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The effect of additives on quality, protein degradability, intestinal digestibility and feed intake of wilted grass silages

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ABSTRACT

First-cut wilted herbage (mainly *Lolium perenne)* was ensiled in barrels (120 l) either untreated (UT), treated with an inoculant, Pioneer 1188 (IN), or with a formic acid-based chemical additive, Foraform (FO). IN silage contained more fermentation products than FO, although applying the inoculant to wilted grass did not result in consistent changes in silage fermentation. Formic acid significantly decreased ($P<0.05$) the content of lactic and acetic acids and of ammonia-N, but increased the water soluble carbohydrates concentration as compared with UT and IN silages. Ruminal effective protein degradability was slightly but not significantly lower for FO silage (71.6%) than for UT (74.3%) and IN (74.0%) silages. Formic acid treatment significantly increased ($P<0.05$) intestinal digestibility of rumen-undegraded protein. Dry matter intake measured with sheep was not affected by treatments.

KEY WORDS: silage, inoculant, formic acid, rumen protein degradability, intestinal digestibility, intake

INTRODUCTION

Organic acids (propionic and formic acids) and inoculants containing lactic acid bacteria can improve fermentation quality and reduce DM losses of grass silages. Many studies have shown the positive effects of such additives on silage intake, animal performance, aerobic stability also when untreated silages were well

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preserved (Selmer-Olsen and Mo, 1997; Huhtanen, 2001). Rooke et al. (1983) and Mc Donald et al. (1991) found that protein in grass silage is rapidly degraded in the rumen and the resulting ammonia-N may be poorly utilized for microbial N synthesis. The requirements for RUP (ruminal undegraded protein) increases with the yield of dairy cows (NRC, 2001). The supply of protein can be increased by reducing the ruminal degradation of dietary protein and increasing the amount of protein digested postruminally. The effects of forage preservation methods and impact of silage additives on ruminal protein degradability were studied (Rooke et al., 1983; Polan et al., 1998). Silage additives may decrease proteolysis during ensiling by stimulating rapid fermentation or by direct reduction of pH. There is no clear evidence that decrease of proteolysis by silage additives is correlated with protein degradability in the rumen. Jaakkola et al. (1993) showed that protein protected from proteolysis in the silo is still degradable in the rumen.

Relatively small differences in silage fermentation characteristics can markedly influence dry matter intake. Huhtanen et al. (2001) suggested that reduced silage intake of badly or extensively fermented silages can be related to low palatability, clearance rate of ruminal digesta, and an imbalanced amino acid-to-energy ratio at the tissue level. Application of formic acids efficiently restricted the extent of fermentation and proteolysis in the silo, which could have resulted in higher DM intake and production compared with untreated silage (Shingfield et al., 2001). Silage inoculation improved feed intake from 5-11% in 25-40% of the studies reviewed (Weinberg et al., 2003).

The objective of this study was to determine the effect of two commercial additives, biological and chemical, on fermentation, protein degradability in the rumen, and intestinal digestibility of silages produced from wilted grass.

MATERIAL AND METHODS

Ensiling procedure and treatments

Silages were made from the first cut of pasture herbage containing 90% grass (mainly *Lolium perenne*) and 10% of legumes. About 900 kg of herbage wilted for 24 h under very good weather conditions was harvested untreated with a forage-loading wagon and transported to the laboratory. Herbage was preserved without additives (UT), with an inoculant (IN; Pioneer 1188; Hi-Bred Services G.m.b.H.), or with a chemical additive, Foraform (FO; A/S NOFO, Norway).

The inoculant contained four strains of *L. plantarum* and two strains of *E.faecium* (Pioneer Brand 1188) and was applied in liquid form by hand sprayer at a rate of 30 ml kg^{-1} and 10^5 CFU g^{-1} of fresh matter. Foraform contained 645 g kg^{-1} formic acid and 60 g kg^{-1} NH_3 was applied at a rate of 4 ml kg^{-1} by hand sprayer.

