Can lactobacilli producing ferulate esterase improve the nutritive value of grass and maize silage?

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Abstract

Pioneer® has patented a silage inoculant containing *Lactobacillus* strains of which *L. buchneri* produces ferulate esterase. The product is claimed to improve silage quality and aerobic stability as well as cell wall digestibility. The effect of the inoculant added to grass and whole-plant maize was studied using micro-silos during two years. Each year, grass was mown at 4 growth stages and maize was harvested at 2 maturity stages. Compared to the grass silage without additive, in the treated silage more sugars were fermented to lactic and acetic acid, resulting in a lower pH, less dry matter (DM) and protein degradation and a better aerobic stability. The inoculant lowered neutral detergent fibre (NDF) content of the grass silage from the early cuts, but not that from the late cuts. *In situ* rumen degradability of NDF (NDFD) was not affected, whereas *in vitro* organic matter digestibility tended to be better for the treated grass silage. In the early harvested maize, treatment resulted in less lactic and more acetic acid, a higher pH and higher DM-losses; the aerobic stability was better. Silage quality of the late-harvested maize was not affected. The additive did not affect chemical composition nor NDFD of the maize silage. It appears that the ferulate esterase in the inoculant is only able to affect less-lignified cell walls.

Keywords: *Lactobacillus buchneri*, grass silage, maize silage, nutritive value

Introduction

Sustainable dairy farms rely on the production and the preservation of high quality forage. There are various preservatives that may be used in case ensiling conditions are unfavourable. Pioneer® has an inoculant on the market which would not only improve silage quality and aerobic stability of grass and maize, but also improve cell wall digestibility. The product 11GFT (used for grass silage) consists of three *Lactobacillus* strains: *L. casei, L. plantarum* and *L. buchneri*, whereas 11CFT (used for maize) contains *L. casei and L. buchneri*. The latter ferments sugars not only to lactate but also to acetate known to inhibit yeasts and moulds (Holzer *et al.*, 2003). Further, *L. buchneri* is able to produce ferulate esterase, an enzyme which breaks down the linkages between (hemi)cellulose and lignin (Donaghy *et al.*, 1998). The objective was to study the claimed effects of the inoculant with grass cut at different growth stages and with maize harvested at a moderate and a late maturity stage by using micro-silos.

Materials and methods

A first cut of perennial ryegrass (*Lolium perenne*) was mown in 2010 and 2011 at 4 growth stages between the end of April and the beginning of June. The grass was wilted to about 35% dry matter (DM) and chopped at a length of 24 mm. Whole-plant maize (*Zea mays* L., cv PR39A98) was harvested in 2010 and 2011 at about 30 and 40% DM and chopped at a length of 8 mm. Half of the wilted grass and half of the maize was treated (T) with 11GFT and 11CFT respectively at the recommended dose of 1 g per ton, whereas the other half was not treated (Control, C). Plastic tubes of 2.75 l were filled with forage (for each stage: 5 tubes C and 5 T) at a density of 180 kg m^{-3} DM and provided with a CO_2-lock. The micro-silos were weighed and stored at ambient temperature in an unheated barn for 60 d. Aerobic stress was induced during 24 h at 18 d before opening. At opening, tubes were weighed again and 4 of the 5 tubes per treatment were selected for further study. From each tube, 100 g sample was extracted with water and

analysed for pH, lactic acid, volatile fatty acids, alcohols and ammonia. DM, crude protein (CP), neutral detergent fibre (NDF), crude ash, sugars for grass silage and starch for maize silage were analysed using EU/ISO methods. The degradability of NDF (NDFD) was determined *in situ* by incubating nylon bags in the rumen of two cannulated cows (Tamminga *et al.*, 2007). Organic matter digestibility (COMD) was determined *in vitro* with the cellulase technique (De Boever *et al.*, 1986).

The results were analysed using ANOVA to study the effect of the inoculant as well as the interaction between treatment and growth/maturity stage. If treatment effect was significant ($P \leq 0.05$), C and T means within stage were compared by a t-test.

