (VFA) were storaged with Ag_2O in a refrigerator for later analysis.

To determine the rumen degradability and subsequent intestinal degradability of the experimental silages, nylon bag techniques were used. Each experimental silage was incubated in the animal fed with that particular silage diet. Five nylon bags (60 x 120 mm) made of polyester (PES 41 µm/33 %, Polymon, Switzerland) containing fresh, chopped (< 10 mm) silage (2.5 g DM) were inserted into the rumen on day 14 and incubated for periods of 2, 4, 8, 16, 24, 48, 72 and 96 h. After removal, the bags were machine-washed and dried at 60 °C in a forced-draught oven.

Fifteen mobile bags (35 x 50 mm, PES 10 µm/2 %/C) per experimental silage were introduced into the duodenum from day 14 onwards. Each bag contained 0.8 g DM freeze-dried or rumen-incubated (30 h) silage, that was milled through a 2 mm mesh. One animal received a maximum of 20 mobile bags per day. Subsequently, the bags were collected from the faeces and machine-washed (40 °C) and dried at 60 °C.

Chemical analyses

The DM content of the grass silages was determined by oven-drying (105 °C), correcting the values for volatile losses according to HUIDA et al. (1986). Organic matter (OM) content was measured by ashing at 500 °C for 2 h and total N by the Kjeldahl method. Determinations of neutral detergent fibre (NDF) and acid detergent fibre (ADF) were made according to VAN SOEST (1963) and VAN SOEST and WINE (1967), calculating the content of hemicellulose as the difference between NDF and ADF and the content of lignin as the difference between ADF and cellulose. Experimental silages as well as rumen liquor and the liquid phase of duodenal digesta were analyzed for ammonia-N (MCCULLOUGH 1967). Silages and rumen liquor were further analyzed for VFAs (HUIDA 1973). Also the lactic acid concentration (BARKER and SUMMERSON 1941) and water soluble carbohydrates (WSC) (SOMOGYI 1945) in the silages were measured. Nucleic acids in the microbial fraction and duodenal digesta were determined according to ZINN and OWENS (1986). Chromium and cobalt concentrations in faecal and digesta samples were determined by atomic absorption spectrophotometry (WILLIAMS et al. 1962). Faecal samples were analyzed also for acid-insoluble ash (VAN KEULEN and YOUNG 1977).

Calculations and statistical analysis

The duodenal flow of nutrients was calculated based on the amounts of Co and Cr excreted in faeces. The liquid outflow rate from the rumen was calculated as the slope of the regression of the natural logarithm of the Co concentration against time. The total outflow of liquid at the duodenum was calculated as the difference between total digesta flow and DM flow. The estimate of rumen volume was calculated as total outflow (l)/[liquid dilution rate (1/h) x 24 (h)].

The rumen degradability of feed N *in vivo* was estimated by assuming an endogenous N flow of 15 % of the duodenal N flow (TAMMINGA et al. 1989).

To characterize the rumen degradability of the experimental silages, their degradability values were fitted in to the following equations (MCDONALD 1981):

p_1=a' up to time t_o
p_2=a+b(1-e^{-ct}) from time t_o onwards,
where a' represents the rapidly degradable fraction of the feed during the washing ('0 h wash'), p_2 is the proportionate disappearance of feed after time t (h) (t>t_o) and a, b and c are the constants for the instantly degradable and slowly degradable fraction of the feed and rate of degradation of the latter from time t_o onwards. The lag time, t_o, was calculated as: t_o=1/c ln[b/(a+b-a')].

The values obtained for N were corrected for microbial contamination as suggested by LINDBERG (1988).

The total tract degradability (TTD) of the feeds was calculated as a sum of the 30-h ruminal degradation and the subsequent intestinal degradation of the undegraded feed residue.

The standard analysis of variance appropriate to the latin square design was applied to digestibility and nylon bag data using the Tukey's test for treatment comparisons.

Rumen fluid data was analyzed by analyses of variance using the following model:

$y_{ijklm} = \mu... + A_i + P_j + T_k + e_{ijk} + H_l + AH_{il} + PH_{jl} + TH_{kl} + e_{ijklm}$,

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

Dilution rate data were analyzed using the same model as with rumen fluid data, with the exception that the effect of sampling site replaced sampling time in the model. Tukey's test was used for the comparison of the treatments.