Silages were prepared in fifteen PCV barrels with a capacity of 120 l (3 treatments, 5 replications) and stored for 90 days at room temperature. Frozen silage samples (one from each barrel) were chopped in a meat chopper and were subsampled prior to chemical analyses and *in sacco* procedure.

Chemical analyses

Silage dry matter (DM) content was determined by drying at 105°C for 24 h, and was not corrected for volatile fatty acids (VFA) or ethanol; ADF and NDF were determined according to Van Soest et al. (1991) on a Tecator apparatus. Crude protein and crude fibre were estimated according to standard methods (AOAC, 1990). Water soluble carbohydrates (WSC) were determined according to the modified method of McDonald and Henderson (1964). Silage ammonia nitrogen in FO silage was corrected for the ammonia-N applied with this additive.

In sacco and mobile bag study

Two non-lactating Jersey cows weighing 475 kg, fitted with ruminal and proximal duodenal cannulas were used. The cows were kept in individual pens and were fed a diet consisting of meadow hay and 2 kg of concentrate.

Quadruplicate nylon bags (2 bags per cow) were filled with 5 g of silage sample (dried at 50°C) and incubated for 0, 2, 4, 8, 12, 16, 24 and 48 h in the rumen. Four extra sample bags were incubated 12 h to determine crude protein intestinal digestibility using the mobile nylon bag method. The bags (200 × 90 mm, pore size 45 μm) were attached to a semi-rigid stalk and in turn were attached to a swivel-connector inside the rumen fistula cap. The bags were placed simultaneously in the rumen just before the animals were offered the first meal of the morning (7.00 a.m.). After removal from the rumen, the bags were washed with cold water and stored frozen. After thawing, the bags were washed, and then dried at 50°C for 48 h and weighed. Zero h bags were not incubated but only washed. Based on dry matter digestibility in particular periods of incubation, the constant rate was calculated using a mathematical model elaborated by Ørskov and McDonald (1979). Data were fitted to a non-linear regression equation, $P = a + b\ (1 - e^{-ct})$, where 'a' represents the rapidly soluble fraction, 'b' represents the less rapidly degradable fraction that disappears at a constant rate 'c' per unit time. The three constants were then used to calculate the effective protein degradability (EPD) by the equation $EPD = a + [(b \times c) / (c + k)]$ assuming a fractional outflow rate (k) of 0·06 h^{-1}. Degradation constants a, b, c were calculated using the Statgraphics (ver. 5.0) statistical package.

Four mobile bags (80 × 25 mm, pore size 9 μm) per treatment were filled with one gram samples of the residue after 12 h ruminal digestion. The bags were incubated for 2 h at 39°C in pepsin-HCl solution (100 mg pepsin-1: 10000 l^{-1} of 0.004 mol l^{-1} HCl

solution, pH 2.4) as recommended by Madsen et al. (1995) and inserted into the intestine *via* a duodenal fistula approximately one h after feeding (8 bags per cow per 1 h). The bags were recovered in the faeces within 26 h. Bags not recovered within 30 h were discarded. After recovery, the bags were washed, then frozen, dried and weighed as described previously. The digestibility of undegradable protein was calculated as the amount of N lost from the bag during the passage through the intestines divided by the amount of N in the bag before incubation. Total tract digestibility was calculated by equation: TD = RD12 + (100-RD12) x IDUP/100, where TD represents total digestibility, RD12 ruminal disappearance for 12 h incubation, and IDUP intestinal digestibility of undegraded protein.

Dry matter intake

Dry matter intakes of silages were determined using four rams (average weight 41 kg) per sample in two 14-day-experimental periods. In the first period the diet was composed of silage given *ad libitum* and 0.5 kg of barley grain-based concentrate (15% CP). In the second period, only experimental silages were fed.

Statistical analysis

The results obtained were subjected to one-way analysis of variance. Significance was declared at $P<0.05$.

