CHEMICAL AND MICROBIOLOGICAL CHARACTERISTIC OF SILOMAIZE ENSILED WITH SOME LACTIC ACID BACTERIA STRAINS

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ABSTRACT
The object of the trial was to study the effect of some lactic acid bacteria strains on the chemical composition, energy- and metabolisable protein (MP) content, microbiological characteristics and in-silo weight and dry matter losses of whole crop maize silages.
The whole plant maize raw material was 32% DM, in soft cheddar stage of grain ripeness. It was ensiled in 4.2 litre capacity glass micro-size silos in 5 replicates /each treatment and stored on constant 25 °C room temperature on day 95. The average packing desity was 211kg DM/m^3
The applied treatments: 1. Untreated control, 2. *Enterococcus faecium* 100.000 CFU/g FM, 3. *Lactobacillus plantarum* 50.000 CFU/g + *Enterococcus faecium* 50.000 CFU/g, 4. *Lactococcus lactis* 100.000 CFU/g, 5. *Lactobacillus plantarum* 50.000 CFU + *Lactococcus lactis* 50.000 CFU/g, 6. *Lactobacillus plantarum* 100.000 CFU
The main experiences are the following:

- Chemical composition of whole crop maize silages treated by lactic acid bacteria strains are significantly differed from the control in some cases on P 5% level but the nutritive value (energy and MP content) of silages did not change significantly compare to the control untreated silage.
- Number of yeast and mould CFU of control silage was the highest (4.5 x 10^4 CFU/g FM) among all kind of treated ones, which was significant on P 1% level.
- Weight loss and DM loss were lower in all of the lactic acid bacteria treated silages in general than it was measured in the control silage. Least weight loss and one-third of DM loss was detected in *Lactobacillus plantarum* 100.000 CFU/g treated silage among all kind of silages.

Keywords: whole crop maize silage, lactic acid bacteria strain, *Lactobacillus plantarum*, chemical composition, weigh and DM loss

INTRODUCTION

The object of the trial was to study the effect of *Lactobacillus plantarum*, *Enterococcus faecium and Lactococcus lactis* lactic acid bacteria (LAB) strains applied them by in itself and with combination of *Lactobacillus plantarum* on the chemical composition, energy- and metabolisable protein (MP) content, microbiological characteristics and in-silo weight- and dry matter loss of whole plant maize silages during the fermentation and 95 days of storage.
Microbiological inoculants are the dominant silage additives in most part of the word nowadays. The aim is to have a rapid and efficient fermentation, minimizing weight and DM loss, to reduce the risk of deterioration, to keep nutritive value similar to that of the crop at ensiling (MUCK 2013)
Most of the bacterial inoculants containing homofermentative lactic acid bacteria (LAB) in most cases such as *Lactobacillus plantarum*, secondly *Enterococcus faecium*, *Lactococcus lactis* are often added to silage because they very quickly produce large quantities of lactic acid, which lowers the pH of the silage (LI AND NISHINO 2011, MARCINAKOVA ET AL., 2008)

Homofermentative strains such as *Lactobacillus plantarum*, *Enterococcus faecium* and *Pediococcus spp*. produce the largest reductions in pH, higher lactate: acetate ratios and lower dry matter losses by 1-2% (WEINBERG AND MUCK, 1996).
The mixture of *Lactobacillus plantarum* and *Enterococcus faecium* used inhibited the development of yeast and mould populations in barley silages, both during ensiling and upon aerobic exposure and increased aerobic stability (MCALISTER ET AL., 1995).
DRIEHUIS ET AL. (1999) showed that LAB affected the activity of yeasts in two ways. Firstly during anaerobic conditions, the survival of yeasts is reduced, and secondly, during the aerobic exposure, yeast growth is reduced. Silages treated with inoculants containing various strains of *Lactobacillus plantarum* had lower yeast, moulds, ethanol, and ammonia-N concentrations than did untreated silages.
Some inoculant lactic acid bacteria strains produce anti-microbial compounds that inhibit mould growth or undesirable bacterial species like *Salmonella sp.*, *Listeria sp.* and *Escherichia coli* (GOLLOP ET AL., 2005).
SCHAEFER ET AL. (1989) found inoculants did not inhibit the growth of yeasts or the aerobic deterioration of corn silage. Other researchers have concluded that inoculants improved the aerobic stability of corn silage as measured by a lower mean temperature and a reduction in the disappearance of WSC and lactic acid (WOHLT, 1989, PHILLIP AND FELLNER, 1992).
The additive containing *Lactococcus lactis* NCIMB 30160 has the potential to improve the production of silage from all forages by increasing lactic acid content and the preservation of dry matter, by reducing the pH and moderately the loss of protein, as determined by ammonia-N. (EFSA, 2011).

