STUDY OF THE EFFECT OF EM SILAGE APPLICATION ON THE FERMENTATION CHARACTERISTICS AND THE AEROBIC STABILITY OF CORN COB MIX AT 66.76% DRY MATTER

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Title:	*Study of the effect of EM Silage application on the fermentation characteristics and the aerobic stability of Corn Cob Mix at 66.76% dry matter.*
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Client:	*Agriton*
Publication date:	*September 10th, 2015*

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1. Aim

Aim of the ensiling trial was to test the effect of treatment of corn cob mix (CCM) (66.76% dry matter) with EM Silage on silage fermentation characteristics and aerobic stability parameters.

2. Experimental setup

CCM (66.76% dry matter) was treated with the appropriate treatment solution for both objects:

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

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 456,71 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. After an ensiled period of 84 days, all microsilos were subjected to aerobic stress during 24 hours. All microsilos were desiled after 99 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 CCM was placed loosely into a recipient (volume 1 liter) for aerobic deterioration at 21,75 ± 1,5°C in insulating boxes during 7 days. Each recipient was covered with a double layer of cheesecloth to prevent drying and contamination, and to allow gas exchange. Two recipients were filled per microsilo. In the geometric center of each recipient, a 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. Per microsilo, the results of both recipients were averaged out for aerobic stability, dry matter after Honig and dry matter loss during the Honig protocol.

The obtained data (Annex 2) were statistically analyzed with SAS 6.1 (Annex 3). Outliers were removed 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.

Corn Cob Mix	
dry matter content (g/kg FM)	667,6
water-soluble carbohydrates (g/kg DM)	44,1
yeasts (cfu/g FM) (N=3)	3,3E+05
moulds (cfu/g FM) (N=3)	3,5E+04
lactic acid bacteria (cfu/g FM) (N=3)	6,1E+03
Counts on EM Silage (batchnr. 49140019)	
yeasts (cfu/g)	2,5E+03
lactic acid bacteria (cfu/g)	1,1E+06

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.

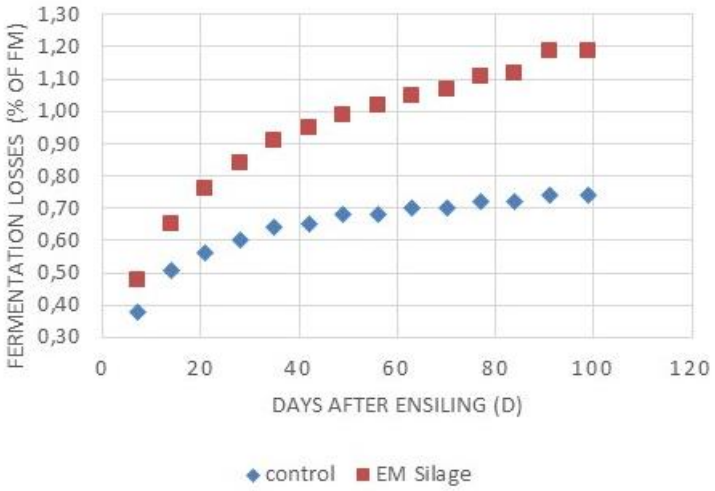

Figure 1. Evolution of fermentation losses during ensiled period.

The fermentation losses were higher after treatment with EM Silage compared to the negative control during the entire ensiled period, which is confirmed statistically in Table 2. However, the fermentation losses are low for both objects.