Table 2. The fermentation characteristics of the silages preserved with different additives.

	Silage additive				Grass
	NA	FA	LFA	E	
pH	4.1	4.2	4.1	4.1	
In dry matter (g/kg dry matter)					
WSC	18	89	18	24	91
Lactic acid	92	11	60	86	
Acetic acid	14	7	20	10	
Formic acid	-	21	8	-	
Total acids	106	39	88	96	
Ethanol	5	11	19	8	
Lactic/Acetic	6.9	1.7	3.1	8.6	
In total N (g/kg)					
Ammonia N	47	18	45	55	
Soluble N	541	432	467	499	22

For silage additives, see text.
WSC, water soluble carbohydrates.
None of the silages contained propionic or butyric acid.

Table 1. The chemical composition (g/kg dry matter) of the silages, grass and concentrate.

	Silage additive				Grass	Concentrate
	NA	FA	LFA	E		
Dry matter, g/kg	177	196	175	187	173	888
Ash	100	93	99	95	95	28
Nitrogen	28	28	29	28	27	19
NDF	531	541	541	508	589	269
ADF	320	319	319	299	305	90
Cellulose	280	280	279	260	262	70
Hemicellulose	211	221	221	207	284	179
Lignin	40	39	40	40	43	20

For silage additives, see text.
NDF, neutral detergent fibre; ADF, acid detergent fibre.

Results

Chemical composition and fermentation of the silages

The chemical composition (Table 1) and fermentation (Table 2) of the silages were clearly affected by the additive used. The DM content was highest in FA silage, and E silage had the lowest contents of cell walls. Compared with E and NA silages, fermentation was restricted during ensiling by the acid-based additives, particularly FA. The content of WSC in FA silage was close to that in original grass, and ammonia-N and total acid content was low compared with the other silages.

Intake and digestion of organic matter and fibre

There was no difference ($P>0.05$) in OM intake or in faecal OM between the silages (Table 3). However, compared with the other silages, the amount of OM entering the duodenum was lower ($P<0.01$) in E silage and the microbial OM higher ($P<0.001$) in LFA silage. Digestibility of OM in the rumen, disappearance of digestible OM before the intes-

Table 3. The effect of silage additive on organic matter (OM) and cell wall digestion.

Silage additive...	NA	FA	LFA	E	SEM	Statistical significance of additive
Organic matter (g/24h)						
In feed	4633	4714	4886	4746	67.9	NS
At duodenum	2907 b	2994 b	3101 b	2339 a	80.7	**
Microbial OM§	580 a	590 a	729 b	520 a	18.3	***
In faeces	1040	1078	1127	978	31.9	NS
Digestibility in the rumen						
Apparent	0.370 a	0.366 a	0.364 a	0.510 b	0.0227	**
True†	0.496 a	0.493 a	0.512 a	0.619 b	0.0213	*
Disappearance of digestible OM before intestine						
Apparent	0.477 a	0.476 a	0.473 a	0.642 b	0.0293	*
True	0.640 a	0.640 a	0.666 ab	0.779 b	0.0275	*
Apparent digestibility	0.774	0.771	0.769	0.794	0.0052	*
Neutral detergent fibre (g/24h)						
In feed	2253 ab	2329 ab	2441 b	2230 a	41.9	*
At duodenum	675	697	711	561	32.8	NS
In faeces	586	595	615	516	23.4	NS
Digestibility						
Rumen	0.699	0.699	0.708	0.750	0.0109	*
Total	0.738	0.743	0.747	0.769	0.0093	NS
Total digestibility						
Hemicellulose	0.709	0.717	0.710	0.741	0.0101	NS
Cellulose	0.799	0.798	0.815	0.823	0.0100	NS

For silage additives, see text.
§ Assuming a N:OM ratio of microbial matter 0.09 (CZERKAWSKI 1986).
† Corrected according to microbial organic matter.
a,b Means in the same row with different superscripts were significantly different ($P<0.05$).
NS, not significant; *, $P\leq0.05$; **, $P\leq0.01$; ***, $P\leq0.001$.

tine, and apparent total OM digestibility were highest (P<0.05) with E treatment. The intake of NDF and ADF (data not shown) was lower and their digestion in the rumen or in the total tract tended to be higher with E-treated silage than with the other silages (Table 3). Total digestibility of hemicellulose and cellulose tended to be highest with E treatment as well.

