STUDY OF THE EFFECT OF EM SILAGE APPLICATION ON THE FERMENTATION CHARACTERISTICS AND THE AEROBIC STABILITY OF FIRST CUT *LOLIUM PERENNE* RYEGRASS SILAGE

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Title:	*Study of the effect of EM Silage application on the fermentation characteristics and the aerobic stability of first cut Lolium perenne ryegrass silage.*
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Client:	*Agriton*
Publication date:	*October 28th, 2015*

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1. Aim

Aim of the ensiling trial was to test the effect of treatment of *Lolium perenne* ryegrass (32.71% dry matter) of the first cut with EM Silage on silage fermentation characteristics and aerobic stability parameters.

2. Experimental setup

Lolium perenne ryegrass from the first cut was mown and prewilted to a dry matter (DM) content of 32.71%. This grass was treated with the appropriate treatment solution prior to ensiling for both objects:

control	plain tap water (untreated)
EM Silage	80 ml/ton fresh matter (dissolved in tap water)

The batch number of the EM Silage product was 1509010004.
All solutions were homogeneously sprayed onto the starting material with different hand-hold sprayers, in a ratio of 10 liter of solution per ton fresh matter.

From the negative control, samples were taken prior to ensiling for determination of the dry matter (DM) content[1], nutritional analysis[2] (NEL, degradability of the organic matter, crude protein, crude ash, crude fat and water-soluble carbohydrates (WSC)) and microbal analysis[3] (counting of yeasts, moulds and lactic acid bacteria on three repetitions). On the undiluted EM Silage product, counts of yeasts and lactic acid bacteria were performed (Annex 1).

Per object eight microsilos (volume 2,75 liter – equipped with a CO_2 valve) were filled at a mean silo density of 145 kg DM/m³. Microsilos were weighed empty and immediately after filling. Weighing was repeated on a weekly basis to allow calculation of the fermentation losses (as percentage of fresh matter (FM)) during the ensiled period. All microsilos were desiled after 102 days.
From six of the eight microsilos per object, samples were taken for determination of the dry matter content, for counting of yeasts and moulds and for determination of the aerobic stability by the Honig protocol. Four microsilos per object were selected for analysis of fermentation characteristics[4] (ammonia, crude protein, pH, lactic acid, acetic acid, propionic acid, ethanol and water-soluble carbohydrates).

[1] Uncorrected dry matter content was determined by air drying at 65°C until constant weight. For silage samples, this dry matter content was corrected according to Dulphy and Demarquilly (1981).
[2] Nutritional parameters were determined according to ISO 17025: the *in vitro* degradability of the organic matter was determined according to De Boever et al. (1986), crude protein according to NF ISO 15670, crude ash according to 71/250/EG and crude fat according to 71/393/EG - 98/64/EG. Water-soluble carbohydrates were determined by the Luff-Schoorl method.
[3] Yeast and mould counts were determined according to ISO 21527, while lactic acid bacteria were counted according to ISO 15214; all under Belac accreditation.
[4] Ammonia and crude protein were determined by Kjeldahl (1883), pH was measured on a 1/10 (w/w) watery extract of fresh silage (Muck et al. 1999) and lactic acid, acetic acid, butyric acid and propionic acid were determined by HPLC (Ohmomo et al. 1993). Ethanol was determined on a watery extract by NIRS absorption based on Sørensen (2004). Water-soluble carbohydrates were determined by the Luff-Schoorl method.

Aerobic stability was determined by the protocol of Honig (1990): desiled grass was placed loosely into a recipient (volume 1 liter) for aerobic deterioration at 20,8 ± 0,5°C in insulating boxes during 8 days. Two recipients were filled per microsilo. Each recipient was covered with a double layer of cheesecloth to prevent drying and contamination, and to allow gas exchange. In the geometric center of each recipient, the temperature was logged every ten minutes. Surrounding temperature was also registered. Per two hours, the average temperature was calculated. A temperature rise of 3°C above surrounding temperature is considered as an indicator of aerobic instability. At the end of the Honig protocol, the dry matter content was determined again, so the dry matter loss per day during the Honig protocol could be calculated.