MATERIAL AND METHOD

Experimental ensilage procedure

The applied treatments:
T1. Untreated, control whole crop maize
T2. *Enterococcus faecium* 100.000 CFU/g FM
T3. *Lactobacillus plantarum* 50.000 CFU/g +
Enterococcus faecium 50.000 CFU/g FM
T4. *Lactococcus lactis* 100.000 CFU/g FM
T5. *Lactobacillus plantarum* 50.000 CFU +
Lactococcus lactis 50.000 CFU/g *FM*
T6. *Lactobacillus plantarum* 100.000 CFU/g FM

Each type of inoculant was individually prepared for application by suspending the appropriate dosage in 100 ml distilled water and then evenly was applying 2 ml solution to 1 kg of fresh forage (FM) repectively. The control untreated silomaize was prepared with the same amount of distilled water to 1 kg FM as well.
The maturity of whole crop maize raw material was in soft cheddar stage of grain ripeness, the dry matter content was 32%. The treated forages were ensiled in airtight 4.2 litre capacity glass micro-size silos, sealed with screw-topped cap. Each treatment prepared in 5 replicates and stored on constant air-conditioned 25 C° room temperature during 95 days. The average packing density was 211 kg DM/m^3.

Chemical-, microbiological and statistical analysis

Dry matter, crude protein, crude fat, crude fiber, crude ash, WSC and NDF content of silages were analysed and the nutritive value (energy and metabolisable protein content) was calculated according to the internationally recognised methods.

Quantity determination of yeast and mould was based on the Hungarian standard MSZ ISO7954 Microbiology: General guidance for enumeration of yeasts and moduls.

Full statistical analyses was using an internationally recognised statistical procedure.

We processed data with the aid of Microsoft Excel program. As method of mathematical statistics, we used the method of comparison of calculated mean values and significance.

RESULTS

The chemical composition of whole crop maize silages and their significance compare to the control one can see on *Table 1*. The calculated nutritive value of he silages see on *table 2.*, while the mould and yeast CFU on silages on 95^{th} day of storage can see on *table 3.*, and the weight and dry matter loss of silages during 95 days storages show *table 4*.

T1. Control silage

The silage seemed suit for feeding, but mild deficiency of quality was predicted according to the sensory test.

The 31% dry matter content was least compare to the other LAB strain treated silages, which was connected with the highest dry matter loss (3.8%) among all silages.

Yeast and mould CFU of fresh silage was the highest (4.5 x 10^4CFU) among all treated silages, and contained mainly yeast colonies.

T2. *Enterococcus faecium* treated silage

Sensory test showed a good quality of silage.

The chemical composition differed from the control: higher DM, crude fat, N- free extract and WSC content characterised while the other components were less.

The significant differences of the chemical compositions compare with control silage was most frequent among all LAB treated silages. There was no significant in crude ash, WSC surplus and less NDF content.

Microbiological profile was more favourable with 3.5 x 10^3CFU

Fermentation loss was less than in control during 95 days storage: with 0,2 % weight loss, and the DM loss was one third only (with high CV%)

T3. *Lactobacillus plantarum + Enterococcus faecium* treated silage

Analyses with sensory test showed not a totally uniform quality, but all samples were unbroken and free from mould.

The differences in chemical composition was significant in case of less protein and fiber and higher N-free extract content compare to the control silages.

DM loss was similar, weight loss was higher a bit than the control during the 95 days of storage.

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Table 1. Chemical composition of whole crop maize silages and their significance compare to the control

Parameters		n	Treatments												Treatments	Level of signif. (P%)
			T1 (Control)		T2		T3		T4		T5		T6			
			Mean	s	Mean	s	Mean	s	Mean	s	Mean	s	Mean	s		
Dry matter	**%**	5	31.15	0.40	31.92	0.72	31.34	0.66	31.67	0.59	32.22	0.42	32.04	0.42	T1-T2 T1-T5 T1-T6	5% 5% 5%
Crude protein	**g/kg DM**	5	85.46	1.49	80.52	2.41	80.28	1.51	80.92	0.53	79.18	2.84	80.94	0.75	T_1-T_2 T_1-T_3 T_1-T_4 T_1-T_5 T_1-T_6	5% 5% 5% 5% 5%
Crude fat	**g/kg DM**	5	29.26	2.00	32.04	2.11	26.72	2.41	29.84	3.46	28.44	2.95	30.30	2.07	T_1-T_2	5%
Crude fiber	**g/kg DM**	5	216.56	9.86	198.40	10.16	200.22	13.05	207.80	13.41	210.16	10.67	189.56	12.30	T_1-T_2 T_1-T_3 T_1-T_6	5% 5% 5%
Crude ash	**g/kg DM**	5	46.20	2.42	44.30	2.54	43.16	0.92	45.08	2.14	46.44	2.17	46.04	1.46	T_1-T_3	5%
N-free extract	**g/kg DM**	5	622.50	10.80	644.72	10.35	649.62	13.13	637.06	14.97	635.78	9.53	653.12	11.68	T_1-T_2 T_1-T_3 T_1-T_6	5% 5% 5%
WSC	**g/kg DM**	5	27.78	10.26	33.08	11.41	19.28	6.01	28.20	6.07	8.90	2.21	4.40	4.71	T_1-T_5	5%
NDF	**g/kg DM**	5	432.80	21.20	427.06	35.20	414.87	25.84	416.94	25.52	442.58	27.39	396.80	35.79	-	ns

