
RESEARCH NOTE

Enzymes as silage additive. Effect on fermentation quality, digestibility in sheep, degradability *in sacco* and performance in growing cattle

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Abstract. Fungal glucose oxidase and cellulase were used as silage additives in laboratory (25 l), pilot (3 m³) and farm scale (250 t) silos. In 3 m³ scale silos, pH and the concentration of acetic acid were lower and the concentrations of lactic acid and sugars were higher in the enzyme treated than in untreated silage. The concentration of butyric acid was equal to or lower than in formic acid treated silage in all experiments. Cell wall constituents were degraded in the silo by cellulase and thus more energy was available for lactic acid bacteria. With increasing levels of cellulase application, the disappearance of organic matter (OM) from nylon bags incubated for 1 to 12 h in the rumen of a dairy cow increased significantly ($P < 0.001$, $P < 0.01$) in two different experiments. The level of cellulase had no effect on the ratio N/OM disappeared from nylon bags. Digestibilities of OM in sheep were 75.8, 76.5 and 77.2 % for untreated, formic acid and enzyme treated silage ($P > 0.05$). On untreated silage diet N retention tended to be reduced in sheep compared with formic acid or enzyme treated silages. In growing bulls there were no differences in feed intake, growth rate, feed conversion rate, carcass characteristics or OM digestibility between formic acid (4 l/t) and enzyme treated silage.

Index words: silage, additives, enzymes, fermentation, degradability *in sacco*, growing cattle

Introduction

Silage additives used to influence fermentation can act either as stimulants by encouraging lactic acid fermentation or as inhibitors by inhibiting completely or partially microbial growth. Fermentation stimulants can be clas-

sified as bacterial cultures or as carbohydrate sources.

The effects of bacterial inoculants on silage fermentation have been variable and in many experiments no benefits at all have been seen. WOOLFORD and SAWCZYC (1984) did not find any notable influence on the rate of acidification or promotion of homolactic fermentation by selected cultures of lactic acid bac-

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teria and these also tended to increase the amount of soluble protein and deamination.

Carbohydrate rich materials such as sugars, molasses and cereals have been added to silage crop in order to supply energy for lactic acid bacteria. In his review, McDONALD (1981) notes that molasses has been shown to decrease pH and the amount of ammonia N in silage. CASTLE and WATSON (1985) did not record any differences in the performance of dairy cows fed formic acid or molasses treated silages. A disadvantage of using carbohydrate rich materials is the relatively high concentrations required.

Forage plants have a large reserve of carbohydrates, but these occur mainly in the form of polysaccharides which cannot be used as an energy source by lactic acid bacteria in silage. In theory, the addition of enzymes able to hydrolyse forage polysaccharides would increase the fermentation capacity of silage by releasing fermentable substrate. The cellulolytic enzymes would also promote cellular breakdown and render cell contents more accessible to silage microflora.

The objectives of the present studies were to investigate the effect of enzymes on silage fermentation, degradation rate *in sacco*, digestibility in sheep and on the performance of growing bulls. The silages were made in laboratory, pilot and farm scale silos.

Materials and methods

In two experiments 5 kg of chopped grass was wilted for 4 h in order to increase dry matter (DM) content to about 25 %, and then ensiled into 25 l laboratory silos. The herbage contained 19.0 % of crude protein and 22.6 % of crude fibre in Exp. 1 and 15.6 and 25.2 % in Exp. 2. The treatments were as follows: untreated control, formic acid (5 l/t), glucose oxidase (GO) and GO + 100, 200, 400, 800, 1600 or 3200 ml/t of cellulase. Formic acid was applied as AIV II solution (80 % formic acid, 2 % orthophosphoric acid) in all the experiments. After 59 d (Exp. 1) and 86 d (Exp. 2) the silos were opened and

analyzed for DM, organic matter (OM), ash, nitrogen (N), true protein, ether extract, crude fibre, neutral detergent fibre (NDF), acid detergent fibre (ADF), lignin (ADL), pH, sugars, lactic acid, volatile fatty acids (VFA), ammonia and soluble N.