The obtained data (Annex 2) were statistically analyzed with SAS 7 (Annex 3). Outliers were removed per treatment based on box-plot results. Normality was tested with Kolmogorov-Smirnov and equality of variances was tested by Levene's test. Normally distributed, homoscedastic variables were subjected to a two-sided one-way Anova with Tukey as *post hoc* test. If these two conditions were not fulfilled, a two-sided non-parametric one-way Anova according to Wilcoxon was performed. Significance was declared at $p<0,05$.

3. Starting material

The nutritional and microbial properties of the starting material are summarized in Table 1, as well as the results of the microbial counts performed on the undiluted EM Silage product.

Table 1. Nutritional and microbial properties of the starting material.

Grass: *Lolium perenne* - first cut	
dry matter (g/kg FM)	327,1
net energy for lactation	6,22
degradability of the organic matter (%)	88,0
crude protein (g/kg DM)	208,5
crude fat (g/kg DM)	23,6
crude ash (g/kg DM)	85,7
water-soluble carbohydrates (g/kg DM)	191,8
yeasts (cfu/g FM) (N=3)	1,3E+06
moulds (cfu/g FM) (N=3)	3,3E+04
lactic acid bacteria (cfu/g FM) (N=3)	7,4E+04

4. Results

- Fermentation losses during the ensiled period

The evolution of the fermentation losses (as % of fresh matter) is visualized in Figure 1, while the detailed figures are presented in Table 2.

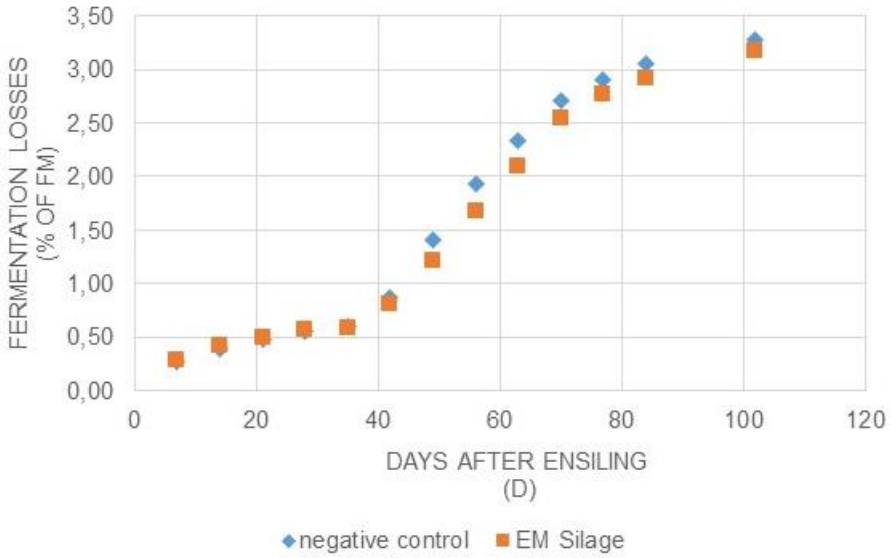

Figure 1. Evolution of fermentation losses during ensiled period.

The fermentation losses were very alike for both objects. This is confirmed in Table 2, showing only few significant differences between the fermentation losses of the negative control and the EM Silage treatment throughout the ensiled period.

Table 2. Fermentation losses (% of fresh matter) during the ensiled period.