RESULTS AND DISCUSSION

Table 1 presents silage composition and fermentation quality. There was no significant effect ($P>0.05$) of silage additives on chemical composition (crude protein, crude fibre, ADF and NDF). All silages were well preserved with low butyric acid and ammonia-N contents. The low level of ethanol indicates a low rate of alcoholic fermentation (Selmer-Olsen and Mo, 1997). There was no significant effect of either additive on the ethanol content in silage. In contrast, O' Kiely (1996) found a significant increase in ethanol concentration in silages with formic acid additives. Jaakkola et al. (1993) stated that the effect of formic acid on ethanol depends very much on the application rate; high rates (about 4 lt^{-1}) sometimes decreased the alcohol content, while low rates (2 lt^{-1}) often increased it.

IN silage contained more fermentation products than FO-treated silage, although applying inoculant did not result in a consistent change in the silage fermentation. The difference in fermentation parameters between IN and UN silages was small, possibly without any biological significance, similarly as found by others (Gąsior and Brzóska, 2000). The high DM content of the silages may explain the small dif-

TABLE 1

Chemical composition of grass and silages

Items	Grass	Silages			SEM
		UT	IN	FO	
Dry matter, g kg^{-1}	171	480	491	473	12
Crude protein, g kg^{-1} DM	165	128	125	123	2.1
Crude fibre, g kg^{-1} DM	268	294	301	304	5.0
ADF, g kg^{-1} DM	314	340	355	353	3.6
NDF, g kg^{-1} DM	476	529	530	539	4.5
Lactic acid, g kg^{-1} DM	-	108.6^{a}	116.4^{a}	87.4^{b}	2.8
Acetic acid, g kg^{-1} DM	-	7.5^{a}	5.3^{b}	5.1^{b}	0.3
Propionic acid, g kg^{-1} DM	-	0.4	0.2	0.3	0.1
Butyric acid, g kg^{-1} DM	-	0.5	0.5	0.3	0.1
Ethanol, g kg^{-1} DM	-	6.1	5.0	5.2	1.3
SFP, g kg^{-1} DM*	-	122.2^{a}	126.3^{a}	98.3^{b}	4.4
NH_3-N, g kg^{-1} total N	-	28.0^{a}	29.2^{a}	12.6^{b}*	0.8
WSC, g kg^{-1} DM*	135	35.8^{a}	32.4^{a}	52.7^{b}	5.6
PH	-	4.75	4.93	4.73	0.2

UT - untreated silage
IN - silage with inoculant
FO - silage with formic acid
SFP - sum of fermentation products - lactic acid + acetic acid + ethanol
* corrected for all the ammonia applied with the additive
WSC water solube carbohydrates
^{a,b} significant differences between treatments at $P< 0.05$
SEM = standard error of the means

ferences between untreated and inoculated silages. O'Kiely (1996) suggested that this may be due partly to apparent domination of fermentation by indigenous homofermentative lactic acid bacteria in the silages made without inoculate, thereby reducing the opportunity for response in major fermentation products to inoculation. Treatment with the inoculant resulted in significantly ($P<0.05$) lower acetic acid concentration than in UT silage. O'Kiely (1996) showed that the effects of inoculation on the concentration of fermentation products and residual WSC were reduced with increased degree of wilting. Driehuis et al. (1997) found that inoculant bacteria posses a high osmotolerance in comparison with the epiphytic flora, giving the inoculant bacteria a growth advantage. Lower acetic acid concentration in the IN silage indicates a more homolactic type of fermentation or inhibition of enterobacteria by chemical treatment. In the present study, acid treatment significantly decreased the sum of fermentation products mainly by lowering lactic and acetic acid contents, which indicates restriction of fermentation. Similarly, O'Kiely (1993) observed a significant decrease in the concentration of lactic and acetic acids and increase in WSC in grass ensiled with formic acid supplementa-

tion in comparison with untreated silage. Gąsior and Brzóska (1999) found that formic acid treatment inhibited mainly lactic acid fermentation, while failing to inhibit the production of other carboxylic acids and ethanol. In the current study, acid treatment preserved the water-soluble carbohydrates efficiently. Jaakola et al. (1993) suggested that a higher water soluble carbohydrates content in silage improves microbial protein synthesis in the rumen. Acid treatment significantly decreased ($P<0.05$) the ammonia-N concentration (after correction) similarly as found by Jaakkola et al. (1993) and Selmer-Olsen and Mo (1997). The greatest restrictions in silage fermentation are obtained with low DM silages by using high levels of formic acid. McDonald et al. (1991) stated that the effect of formic acid on restricting fermentation is enhanced with higher content of DM.