Nylon bag (porosity 40 μm) incubations were made in the rumen of a dairy cow fed at the level of 2.8 times maintenance, with forage 60 % of DM. The silages incubated in triplicate were withdrawn after 2, 5, 9, 24 and 48 h in Exp. 1 and after 1, 3, 6, 12, 24 and 48 h in Exp. 2. Residues were analysed for DM, OM and N and some treatments also for NDF. The values for degradation constants were calculated using the formula of ØRSKOV and McDONALD (1979)

$$p = a + b(1 - e^{-ct}).$$

For the digestibility experiment (Exp. 3) silages were made in 3 m³ glass fibre silos from first cut timothy sward. The grass was wilted for 4 h except the direct cut formic acid treated silage. The treatments were untreated control, formic acid direct cut (4.5 l/t), formic acid pre-wilted (4 l/t), GO + hemicellulase (150 ml/t) + cellulase (200 ml/t) (Enzyme A) and hemicellulase (150 ml/t) + cellulase (200 ml/t) (Enzyme B). The digestibility trial was carried out with 5 sheep using a 5 \times 5 Latin square design.

The material for the feeding trial (Exp. 4) was second cut timothy red clover sward (red clover 35–40 % of DM). After wilting for 4 h the grass was ensiled into a 250 t bunker silo either with formic acid (4 l/t) or 2–3 d later with enzyme mixture (GO + cellulase 150 ml/t). A feeding trial of 210 d was carried out with 24 bulls using 2 \times 2 factorial design. Both silages were supplemented with barley or barley + fishmeal (FM). Barley was fed at the level of 40 g/kg W^{0.75} on an air dry basis. In the FM group 0.25 kg of barley was replaced by FM. *In vivo* digestibility was determined at the liveweight of 250–300 kg using acid-insoluble ash as digestibility marker (VAN KEULEN and YOUNG 1977).

Results and discussion

The breakdown of various fibre fractions increased during the ensilage period with increasing level of cellulase (Fig. 1). The effect was proportional to the square root of cellulase activity. In Exp. 2 with longer ensilage period and more mature material, the effect of cellulase on ADF, cellulose and crude fibre was like that in Exp. 1, but the effect on NDF was greater indicating the hydrolysis of hemicellulose and the hemicellulose activity of the enzyme mixture. In the normal ensilage process the breakdown of hemicellulose is caused also by plant enzymes and later, when pH is low, by acid hydrolysis (McDONALD 1981).

All the silages were well preserved. Formic acid almost completely inhibited fermentation in the laboratory silos (4 kg). Presumably at

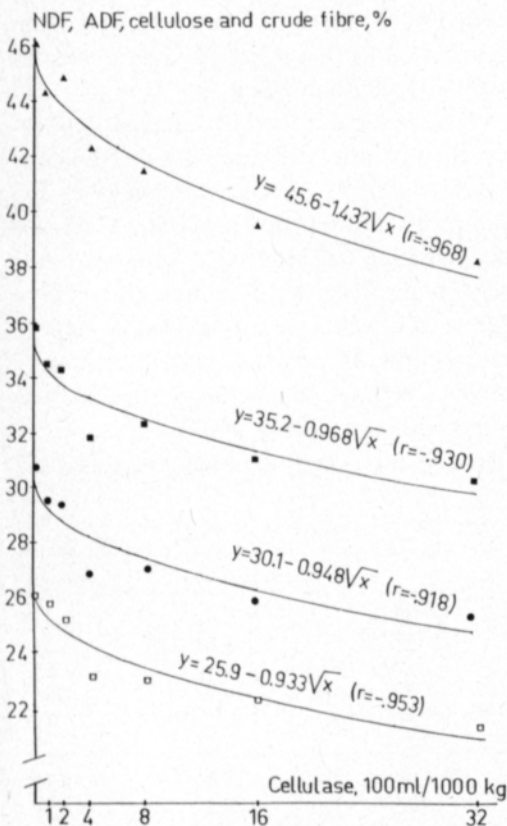


Fig. 1. The effect of enzyme level on NDF Δ , ADF \blacksquare , cellulose \bullet and crude fibre \square contents of silage (% of DM) in Exp. 2.