Intake and digestion of nitrogen

Nitrogen intake, faecal N and apparent digestibility of N were similar (P>0.05) for all the diets (Table 4). Non-ammonia-N (NAN) and feed N entering the small intestine were lower (P<0.05) for E treatment as compared with the others, while microbial N was highest (P<0.001) and microbial N synthesis most efficient (P<0.05) for LFA treatment. Silage-N degradability was lowest in FA silage.

Ruminal and intestinal degradability of silages

Except for the potential degradability *(a+b)* of cell walls (P<0.05), no statistically significant differences were found between the silages in the constants describing the degradability of OM, NDF or N in the rumen. However, there was a tendency towards lower *a* and higher *b* values, and effective protein degradability (EPD) was always lower for silages ensiled with acid-based additives (Table 5). The correction of EPD values for microbial protein according to LINDBERG (1988) increased all the values notably, the EPD for FA silage being significantly (P<0.001) lower than that of the other silages.

No statistically significant differences (P>0.05) were found in lag time, 30-h rumen degradation, intestinal degradation, or total degradation of the nutrients between the silages.

Table 4. The effect of silage additive on N intake, flow of N to the duodenum and efficiencies of microbial protein synthesis.

Silage additive...	NA	FA	LFA	E	SEM	Statistical significance of additive
Nitrogen (g/24h)						
In feed	127	129	138	132	2.6	NS
At duodenum						
Total-N	174 b	179 b	188 b	138 a	5.1	**
Ammonia-N	4	5	6	3	0.5	NS
NAN	169 b	174 b	182 b	135 a	5.0	**
Microbial N	52 a	53 a	66 b	47 a	1.7	***
Feed N§	91 b	94 b	89 b	67 a	4.5	*
In faeces	30	32	32	30	1.3	NS
Apparent digestibility	0.762	0.753	0.765	0.772	0.0090	NS
Silage-N degradability	0.284	0.271	0.353	0.492	0.0050	*
NAN at duodenum/N intake	1.34 b	1.35 b	1.32 b	1.02 a	0.005	*
Microbial N g/kg OMADR†	31 ab	31 ab	38 b	20 a	2.5	*
Microbial N g/kg OMTDR‡	23 b	23 b	26 b	16 a	1.3	**
Microbial N g/kg DCHO*f*	19 b	19 b	23 c	16 a	0.5	***

For silage additives, see text.
§ assuming endogenous flow of N to be 15 % of duodenal N flow (Tamminga et. al. 1989).
† Organic matter apparently digested in the rumen.
‡ Organic matter truly digested in the rumen.
f Digestible carbohydrates.
a,b Means in the same row with different superscripts were significantly different (P≤0.05).
NS, not significant; *, P≤0.05; **, P≤0.01; ***, P≤0.001.

Table 5. The rumen degradation characteristics (a, constant for the instantly degradable fraction; b, constant for the slowly degradable fraction; c, rate of degradation of b) of silage organic matter, neutral detergent fibre and nitrogen and intestinal degradation of their rumen undegraded feed residues.