Results and discussion

The use of 11GFT for wilted grass had a significant effect on silage quality and chemical composition in both years (Tables 1 and 2). Treatment resulted in less DM losses, a lower pH, more lactic and acetic acid, less alcohols and a lower ammonia fraction. A better aerobic stability was only observed in year 1. Although there was a significant interaction with growth stage for most parameters, the better silage quality of treated grass was clear at all stages. Treated grass silage contained more DM and clearly less sugars, less NDF and also somewhat less CP. The reduced NDF content was only significant at the early growth stages. Treatment had no effect on NDF degradability in the rumen, whereas COMD tended to be better.

The use of 11CFT for maize only showed effects at the first but not at the second maturity stage (Table 3). Treatment resulted in higher DM loss and pH, lower lactic acid and more acetic acid and alcohols, indicating a moderate silage quality. On the other hand, aerobic stability was better. Treatment did not affect NDF and starch content, nor NDFD or COMD.

Table 1. The effect of 11GFT (control C versus treatment T) on silage quality, chemical composition and nutritive value of grass silage mown at 4 growth stages – year 1.[1]

Harvest date	28/04/10		17/05/10		25/05/10		2/06/10		SEM	Significance[2]	
	C	T	C	T	C	T	C	T		T	S×T
DM (g kg^{-1})	387	397**	342	362**	370	380**	359	365**	2.9	**	**
DM loss (%)	1.6	0.8**	1.4	1.2^{ns}	1.9	1.4**	2.2	1.5**	0.08	**	**
pH	4.93	3.93**	4.60	3.84**	4.41	3.93**	4.42	4.04**	0.07	**	nd
Lactic acid (g kg^{-1} DM)	32	87**	40	83**	46	71**	45	52^{ns}	3.5	**	**
Acetic acid (g kg^{-1} DM)	26	24^{ns}	11	32**	11	27**	11	34**	1.6	**	**
Alcohols (g kg^{-1} DM)	38	21**	27	21*	34	22**	42	26**	1.4	**	**
NH_3-N/N (%)	4.5	2.7**	6.3	3.8**	7.6	6.3**	8.3	5.5**	0.32	**	**
Aerobic stability (h)	30	127*	24	153**	31	150**	32	>170**	12.0	**	ns
NDF (g kg^{-1} DM)	344	317**	397	377**	491	484^{ns}	513	506^{ns}	15.3	**	**
CP (g kg^{-1} DM)	231	226	169	167	141	138	135	129	13.5	*	nd
Sugars	119	70	153	38	58	17	52	14	16.1	*	nd
NDFD (%)	67.3	65.2^{ns}	63.0	61.0^{ns}	53.1	53.9^{ns}	52.2	50.7^{ns}	1.30	ns	ns
COMD (%)	91.8	92.4	88.8	88.1	76.9	79.1	73.1	74.6	2.64	ns	nd

[1] DM = dry matter; NDF = neutral detergent fibre; CP = crude protein; NDFD = NDF degradability; COMD = cellulase digestibility of organic matter; SEM = standard error of the mean.
[2] Significance of treatment effect (T) and of interaction between treatment and growth stage (S×T); nd = not determined; ns = not significant ($P>0.05$); * significant at $P \leq 0.05$; ** significant at $P \leq 0.01$.

Table 2. The effect of 11GFT (control C versus treatment T) on silage quality, chemical composition and nutritive value of grass silage mown at 4 growth stages – year 2.[1]

Harvest date	26/04/11		23/05/11		30/05/11		8/06/11		SEM	Significance[2]	
	C	T	C	T	C	T	C	T		T	S×T
DM(g kg^{-1})	322	335**	337	343**	403	414**	348	353**	5.6	**	**
DM loss (%)	0.4	0.7**	1.8	0.9**	2.5	1.0**	2.1	0.8**	0.34	**	**
pH	4.42	3.95*	4.51	3.87**	4.31	3.90**	4.45	3.80**	0.05	**	**
Lactic acid (g kg^{-1} DM)	74	118**	60	105**	58	87**	48	85**	4.1	**	*
Acetic acid (g kg^{-1} DM)	21	28^{ns}	20	20^{ns}	12	18**	14	15^{ns}	0.9	**	*
Alcohols (g kg^{-1} DM)	14	24**	32	20*	38	16*	32	17**	1.6	**	**
NH_3-N/N (%)	8.1	3.1**	11.1	3.0**	7.9	3.4**	12.5	4.5**	0.64	**	**
Aerobic stability (h)	43	32^{ns}	40	34^{ns}	37	60**	38	71^{ns}	3.5	ns	*
NDF (g kg^{-1} DM)	397	384**	494	485^{ns}	nd	nd	578	581^{ns}	23.3	*	*
CP (g kg^{-1} DM)	238	216^{ns}	146	133*	nd	nd	110	103*	15.5	**	*
Sugars	71	15**	65	66^{ns}	nd	nd	11	32**	7.6	*	**
NDFD (%)	55.5	55.4^{ns}	45.0	47.3^{ns}	nd	nd	35.6	37.9^{ns}	2.34	ns	ns
COMD (%)	86.5	86.7^{ns}	74.8	75.6^{ns}	nd	nd	58.3	61.5**	3.31	**	**