The effect of silage additives on ruminal protein degradability is not consistent. In the present study the effective protein degradability (EPD) was low (71-74%) but not significantly affected by silage additives (Table 2). It appears that protein degradability

TABLE 2

Dry matter and protein degradation *in sacco* in the rumen, intestinal and total digestibility of the experimental silages

Items	Silages			SEM
	UT	IN	FO	
Dry matter				
fraction *a,* %	18.44	18.20	17.55	1.5
fraction *b,* %	50.85	50.47	53.11	2.3
degradation rate *c,* $\%h^{-1}$	9.0	8.7	8.4	0.5
EDMD	51.20	50.34	50.83	0.6
Protein				
fraction *a,* %	45.55	46.10	42.62	2.7
fraction *b,* %	47.50	46.53	49.80	4.6
degradation rate *c,* $\%h^{-1}$	7.7	7.5	6.9	0.8
EPD %	74.37	74.01	71.63	3.2
IDUP %	67.6^{a}	64.2^{b}	70.3^{c}	0.8
TD %	86.7	85.7	87.3	1.2

UT - untreated silage
IN - silage wit h inoculant
FO - silage with formic acid
a = instantly degradation fraction
b = slowly degradable fraction
c = rate of degradation
EDMD = effective dry mater degradability
EPD = effective protein degradability
IDUP = intestinal digestibility of rumen undegraded protein
TD = total digestibility = RD12 + (100-RD12) × IDUP/100
^{a,b,c} at $P<0.05$
SEM = standard error of the means

in the rumen was not associated with the extent of proteolysis during ensiling. Formic acid and inoculant treatment did not protect silage protein from ruminal degradation. A previous study demonstrated lowering of CP degradability by wilting (Rooke et al., 1983; Polan et al., 1998). Broderick et al. (1993) suggested that heat production during fermentation may bind some protein and reduce ruminal protein degradation. In the present study, the soluble CP fraction (42.6), degradation rate c (6.9% h^{-1}), and effective protein degradability (EPD) (71.6%) were lowest for formic acid silage. EPD of 71.6% for FO silage was similar to the results of Selmer-Olsen and Mo (1997) and higher than the value (65%) obtained by Thomas (1982) for formic-treated silage when using diaminopimelic acid as a marker for microbial protein contamination. Rooke et al. (1983) and Polan et al. (1998) found no significant effects of silage additives on rumen protein degradation measured by the *in sacco* method. On a laboratory scale, Gąsior and Brzóska (1999) showed that formic acid supplementation decreased protein degradability both in the silo during fermentation and in the rumen, suggesting that ruminal protein degradation is associated with the extent of nitrogen compound degradability during silage fermentation in the silo. On the other hand, Jaakola et al. (1993) suggested that protein protected from proteolysis in the silo is still degradable in the rumen. Gąsior and Brzóska (2000) in experiments conducted on a commercial scale showed no significant effect of formic acid and inoculant on effective protein degradability of grass silages.

Intestinal digestibility of undegraded protein measured by the mobile bag technique increased significantly ($P<0.05$) by FO treatment and decreased for IN silage compared with UT silage (Table 2). The higher intestinal digestibility of rumen undegraded protein in silage prepared with formic acid may have resulted from the lower protein degradability. It is difficult, however, to explain the lower digestibility of silage treated with inoculant. A significant increase of *in vivo* digestibility associated both with inoculant and formic acid treatment was found by O'Kiely (1996). Improvement in *in vivo* total digestibility associated with inoculant treatment has been shown previously, but the precise mechanism of the increase is hard to explain (Keady and Steen, 1994). In contrast, Rooke et al. (1993) found no significant effect of silage additives on digestibility measured in *in vivo* and *in vitro* studies. In the present study, total tract protein digestibility was not significantly affected by silage additives.