Silage additive...	NA	FA	LFA	E	SEM	Statistical significance of additive
Organic matter						
Rumen degradation parameters						
a	0.240	0.165	0.192	0.227	0.0212	NS
b	0.622	0.685	0.659	0.626	0.0196	NS
a+b	0.862	0.850	0.851	0.853	0.0038	NS
c	0.062	0.058	0.056	0.064	0.0059	NS
Lag time (h)	1.7	1.8	1.9	2.3	0.59	NS
Degradability of feed						
in the rumen*f*	0.720	0.685	0.695	0.703	0.0160	NS
in the intestine§	0.102	0.112	0.102	0.112	0.0076	NS
in the total tract	0.749	0.720	0.727	0.737	0.0132	NS
Neutral detergent fibre						
Rumen degradation parameters						
a	-0.035	-0.110	-0.091	-0.133	0.0274	NS
b	0.842	0.902	0.882	0.910	0.0251	NS
a+b	0.807b	0.792ab	0.791ab	0.777a	0.0050	*
c	0.059	0.057	0.054	0.062	0.0054	NS
Lag time (h)	2.0	2.1	2.2	2.9	0.45	NS
Degradability of feed						
in the rumen*f*	0.592	0.556	0.554	0.542	0.0254	NS
in the intestine§	0.061	0.064	0.063	0.063	0.0065	NS
in the total tract	0.614	0.584	0.581	0.574	0.0228	NS
Nitrogen						
Rumen degradation parameters						
a	0.435	0.315	0.398	0.452	0.0436	NS
b	0.487	0.607	0.528	0.468	0.0378	NS
a+b	0.922	0.922	0.926	0.920	0.0079	NS
c	0.085	0.072	0.074	0.077	0.0071	NS
EPD†	0.779	0.730	0.757	0.777	0.0123	NS
EPD‡	0.912b	0.875a	0.896b	0.912b	0.0047	***
Lag time (h)	4.1	1.9	1.8	2.0	1.07	NS
Degradability of feed						
in the rumen*f*	0.864	0.829	0.843	0.842	0.0105	NS
in the intestine§	0.541	0.566	0.527	0.564	0.0205	NS
in the total tract	0.939	0.926	0.927	0.931	0.0045	NS

For silage additives, see text.
f Incubated in the rumen for 30 h.
§ To prepare the rumen undegraded feed residue rumen incubation period of 30 h was used.
† Effective protein degradability calculated with k=0.033.
‡ EPD corrected for microbial contamination as suggested by LINDBERG (1988).
a,b Means in the same row with different superscripts were significantly different (P≤0.05).
NS, not significant; *, P≤0.05; ***, P≤0.001.

Rumen fermentation and liquid dilution rate

The average rumen pH was not affected by the silage additive (Table 6). The concentration of ammonia-N in the rumen was significantly ($P<0.05$) higher for LFA and NA diets as compared to the other diets.

The concentration of total VFA was not affected by the silage additive, but the molar proportion of acetate was significantly ($P<0.001$) higher and that of propionate lower with FA and LFA than with E and NA silages (Table 6). The proportion of butyrate was significantly higher with E treatment compared to the others. Statistically significant differences between the curve patterns were not found for any of the fermentation parameters measured.

The liquid dilution rate tended to be higher with FA and LFA silages compared to NA and E silages (Table 7). The estimate of rumen volume was smallest with E silage and largest with NA silage. The liquid outflow rate from the rumen was slowest with E silage, the value being significantly ($P<0.05$) different from those with LFA and NA silages.

Table 6. The effect of silage additive on rumen fermentation.

Silage additive...	NA	FA	LFA	E	SEM	Statistical significance of additive
pH	6.75	6.73	6.80	6.73	0.065	NS
Ammonia N (mmol/l)	8.88 b	8.86 b	10.51 a	10.17 a	0.969	***
Total VFA (mmol/l)	96.9 b	100.3	97.4	100.0	3.76	NS
Molar proportion of VFAs (mmol/mol)						
Acetic acid	655 b	695 a	690 a	649 b	7.5	***
Propionic acid	221 a	169 d	181 c	206 b	7.9	***
Butyric acid	96 c	107 b	96 c	112 a	3.3	***
Isovaleric acid	18.1 b	18.8 cb	22.2 a	20.6 ab	1.87	***
Valeric acid	10.8 b	10.8	11.9	11.5	1.24	NS
Ratio (Ac+Bu)/Pr	3.5 d	4.8 a	4.4 b	3.8 c	0.19	***
Ratio Pr/Bu	2.4 a	1.6 c	1.9 b	1.9 b	0.12	***

For silage additives, see text.
Ac, acetic acid; Pr, propionc acid; Bu, butyric acid.
a,b,c,d Means in the same row with different superscripts were significantly different ($P\leq0.05$).
NS, not significant; ***, $P\leq0.001$.