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

Fermentation losses (% of FM) (N=8)	**control**		**EM Silage**		***p-value***
	mean	**st.dev.**	**mean**	**st.dev.**	
7 days	0,38	0,01	0,48	0,01	***<0,0001***
14 days	0,51	0,02	0,65	0,04	***<0,0001***
21 days	0,56	0,02	0,76	0,04	***<0,0001***
28 days	0,60	0,03	0,84	0,04	***<0,0001***
35 days	0,64	0,02	0,91	0,05	***<0,0001***
42 days	0,65	0,04	0,95	0,06	***<0,0001***
49 days	0,68	0,03	0,99	0,06	***<0,0001***
56 days	0,68	0,02	1,02	0,06	***<0,0001***
63 days	0,70	0,02	1,05	0,06	***<0,0001***
70 days	0,70	0,02	1,07	0,06	***<0,0001***
77 days	0,72	0,02	1,11	0,07	***<0,0001***
84 days	0,72	0,01	1,12	0,07	***0,0034****
91 days	0,74	0,01	1,19	0,04	***0,0034****
99 days	0,74	0,01	1,19	0,04	***0,0034****

* non-parametric one-way Anova

- Fermentation characteristics

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

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

Fermentation characteristics	control		EM Silage		*p-value*
	mean	st.dev.	mean	st.dev.	
DM at desiling (g/kg FM) (N=4)	669,20	0,86	659,34	27,73	*0,3123**
crude protein (g/kg DM) (N=4)	71,74	1,06	74,35	3,12	*0,1644*
ammonia (g/kg DM) (N=4)	0,526	0,001	0,397	0,025	***0,0304****
ammonia-N / total N (N=4)	3,71	0,06	2,71	0,07	***<0,0001***
pH (N=4)	3,94	0,01	4,06	0,01	***0,0247****
lactic acid (g/kg DM) (N=4)	26,73	0,66	18,74	1,53	***<0,0001***
acetic acid (g/kg DM) (N=4)	6,22	0,27	9,00	0,96	***0,0014***
butyric acid (g/kg DM) (N=4)	0,00	0,00	0,00	0,00	-
propionic acid (g/kg DM) (N=4)	0,00	0,00	0,00	0,00	-
ethanol (g/kg DM) (N=4)	3,24	0,34	6,59	0,55	***<0,0001***
WSC (g/kg DM) (N=4)	3,84	0,01	3,37	0,96	*0,8852**
yeasts (log cfu/g FM) (N=6)	4,74	0,50	4,25	0,78	*0,2154*
aerobic stability +3°C (h) (N=6)	65,54	22,89	154,08	19,72	***<0,0001***
DM after Honig (g/kg FM) (N=6)	663,66	3,09	668,00	2,77	***0,0379***
DM loss during Honig (g/day) (N=6)	0,92	0,48	0,37	0,19	***0,0412***

* non-parametric one-way Anova

The **dry matter content at desiling** did not differ significantly between the negative control and the EM Silage treatment, nor did the **crude protein** content. EM Silage application did lower the **ammonia** content and the **ammonia fraction** significantly compared to the negative control. Treatment with EM Silage resulted in a significantly higher **pH t**han the negative control, due to a significantly lower level of **lactic acid** and a significantly higher **acetic acid** level in case of EM Silage application. **Butyric acid** and **propionic acid** were not detected in any sample. The **ethanol** content of the negative control was significantly lower than after EM Silage treatment. The **water-soluble carbohydrates** did not differ significantly between both objects, nor did the **yeast counts**. Mould numbers were below detection limit in all samples. EM Silage application significantly improved the **aerobic stability** compared to the negative control and resulted in significantly higher **dry matter content after the Honig protocol** and significantly lower **dry matter losses during the Honig protocol**.

5. Conclusion

Application of EM Silage resulted in a more heterofermentative fermentation pattern compared to the negative control: homofermentative lactic acid bacteria are very efficient producers of lactic acid, thus improving silage fermentation – heterofermentative lactic acid bacteria on the other hand are less efficient in producing lactic acid and imply also production of carbon dioxide, acetic acid and ethanol. The produced acetic acid however improves aerobic stability (McDonald et al., 1991).

Treatment of the CCM with EM Silage increased the fermentation losses significantly, but in absolute values the losses were at a low level for the negative control as well as for the EM Silage treatment. EM Silage application resulted in elevated levels of acetic acid and ethanol and in a reduced lactic acid level compared to the negative control. Aerobic stability parameters were significantly improved by EM Silage treatment.

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.