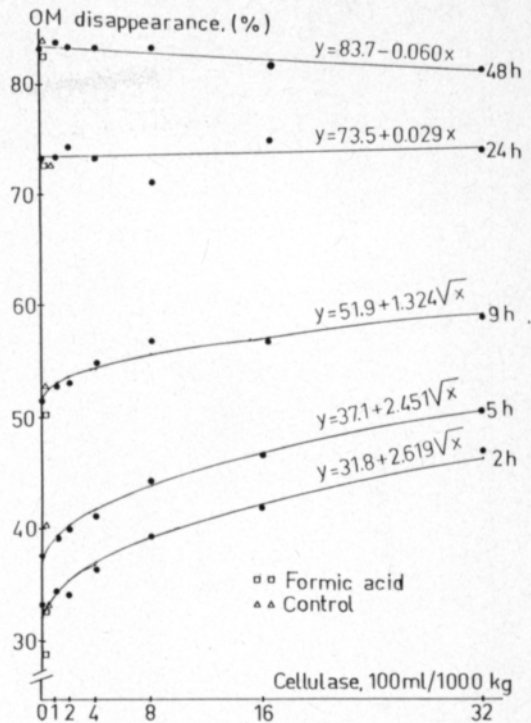


Fig. 2. The effect of enzyme level on silage OM disappearance (% of total OM) from the nylon bags in Exp. 1.

the high levels of addition the bacteriostatic action of formic acid and consequent suppression of lactic acid production allowed the pH to increase in response to the release of buffering constituents from plant tissue. With increasing levels of cellulase the concentration of residual sugars increased and there was also some increase in the concentration of lactic acid. No effect on true protein contents or the ratio ammonia N: total N was found with increasing levels of cellulase, but the ratio soluble N: total N increased, presumably because of the breakdown of cell walls and subsequent release of cell contents.

The disappearance of OM from nylon bags increased with the level of cellulase addition, especially after shorter incubations (Figs. 2 and 3). After an incubation of 48 h there were no differences in the extent of OM degradation between the treatments. The very rapidly degradable fraction a increased with the level of cellulase and in time degradable fraction

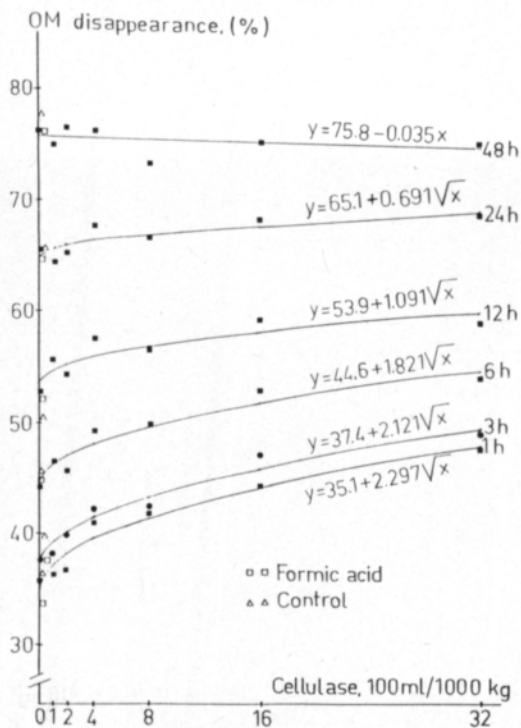


Fig. 3. The effect of enzyme level on silage OM disappearance (% of total OM) from the nylon bags in Exp. 2.

b decreased. The effect of treatments on *c* (the rate of degradation of fraction *b*) was inconsistent in Exp. 1, but in Exp. 2 all enzyme treated silages had a higher rate of OM degradation than untreated or formic acid treated silages. Cellulase treatment had the same pattern of response on N as on OM disappearance. The ratio N:OM that disappea-

red during the first 2 or 3 hours was 55–63 g N/kg OM in Exp. 1 and 43–51 g in Exp. 2 being slightly lower in formic acid treated silage than in other silages. This ratio was not affected by the level of cellulase. The effect of increased N loss after short incubations on N or microbial N flow might be negative. However, CHAMBERLAIN et al. (1982) did not record any response of N flow into the duodenum when the level of formic acid was increased from 0 to 5.9 l/t, and the undegradable fraction of silage N was increased only from 22 to 28 %. It seems that partial limitation of proteolysis can be achieved in the silo by the addition of high levels of formic acid, but protein spared from hydrolysis in the silo seems to be degraded by the rumen microbes.

Part of the response in the OM loss can be explained by increased N loss with higher levels of cellulase. However, the loss of N-free OM after incubations for 3, 6 and 12 h increased from 33.0 to 41.7 % ($P < 0.001$), from 39.9 to 46.8 % ($P < 0.001$), and from 48.4 to 54.0 % ($P < 0.01$) in Exp. 2.