Table 7. The effect of silage additive on rumen outflow rate (D), rumen volume and liquid outflow in cattle.

Silage additive...	NA	FA	LFA	E	SEM	Statistical significance of additive
Liquid D (1/h)	0.075	0.092	0.091	0.082	0.0054	NS
Rumen volume (l)	54.6	46.6	46.9	40.1	3.83	NS
Liquid outflow (l/d)	95.6 b	91.8 ab	98.7 b	75.3 a	3.91	*

For silage additives, see text.
a,b Means in the same row with different supercripts were significantly different ($P\leq0.05$).
NS, not significant; *, $P\leq0.05$.

Discussion

Silage quality

In accordance with previous results (HENDERSON et al. 1982, KENNEDY 1990, HEIKKILÄ et al. 1989, VAN VUUREN et al. 1989, JAAKKOLA 1990, JAAKKOLA et al. 1990, JACOBS and MCALLAN 1991, HEIKKILÄ et al. 1991), the cell wall content of E silage found to be clearly reduced compared to others indicating the activity of enzymes to hydrolyze cell walls to WSC.

Dry matter content was highest in FA silage reflecting higher effluent losses compared to those from other silages (4.1 vs. average 1.7 % DM, see TOIVONEN 1989). The E and NA silages were notably more fermented than the LFA and especially FA silages preserved with acid-based additives. Similar differences between silages have been found in previous comparative studies (RAURAMAA et al. 1987, JAAKKOLA et al. 1991, JACOBS and MCALLAN 1991). In accordance with RAURAMAA et al. (1987), only minor differences in fermentation patterns were found between E and NA silage. However, the use of enzymes in silage making especially under poor weather conditions has resulted in more favourable fermentation than in ensiling without additives (JAAKKOLA 1990, JAAKKOLA et al. 1991). Obviously the use of pilot silos in making the experimental silages resulted in good-quality fermentation in NA silage, too.

Digestion of organic matter and fibre

The proportion of digestible OM apparently digested before the intestine was rather low (average 0.52) and the total N entering the duodenum was rather high (Tables 3 and 4). These values may be affected by duodenal digestive juices, since the duodenal cannulas were checked at slaughter and found to be located posterior to the pancreatic duct. However, the disappearance of digestible fibre before the small intestine indicated no overestimation (Table 3). Hence, the possible contamination should have no influence on the comparison of the treatments.

JAAKKOLA et al. (1991) could find no marked improvements in the digestibility of grass silage OM through the use of cell wall degrading enzymes. However, in the present study, in agreement with the experiment of JACOBS et al. (1991) with growing steers, the OM digestibility of E silage was significantly higher than that of untreated or acid treated silages (Table 3). In accordance with the results of JAAKKOLA et al. (1991), the digestibility of cell walls also tended to be higher for the E silage diet as compared to the others. Several previous reports have pointed out that enzyme treatment impaired rather than improved the fibre digestibility of grass silage with sheep (HEIKKILÄ et al. 1989, TOIVONEN 1989, JAAKKOLA 1990, HEIKKILÄ et al. 1991), and also with growing cattle (JAAKKOLA and HUHTANEN 1990, JAAKKOLA et al. 1990).

It has been suggested that the higher digestibility of cellulose in E silage compared to FA and NA silages with cattle is a consequense of a longer rumen residence time (RRT) (JAAKKOLA et al. 1991). A longer rumen retention time has been observed for cattle given forage diets than for sheep (REES and LITTLE 1980), especially at low levels of feeding (COLUCCI et al. 1984).

The RRT of the silages was not measured in the present experiment, but as the proportion of digestible OM and NDF digested in the rumen was highest with E silage, the higher digestibility of E silage may be related to possible differences in RRT between the silages. The contradiction in results between the experiments may be related at least partly to the different feeding levels. In the present experiment, the cattle were fed near maintenance level in contrast to experiments performed with growing cattle, or with another species, sheep.

