Evaluation of fermentative parameters, aerobic stability and *in vitro* gas production of whole crop maize silage treated with a microbial inoculant containing *Pediococcus pentosaceus* and *Lactobacillus plantarum*

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Introduction Homofermentative lactic acid bacteria (^{ho}LAB) are used as silage additives to improve silage fermentation and preservation of energy value. ^{ho}LAB have been used because they are fast and efficient producers of lactic acid, improving natural silage fermentation. Literature review (Muck and Kung 1997) reported that in 60% of the studies reviewed, the inoculated silages, compared with untreated samples, show a lower pH, greater lactic acid content and less ammonia nitrogen. Furthermore, research (Kung and Muck 1997) indicates that microbial inoculation may have a role in increasing animal performances affecting the nutritive value of the silages. The aim of this trial was to determine the effects of a microbial inoculant containing *Pediococcus pentosaceus* and *Lactobacillus plantarum* as maize silage additive (L) in comparison with untreated silage (C) on the fermentative profile at 2, 5, 40 and 110 days of ensiling, and temperature, aerobic stability and multiple points *in vitro* Gas Production (GP) at 110 days of ensiling.

Material and methods Whole crop maize was harvested at milk-dough stage (276 g DM/kg as fed, 55, 240 and 487 g/kg DM, of WSC, Starch and aNDFom, respectively), with a theoretical length of cut of 15 mm. According to the European Food Safety Authority Guidance on technological additives (EFSA 2008), about 1500 g of fresh forage were ensiled in 2 L micro-silos with lid seal and bleed valve, with an average packing dry matter (DM) density of 246 kg/m^3. The inoculation of the fresh forage was made in order to obtain a mixture of lactic acid bacteria concentration of 1.0 x 10^5 CFU g^{-1} of fresh maize, with a ratio of 4 to 1 CFU between *Pediococcus pentosaceus* and *Lactobacillus plantarum.*

Negative control was treated with the same amount of water used to inoculate the treated samples to ensure a similar DM content. Seven replicates for each incubation time (2, 5, 40 and 110 d) and each treatment were prepared. VFA, lactic acid and alcohols were determined by gas chromatography. *In vitro* GP was determined following the method of Menke and Steingass (1988) at 2, 4, 6, 8 and 24 hours of incubation, on the silages after 110 days of ensiling.

Data were analyzed using the General Linear Model procedure of SAS (SAS Institute, Inc. 2001) for the evaluation of treatment effect.

Results and discussion The evaluation of fermentative parameters for C and L silages showed that microbial inoculant utilization significantly reduced pH values at 2, 5 and 40 days of incubation (Table 1). No differences were registered at 110 days.

Lactic acid content resulted higher for C silage after 5 days of ensiling (109 and 92.4 g/kg DM for C and L, respectively; P<0.01); however, the additive determined higher values at 40 (97.2 and 104 g/kg DM for C and L, respectively; P<0.05) and 110 days of incubation (93.9 and 102 g/kg DM for C and L, respectively; P<0.05).

Considering the other fermentative parameters, differences between treatments were detected only for acetic acid and ammonia nitrogen contents at 40 days of incubation. Particularly, lower acetic acid (21.4 and 18.3 g/kg DM for C and L, respectively; P<0.05) and higher N-NH_3 concentration (2.08 and 2.24 g/kg DM for C and L, respectively; P<0.01) were found in the silage treated with microbial inoculant. Propionic, isobutyric and butyric acids were detected only in trace in the samples, with the exception for butyric acid during the first week of incubation; however, at 2 and 5 days, only a very few amount of butyric acid was found (0,20 g/kg DM, on average). Small amounts of ethanol were detected in the silages until forty days of incubation (3.4 g/kg DM, on average); in the last period it increased at 7.49 and 5.75 g/kg DM, for C and L, respectively.

DM losses resulted similar up to 40 days of incubation (1.14 *vs* 2.70% for C and L, respectively), but a significant difference was found at 110 days, with lower DM losses for L treatment (7.12 *vs* 3.53%, P<0.01). Aerobic stability data did not show any difference due to the treatment applied to the forage. In fact, both treatments determined a rise in temperature higher than 3°C above the background temperature (20°C) after 3 days.