As shown in Table 3, silage additives did not significantly affect silage dry matter intake measured on sheep. Daily dry matter intake of silages UT, IN and FO fed as the only feed were 0.84, 0.85 and 0.86 kg, respectively. This result does not confirm the results of the study of Selmer-Olsen and Mo (1997) who observed significantly higher intake of restrictively fermented silage, suggesting that high contents of silage fermentation products reduce feed intake. For estimation of grass silage intake, Huhtanen et al. (2002) proposed the silage dry matter intake index (SDMI), which is negatively correlated with the concentration of ammo-

TABLE 3

Silage DM intake, kg

Items	Silages			SEM
	UT	IN	FO	
With concentrate				
total DMI, kg DM	1.15	1.18	1.19	0.4
silage intake, kg DM	0.70	0.73	0.74	0.2
silage DMI / 100 kg Lw	1.66	1.76	1.76	0.3
Without concentrate				
silage intake, kg DM	0.84	0.85	0.86	0.5
silage DMI / 100 kg Lw	2.00	2.06	2.05	0.3

UT - untreated silage
IN - silage with inoculant
FO - silage with formic acid
SEM = standard error of the means

nia-N, lactic acid, individual and total fatty acids, and total fermentation acids, and positively correlated with the concentration of residual water-soluble carbohydrates. In the present study there were no marked differences between intakes of IN and FO silage. Similarly, Philip et al. (1990) found no effect of bacterial supplements to ensiled wilted grass on dry matter intake by sheep. However, Gordon (1989) obtained higher dry matter intake of silage prepared with lactic acid bacteria additives. In the present study IN and FO slightly improved fermentation, but provided no significant benefit in terms of silage DM intake.

In conclusion, this study showed that when formic acid was applied to wilted grass, a higher level of WCS and lower level of fermentation products compared with untreated silage were obtained. Lactic acid bacteria did not significantly affect the quality of the silage. Both silage additives had no significant effects on effective protein degradability in the rumen. Formic acid treatment significantly increased intestinal digestibility of rumen-undegraded protein. These results indicate that intensive prewilting of grass (high dry matter) may restrict the effectiveness of chemical and biological silage additives.

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STRESZCZENIE

Wpływ różnych dodatków na jakość kiszonek, rozkład białka w żwaczu, jelitową strawność białka oraz spożycie kiszonek przygotowanych z podwiędniętych traw

Podwiędnięty (ok. 48% s.m.) pierwszy pokos porostu pastwiskowego zakiszono w beczkach o pojemności 120 l (5 powtórzeń): bez dodatku (UT), z inokulantem bakteryjnym Pioneer 1188 (IN) lub z kwasem mrówkowym w preparacie Foraform (FO).

Inokulant oprócz zmniejszenia zawartości kwasu octowego w porównaniu z kiszonką bez dodatku, nie miał istotnego wpływu na zawartość pozostałych produktów fermentacji. Kwas mrówkowy (FO) w porównaniu z UT zmniejszył istotnie ($P<0,05$) zawartość kwasu mlekowego i octowego oraz azotu amoniakalnego, a jednocześnie zwiększył koncentrację cukrów rozpuszczalnych w wodzie (WCS). Dodatek IN i FO nie miał istotnego wpływu na efektywny rozkład białka w żwaczu. Foraform spowodował statystycznie istotne ($P<0,05$) zwiększenie, o 2,7% w porównaniu z kiszonką kontrolną, strawności jelitowej białka nie ulegającego degradacji w żwaczu. Nie wykazano istotnego wpływu IN i FO na spożycie suchej masy kiszonki przez owce.