Digestion of nitrogen

The NAN flow entering the duodenum was significantly lower with the E diet than the other diets (Table 4), mainly due to the significantly lower feed N flow. The flow of microbial N was clearly highest for the LFA diet. In the study of JACOBS and

McAllan (1991), the flow of nitrogenous compounds was similar for untreated, FA and enzyme silages. On the other hand, Jaakkola et al. (1991) found that the quantity of microbial N entering the duodenum was significantly higher with FA silage than with E silage. The contradiction between the experiments may be due to the different application rate of formic acid used, resulting in differences in the extent of fermentation. When feeding restrictively fermented silages, more fermentable carbohydrates are available for rumen microbes than from extensively fermented silages (Chamberlain 1987). This is possibly one reason for the higher microbial N obtained with the LFA diet in the present experiment. The advantage of restricted fermentation of FA silage in the present experiment was probably lost because of considerable effluent losses in the silo (Toivonen 1989) due to the reduced moisture-holding capacity of FA silage (Woolford 1978).

Supplementation of grass silage with concentrate has generally increased the efficiency of microbial protein synthesis in the rumen (ARC 1984). The present average efficiency of 30 g microbial N/organic matter apparently digested in the rumen (OMARD) is consistent with the value given for similar feeding by ARC (1984). The lowest efficiency, which was obtained for the E diet (Table 3), contrasts with the results of Jacobs and McAllan (1989, 1991), who reported higher efficiency for E feeding compared with FA feeding, especially when the E diet was supplemented with rapeseed meal. Jacobs and McAllan (1989) concluded that enzyme treatment of the grass during ensiling had a marked effect on the availibility or utilizability of structural carbohydrates. Nevertheless, the present results rather support the suggestion by Jaakkola et al. (1991) that not even a high-quality supplement compensates for the difference in silage quality caused by lactic acid fermentation.

Degradability of silages in the rumen and the intestine

Compared with the acid-based additives, the E additive tended to increase the instantly degradable fraction *a* of OM and N of E with no effect on potential degradation, but it decreased the potential degradability of the cell walls. This finding was in line with the earlier results of Huhtanen et al. (1985) and Van Vuuren et al. (1989) indicating that the breakdown of cell walls occurs in the silo rather than in the rumen.

The EPD values and rate of degradation *c* of silages preserved with acid additives tended to be lower than those of other silages, in accordance with the results of Setälä et al. (1985) and Vik-Mo (1989) which indicate that the rate of crude protein degradability was regulated mainly by the proteolysis in the silage. Nevertheless, the EPD values of grass silages were high and the correction for microbial contamination still increased them notably, in agreement with the results of Lindberg (1988). This emphasizes the need to take the correction into account when calculating the EPD values of forages. The degradability of silage-N *in vivo* (Table 4) was notably lower than the respective values obtained with the nylon bag method. As the *in vivo* value was calculated by difference, possible errors in the determination of microbial or endogenous N will be accumulated in the fraction of feed N. However, both methods ranked the value for FA silage as lowest.

Intestinal OM, NDF and N degradability of rumen-undegraded feed was rather low regardless of the additive used, on an average 10.7 % for OM, 6.3 % for NDF and 55.0 % for N. In a previous experiment (Varvikko and Vanhatalo 1988), the respective value for grass silage N was also low, 57 % on an average, suggesting that the values used in protein evaluation systems, e.g. 85 % by ARC (1984), overestimate the intestinal degradability of grass silage N.

Rumen fermentation and liquid dilution rate

Consistent with the results of Gordon (1989), but not with those of Jaakkola et al. (1991), the molar proportion of acetate in the rumen fluid was found to be significantly higher with acid-treated silages

than with E or NA silages. In accordance with previous reports (GORDON 1989, JAAKKOLA et al. 1991), the molar proportion of propionate in the rumen fluid was significantly higher in E and NA feeding, i. e., in silages with a high content of lactic acid, than in FA and LFA feedings. Actually, the propionate increased along with the increasing lactate content in the silage, indicating the conversion of silage lactate to propionate in the rumen (CHAMBERLAIN et al. 1983, NEWBOLD et al. 1987, JAAKKOLA and HUHTANEN 1989). Contrary to the results of GORDON (1989) and JAAKKOLA et al. (1991), the proportion of butyric acid for diet E was higher than for diet FA. When compared with untreated silage, a small increase in the proportion of butyric acid was found with E silage (VAN VUUREN et al. 1991). However, the changes in rumen VFAs found in the present experiment were commensurate to the reduction in milk fat content with E silage feeding compared to FA silage feeding in dairy cows (HEIKKILÄ et al. 1989, 1991).