L treatment increased significantly GP at 6 and 8 hours (+10%); the same trend, even not significant, was also registered at 4 (+10%; P=0.062) and 24 hours (+5%; P=0.093) (Table 2). These results indicate a possible effect of the inoculum on rumen fermentability of organic matter after 110 days of ensiling.

Conclusions The results of this study confirm that the addition of ^{ho}LAB has positive effects on the silage fermentative pattern and on the reduction of DM loss. Moreover, ^{ho}LAB seems to improve the nutritive value of maize silage increasing *in vitro* rumen fermentability**.**

References

EFSA 2008. Guidance for the preparation of dossiers for technological additives. *The EFSA Journal* 774: 1-21.

Menke, K.H. & Steingass, H. 1988. Estimation of the energetic feed value obtained from chemical analysis and in vitro gas production using rumen fluid. *Animal. Research. & Development.* 28: 7-55.

Kung Jr., L. & Muck, R.E. 1997. Animal response to silage additives. In: *Silage: Field to Feedbunk*. NRAES-99. Northeast Regional Agric. Eng. Service. Ithaca. NY. USA. pp. 200–210.

Muck. R. E. & Kung Jr. L. 1997. Effects of silage additives on ensiling. Proc. *Silage: Field to feedbunk*. NRAES-99. Northeast Regional Agric. Eng. Service. Ithaca. NY. USA. pp. 187-199.

Table 1. Fermentative parameters of maize silage treated with the additive (L) compared with untreated control (C) at different days of ensiling.

Days of ensiling	2			5			40			110		
Treatment	C	L	SEM	C	L	SEM	C	L	SEM	C	L	SEM
pH	3.80^{A}	3.76^{B}	0.01	3.64^{A}	3.59^{B}	0.01	3.47^{A}	3.44^{B}	0.01	3.56	3.55	0.02
Temperature, °C *	20.0	20.1	0.14	20.1	20.0	0.16	19.8	20.2	0.15	20.7^{A}	19.2^{B}	0.30
Dry matter1, g/kg	275	273	1.66	275	273	2.41	273	269	1.60	256^{B}	266^{A}	2.15
DM losses, %	0.33	1.00	0.60	0.36	1.04	0.87	1.14	2.70	0.58	7.12^{A}	3.53^{B}	0.78
Lactic acid, g/kg DM	102	95.6	2.14	109^{A}	92.4^{B}	2.08	97.2^{b}	104^{a}	1.75	93.9^{b}	102^{a}	1.99
Acetic acid, g/kg DM	10.4	11.5	0.40	12.2	12.0	0.37	21.4^{a}	18.3^{b}	0.84	22.0	23.2	0.51
Propionic ac., g/kg DM	trace	trace	.	trace	trace	.	trace	trace	.	trace	trace	.
Isobutyric ac., g/kg DM	trace	trace	.	trace	trace	.	trace	trace	.	trace	trace	.
Butyric acid, g/kg DM *	0.20	0.20	0.09	0.26	0.14	0.10	trace	trace	.	trace	trace	.
Ethanol, g/kg DM	3.12	3.56	0.16	3.67	3.54	0.13	3.54	3.04	0.17	7.49	5.75	0.59
N-NH3, g/kg DM	1.52	1.42	0.05	1.59	1.58	0.05	2.08^{B}	2.24^{A}	0.01	2.61	2.57	0.08

[1] Dry matter content corrected for volatiles.
*Variables not normally distributed. In this case the P value reported is that obtained with Kruskal-Wallis Test.
$^{A, B}$: capital letters differ for P<0.01; $^{a, b}$: lower case letters differ for P<0.05.

Table 2. Gas Production (GP) of maize silage treated with inoculants (L) in comparison with untreated control (C) at 110 days of ensiling.

	C	L	SEM	P value
2h GP, mL/200 mg DM	8.8	9.7	0.39	0.139
4h GP, mL/200 mg DM	16.9	18.6	0.57	0.062
6h GP, mL/200 mg DM	23.3	26.0	0.65	0.019
8h GP, mL/200 mg DM	28.4	31.4	0.66	0.005
24h GP, mL/200 mg DM	46.1	48.5	0.92	0.093