Fermentation losses	**negative control**		**EM Silage**		*p value*
(% of FM) (N=6)	**mean**	**st.dev.**	**mean**	**st.dev.**	
7 days	0,27	0,01	0,28	0,02	*0,1263*
14 days	0,39	0,00	0,42	0,00	***0,0002***
21 days	0,48	0,02	0,50	0,01	***0,0353***
28 days	0,55	0,01	0,57	0,05	*1,0000**
35 days	0,60	0,02	0,59	0,04	*0,4356*
42 days	0,87	0,09	0,81	0,17	*0,4870*
49 days	1,41	0,17	1,21	0,29	*0,1693*
56 days	1,93	0,18	1,68	0,32	*0,1210*
63 days	2,33	0,17	2,10	0,31	*0,1344*
70 days	2,71	0,15	2,55	0,23	*0,1820*
77 days	2,90	0,14	2,77	0,19	*0,1852*
84 days	3,05	0,14	2,92	0,21	*0,2135*
102 days	3,28	0,12	3,17	0,15	*0,2101*

* non-parametric one-way Anova

- Fermentation characteristics

The fermentation characteristics, microbial counts and the aerobic stability parameters are summarized in Table 3.

Table 3. Fermentation characteristics, microbial analyses and aerobic stability parameters.

Fermentation characteristics	negative control		EM Silage		*p value*
	mean	st.dev.	mean	st.dev.	
DM at desiling (g/kg FM) (N=4)	328,53	7,26	337,05	10,89	*0,2406*
crude protein (g/kg DM) (N=4)	132,92	8,48	149,77	4,44	***0,0125***
ammonia (g/kg DM) (N=4)	0,340	0,029	0,315	0,030	*0,2766*
ammonia-N / total N (N=4)	10,22	2,59	8,41	0,61	*0,2228*
pH (N=4)	4,44	0,05	4,56	0,12	*0,1167*
lactic acid (g/kg DM) (N=4)	36,10	8,79	62,07	20,94	*0,0606**
acetic acid (g/kg DM) (N=4)	6,53	2,08	9,53	4,04	*0,2343*
butyric acid (g/kg DM) (N=4)	1,75	0,90	1,96	1,04	*0,7317*
propionic acid (g/kg DM) (N=4)	not detected		not detected		-
ethanol (g/kg DM) (N=4)	74,50	4,29	70,26	1,48	*0,1108*
water-soluble carbohydrates (g/kg DM) (N=4)	17,08	4,22	13,98	3,69	*0,3105*
yeasts (log cfu/g FM) (N=6)	3,94	0,88	3,37	0,40	*0,1840*
moulds (log cfu/g FM) (N=6)	2,52	0,64	1,96	0,02	*0,2448**
aerobic stability +3°C (h) (N=6)	140,30	29,12	165,61	19,78	***0,0303***
DM after Honig (g/kg FM) (N=6)	288,48	7,67	291,36	5,89	*0,3503*
DM loss during Honig (g/day) (N=6)	0,85	0,38	0,83	0,26	*0,8530*

* non-parametric one-way Anova

The **dry matter content at desiling** did not differ significantly between the negative control and the EM Silage treatment. Application of EM Silage increased the **crude protein** content significantly compared to the negative control. The **ammonia** and **ammonia fraction** were not significantly influenced by EM Silage treatment, nor was the **pH**. There was a tendency towards more **lactic acid** after EM Silage application, but this was not significantly different from the negative control. The levels of **acetic acid** did not differ significantly between both objects. **Butyric acid** was detected at similar levels for both objects, while **propionic acid** was below detection limit in all samples. **Ethanol** levels were not significantly different between both objects, nor were the **water-soluble carbohydrates**.

Yeast counts and **mould counts** were not significantly different from the negative control than after EM Silage treatment.

EM Silage treatment increased the **aerobic stability** significantly compared to the negative control. The **dry matter content after Honig** and the **dry matter losses during the Honig protocol** did not differ significantly between both objects.

5. Conclusion

Application of EM Silage did not have a significant influence on most of the fermentation parameters of the first cut *Lolium perenne* ryegrass silage. There was a tendency towards a higher lactic acid content, but the difference was not significant because of high standard deviation. EM Silage treatment did increase the crude protein content of the silage significantly and significantly improved the aerobic stability compared to the negative control.

6. References

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7. Annexes

Annex 1. Results of counting of lactic acid bacteria and yeasts on undiluted EM Silage product.

Annex 2. Crude data.

Annex 3. Statistical analysis.