[1] DM = dry matter; NDF = neutral detergent fibre; CP = crude protein; NDFD = NDF degradability; COMD = cellulase digestibility of organic matter; SEM = standard error of the mean.
[2] Significance of treatment effect (T) and of interaction between treatment and growth stage (S×T); nd: = not determined; ns = not significant (P>0.05); * significant at P≤0.05; ** significant at P≤0.01.

Table 3. The effect of 11CFT (control C versus treatment T) on silage quality, chemical composition and nutritive value of maize silage harvested at 2 maturity stages during 2 years.[1,2]

Year	2010							2011						
	Stage 1		Stage 2		SEM	Significance[2]		Stage 1		Stage 2		SEM	Significance[3]	
	C	T	C	T		T	T×S	C	T	C	T		T	T×S
DM (g kg^{-1})	303	297*	406	389**	12.6	**	*	307	312^{ns}	421	421^{ns}	14.5	ns	ns
DM loss (%)	0.6	1.1^{ns}	0.9	0.8^{ns}	0.05	**	**	0.5	0.6*	0.5	0.5^{ns}	0.01	*	*
pH	3.81	4.00**	3.92	3.92^{ns}	0.02	**	**	3.77	3.79^{ns}	3.88	3.87^{ns}	0.01	ns	ns
Lactic acid (g kg^{-1} DM)	53	31**	54	50^{ns}	2.5	**	**	60	55*	47	46^{ns}	1.5	*	ns
Acetic acid (g kg^{-1} DM)	18	39**	15	13*	2.7	**	**	18	25**	15	15^{ns}	1.2	**	**
Alcohols (g kg^{-1} DM)	12	20**	17	14^{ns}	0.8	*	**	12	14*	12	12^{ns}	0.2	*	**
NH_3-N/N (%)	4.9	4.8^{ns}	4.7	4.4**	0.06	*	ns	4.6	4.4^{ns}	4.6	4.6^{ns}	0.05	ns	ns
Aerobic stability (h)	97	220**	104	70^{ns}	15.4	**	**	36	200**	91	123^{ns}	16.5	**	**
NDF (g kg^{-1} DM)	347	379*	377	381^{ns}	6.0	ns	ns	359	353^{ns}	347	352^{ns}	3.6	ns	ns
Starch (g kg^{-1} DM)	321	294	317	320	6.3	nd	nd	325	332^{ns}	366	358^{ns}	7.2	ns	ns
NDFD (%)	28.4	29.2^{ns}	28.6	28.7^{ns}	0.61	ns	ns	24.7	27.4^{ns}	nd	nd	3.9	nd	nd
COMD (%)	75.7	72.2	72.2	72.0	0.89	nd	nd	72.1	72.2^{ns}	72.2	71.8^{ns}	0.30	ns	ns

[1] DM = dry matter; NDF = neutral detergent fibre; NDFD = NDF degradability; COMD = cellulase digestibility of organic matter; SEM = standard error of the mean.
[2] Growth stage 1: 30% DM; growth stage 2: 40% DM.
[3] Significance of treatment effect (T) and of interaction between treatment and growth stage (S×T); nd: = not determined; ns = not significant (P>0.05); * significant at P≤0.05; ** significant at P≤0.01.

Conclusions

Treatment of wilted grass with 11GFT clearly gives a better silage quality, tends to improve aerobic stability and organic matter digestibility, but has no effect on cell wall digestibility. The use of 11CFT in maize silage only showed a positive effect on aerobic stability.

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