With the highest level of cellulase application the potential degradability of NDF was decreased (Table 1), but this could be explained by the breakdown of cell walls in the silo. Lag time was reduced by cellulase treatment, but in Exp. 1 with young grass a very high level of cellulase was needed to achieve this response. By contrast, with more mature material in Exp. 2 the same response was found with the level of 400 ml/t.

In Exp. 3 enzyme treatment decreased pH

Table 1. The degradation constants of NDF disappearance from the nylon bags in Exp. 1 and Exp. 2.

Exp. 1	<i>a</i> + <i>b</i>	<i>c</i>	<i>r</i>	Lag time (h)	Res. s.d.
Control	76.0	.0608	.9999	2.6	0.92
Formic acid	74.4	.0677	.9977	3.0	3.23
GO + C 400 ml	73.5	.0620	.9998	2.5	1.54
GO + C 3200 ml	65.5	.0644	.9987	0.9	2.96
Exp. 2					
Control	68.3	.0385	.9984	2.8	4.21
Formic acid	62.5	.0376	.9996	4.2	1.57
GO + C 400 ml	61.5	.0403	.9993	1.7	1.26
GO + C 3200 ml	55.5	.0321	.9990	1.6	2.30

GO = glucose oxidase, C = cellulase

Table 2. Fermentation quality of experimental silages in Exp. 3.

	Formic acid direct-cut	Formic acid pre-wilted	Control	Enzyme A	Enzyme B	SEM
pH	4.06 ^c	3.98 ^c	4.40 ^d	3.95 ^c	3.96 ^c	0.03
In dry matter, %						
Sugars	1.53 ^d	3.62 ^c	0.17 ^c	0.80 ^{de}	0.89 ^{de}	0.18
Lactic acid	6.34 ^e	6.38 ^e	9.31 ^d	13.26 ^c	13.05 ^c	0.54
Acetic acid	1.82 ^{de}	1.38 ^e	3.03 ^c	2.33 ^{cd}	2.33 ^{cd}	0.17
Propionic acid	0.08	—	0.07	—	—	0.03
Ratio lactic:acetic	4.1 ^{ab}	4.8 ^{ab}	3.1 ^b	5.7 ^a	5.6 ^a	0.47
In total N, %						
Ammonia N	6.3 ^{de}	5.2 ^e	11.3 ^c	7.2 ^d	7.2 ^d	0.35
Soluble N	60.5 ^{cd}	58.9 ^d	70.4 ^c	67.9 ^{cd}	66.9 ^{cd}	2.0

Means with different letters were significantly different a, b ($P < 0.05$), c, d, e ($P < 0.01$)

Table 3. Feed intake and the performance data in growing bulls in Exp. 4.

	Formic		Enzyme		Silage		Protein		SEM
	Contr.	FM	Contr.	FM	FA	Enzyme	Contr.	FM	
DM intake ¹ , kg/d									
Silage	3.68	3.81	3.57	3.69	3.74	3.63	3.63	3.75	0.11
Barley	2.29	2.09	2.23	2.11	2.19	2.17	2.26	2.10	0.07
Fishmeal	—	0.22	—	0.22	0.11	0.11	—	0.22	0.00
Total	5.97	6.12	5.80	6.02	6.05	5.91	5.88	6.07	0.16
DM g/kg W ^{0.75}	86.7	88.2	86.8	85.8	87.5	86.3	86.8	87.0	1.2
Initial weight, kg	181.0	175.5	179.3	178.0	178.2	178.7	180.2	176.8	12.1
Final weight, kg	401.0	406.2	386.7	411.3	403.6	399.0	393.8	408.7	11.5
Daily gain, g	1064	1137	1004	1141	1100	1072	1034 ^a	1139 ^b	35
Feed conversion									
Kg DM/kg gain	5.63	5.40	5.85	5.31	5.52	5.58	5.74	5.36	0.18
ME MJ/kg gain	63.6	61.2	64.9	59.2	62.4	62.1	64.2	60.2	2.1
Carcass weight, kg	205.2	206.7	197.0	210.8	205.9	203.9	201.1	208.8	7.2
Dressing-%	50.8	50.7	50.9	51.2	50.7	51.0	50.9	50.9	0.5

¹ On oven DM basis, FM = fishmeal, FA = formic acid
Means with different letters significantly different a, b ($P < 0.05$)

and the concentrations of acetic acid and ammonia N and increased the concentrations of lactic acid and residual sugars compared with untreated control (Table 2). Enzyme treatment encouraged homofermentative lactic acid bacteria and the ratio lactic acid:acetic acid was the most favourable in enzyme silages. Apparently in the absence of fermentation inhibitors the sugars from cell wall hydrolysis are used mainly for lactic acid production.