Again in contrast to previous results (JAAKKOLA et al. 1991, JACOBS and MCALLAN 1991), the liquid dilution rate, rumen volume and liquid outflow rate from the rumen were different for the E diet compared to the other diets in the present study. However, also in the experiment by JACOBS and MCALLAN (1991), the estimated rumen volume tended to be smaller for the E diet compared to the FA and NA diets. The liquid dilution rate did not seem to be associated with microbial protein synthesis either in this study or in the study by JAAKKOLA et al. (1991), even though the differences between the diets in synthesized microbial protein were quite clear in both of experiments.

In conclusion, acid-based additives resulted in more restricted fermentation during ensiling than did enzymes or the absence of additive. The improved OM and cell wall digestibility *in vivo* of the E diet as compared to the other diets were obviously a consequence of the low feeding level in the present experiment. The highest efficiency of microbial protein synthesis in the rumen obtained for LFA feeding was attributed to the small effluent losses and the restricted fermentation of silage in the silo. Regardless of the additive used, the EPD values of the silages were high, especially when corrected for microbial contamination, and the values for intestinal degradation of rumen-undegraded N were low compared to the values used in protein evaluation systems.

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SELOSTUS

Eri säilöntäaineiden vaikutus säilörehun laatuun sekä säilörehudieetin sulavuuteen, mikrobisynteesiin ja pötsifermentaatioon naudalla

AILA VANHATALO, TUOMO VARVIKKO ja ILMO ARONEN
Maatalouden tutkimuskeskus

Tutkimuksessa verrattiin eri säilöntäaineilla valmistettujen säilörehujen vaikutusta naudan pötsifermentaatioon, ravintoaineiden virtaukseen ohutsuoleen ja mikrobisynteesiin. Säilörehut tehtiin koiranheinä-timotei-nurmen toisesta sadosta seuraavilla säilöntäaineilla: ilman säilöntäainetta (NA), muurahaishappo (FA), lignosulfonaattien, muurahais- ja etikkahapon seos (LFA) ja sellulaasia ja glukoosioksidaasia sisältävä entsyymiseos (E). Koe-eläiminä oli neljä ylläpitotasolla ruokittua pötsi- ja ohutsuolifistelöityä hiehoa (elopaino 550 kg). Koe järjestettiin 4x4 latinalaisen neliön mukaan ja koeruokintoihin sisältyi tutkittavan säilörehun lisäksi väkirehua (ohra-kaura 1:1) 30 % dieetin kuiva-aineesta.

Kaikki säilörehut olivat hyvälaatuisia, mutta hapoilla säilötyt rehut olivat vähemmän käyneitä kuin entsyymi- ja painorehu. Entsyymirehun kuitupitoisuus oli alempi kuin muiden rehujen. E dieetin orgaanisen aineen ja kuidun näennäiset sulavuudet olivat merkitsevästi korkeampia ($P<0.05$) kuin muiden dieettien. Mikrobivalkuaisen virtaus ohutsuoleen oli merkitsevästi korkeampi ($P<0.001$) LFA dieetillä (NA 52; FA 53; LFA 66 ja E 47 g N/d) ja mikrobisynteesin tehokkuus alempi E dieetillä muihin verrattuna (NA 31; FA 31; LFA 38 ja E 20 g N / pötsissä näennäisesti sulanut orgaaninen aine). Etikkahapon osuus pötsin haihtuvista rasvahapoista oli FA ja LFA dieeteillä merkitsevästi korkeampi ($P<0.001$) ja propionihapon osuus merkitsevästi alempi ($P<0.001$) kuin E ja NA dieeteillä. Voihapon osuus oli E dieetillä merkitsevästi ($P<0.001$) muita korkeampi.