Table 2. Calculated nutritive value of whole crop maize silages on DM basis

Parameters	Mean of treatments (n=5)					
	T1 control	T2	T3	T4	T5	T6
Dry matters %	31.15	31.92	31.34	31.67	32.22	32.04
MPE g/kg DM	71.8	71.4	71.4	71.4	71.3	71.5
MPN g/kg DM	50.8	47.9	47.8	48.1	47.1	48.1
ME g/kg DM	10.53	10.62	10.55	10.57	10.53	10.59
NE(m) MJ/kg DM	6.78	6.87	6.81	6.82	6.79	6.84
NE(g) MJ/kg DM	4.24	4.33	4.28	4.29	4.26	4.30
NE(l) MJ/kg DM	6.42	6.48	6.4	6.45	6.43	6.46
UDP g/kg DM	24.8	23.4	23.3	23.6	23.0	23.5

Table 3. Mould and yeast CFU of silages on 95th day of storage

Treatment	Yeast- and mould CFU (n=5)		Treatment	Yeast- and mould CFU (n=5)	
	CFU/g	**Mean**		**CFU/g**	**Mean**
T1	1. 3.3×10^4	4.5×10^4	**T4**	1. 1.6×10^3	1.2×10^3
	2. 4.4×10^4			2. 1.2×10^3	
	3. 3.7×10^4			3. 1.2×10^3	
	4. 5.9×10^4			4. 1.0×10^3	
	5. 5.1×10^4			5. 1.3×10^3	
T2	1. 3.2×10^3	3.5×10^3	**T5**	1. 3.9×10^3	3.3×10^3
	2. 3.3×10^3			2. 2.9×10^3	
	3. 3.3×10^3			3. 4.5×10^3	
	4. 3.2×10^3			4. 3.1×10^3	
	5. 4.4×10^3			5. 2.3×10^3	
T3	1. -	4.1×10^3	**T6**	1. 3.0×10^3	3.1×10^3
	2. 3.3×10^3			2. 3.5×10^3	
	3. 3.6×10^3			3. 2.7×10^3	
	4. 4.4×10^3			4. 3.3×10^3	
	5. 4.9×10^3			5. 3.2×10^3	

Table 4. Weight and dry matter loss of fermentation during 95 days storage

Treatment	Loss of Fermentation		Treatment	Loss of Fermentation	
	Loss of weight %	Loss of dry matter %		Loss of weight %	Loss of dry matter %
T1 control			***T4***		
mean	**0.9**	**3.8**	mean	**1.1**	**2.5**
s	0.2	1.3	s	0.4	1.6
CV %	18.0	33.8	CV %	37.0	66.5
T2			***T5***		
mean	**0.7**	**1.2**	mean	**0.9**	**0.5**
s	0.3	2.2	s	0.1	1.3
CV %	46.8	185.4	CV %	10.3	245.4
T3			***T6***		
mean	**1.5**	**3.6**	mean	**0.6**	**1.0**
s	0.9	2.0	s	0.1	1.3
CV %	61.3	55.6	CV %	9.7	135.8

CONCLUSIONS

Comparison of the efficiency of some homofermentative lactobacillus strains such as *Lactobacillus plantarum, Enterococcus faecium and Lactococcus lactis* applied in itself or combined them with *Lactobacillus plantarum* on ensiling of whole crop maize has given opportunity to draw a lesson as follows:

We expected that the applied LAB strains protect nutritive value, decrease the risk of deterioration throught a favourable microbiological profile, promote to reduce weight and DM loss better than in untreated silage as it was introduced by number of authors (see chapter Introduction).

Our experiments proven them right in most of the case:

- Chemical composition of whole crop maize silages treated by lactic acid bacteria strains are significantly differed on P 5% level from the control in some cases but the nutritive value (energy and MP content) of silages did not change significantly compare to the control untreated silage.
- Number of yeast and mould CFU of control silage was the highest (4.5 x 10^4 CFU/g FM) among all kind of LAB treated ones.
- Weight loss and DM loss were lower in all of the lactic acid bacteria treated silages in general than it was measured in the control silage. Least weight loss and one-third of DM loss was detected in *Lactobacillus plantarum* 100.000 CFU/g treated silage among all kind of silages.
- There was no synergetic effect of the combination of *Enterococcus faecium* or *Lactococcus lactis* with *Lactobacillus plantarum* inoculant for chemical composition and nutritive value of silages.

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