The digestibility of OM was 76.2, 76.5, 75.8, 77.1 and 77.2 % for direct cut formic acid, pre-wilted formic acid, untreated, enzyme A and enzyme B treated silages and N retentions were 2.5, 2.9, 1.4, 2.0 and 2.9 g/d,

respectively.

Both silages in Exp. 4 were well preserved in the bunker silo. The enzyme treated silage had a higher concentration of lactic acid (11.2 vs. 8.6 %), acetic acid (3.8 vs. 2.1 %), ammonia N (8.9 vs. 5.9 % of total N) and soluble N (49.4 vs. 41.8 % of total N) and lower concentration of sugars (1.1 vs. 0.5 %). However, DM intakes of the two silages were similar on oven DM basis (Table 3). Differences between the silages in growth rate, feed conversion and carcass characteristics were small and insignificant. FM, on the other hand, improved significantly ($P < 0.05$) the liveweight gain (Table 3). One animal in the

enzyme silage + barley group had leg injuries, which partly explains the insignificant ($P > 0.05$) interaction between the type of silage and protein supplementation.

Enzyme treatment reduced significantly ($P < 0.001$) the digestibility of crude fibre and improved ($P < 0.001$) that of the ether extract compared with formic acid treated silage. The lower crude fibre digestibility is most likely explained by enzymatic cell wall digestion in the silo, but probably the slightly later harvesting time also had some effect. It seems that the cellulase system first attacks the most easily digestible fraction of fibre in the silo and the fraction which is left is less digestible in the animal than the original fibre. There was a significant ($P < 0.01$) interaction between the

silage and protein supplementation: FM clearly improved the digestibility of enzyme treated silage, but had a slightly negative effect with formic acid silage. Possibly enzyme silage requires a different supplement than acid treated silage to optimize cell wall digestion in the rumen.

In conclusion, enzyme treatment encouraged lactic acid fermentation and also reduced ammonia N compared with untreated control silage. No adverse effects relative to formic acid silage were found on N retention in sheep or feed intake and liveweight gain in growing bulls. The advantages of biological additives over acids are that they are safer to handle, less corrosive to machinery and easier to transport.

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Ms received December 18, 1985

SELOSTUS

Entsyymit nurmirehun säilöntäaineena. Vaikutus käymiseen, sulavuuteen lampailla, rehun hajoamisnopeuteen *in sacco* sekä tuotantoon lihanaudoilla

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Homeiden tuottamaa glukoosioksideasia ja sellulaasia käytettiin nurmirehujen säilöntäaineena laboratorio-

(25 l), pilot (3 m³) ja maatilasiiloissa (250 t) suoritetuissa kokeissa. Entsyymikäsiteltyjen rehujen pH ja etikkahappopitoisuus olivat alhaisempia ja maitohappo- ja sokeripitoisuus korkeampia kuin painorehulla 3 m³:n siiloissa. Voihappopitoisuus oli kaikissa kokeissa entsyymirehuiltä

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la sama tai alhaisempi kuin muurahaishapporehulla (AIV-2). Sellulaasi pilkkoi rehun solunseinämäaineita siiossa ja vapautti siten sokereita maitohappobakteereiden käytettäväksi. Kahdessa eri naitonpussikokeessa lypsylehmällä orgaanisen aineen pötsihajoavuus kohosi merkitsevästi ($P < 0.001$, $P < 0.01$) 1-12 tunnin inkuboinneilla entsyymitasoin kohotessa. Typen ja orgaanisen aineen hajoavuuksien suhteeseen ei sellulaasitasolla ollut vaikutusta. Painorehun, muurahaishapolla sekä entsyymeillä säilöt-

tyjen rehujen orgaanisen aineen sulavuudet päseillä olivat vastaavasti 75.8, 76.5 ja 77.2 % ($P > 0.05$). Typen piddättyminen oli painorehulla hieman alhaisempi kuin muurahaishapolla tai entsyymeillä säilötyillä rehuilla. Liha-naudoilla rehun syönnissä, kasvussa, rehun hyväksikäytössä, teuraslaadussa ja orgaanisen aineen sulavuudessa ei ollut eroa AIV-2:lla (4 l/t) tai entsyymeillä säilöttyjen rehujen välillä.