Effects of inoculant type and composition on fermentation, aerobic stability and volatile organic compounds in grass silage

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Introduction Inoculants have been widely used to improve silage production. However, due to the use of various lactic acid bacteria types – homo- (LAB_{ho}) and heterofermentative (LAB_{he}) – and strain combinations, the effects on silage quality may be different. This study aimed at determining the impact of inoculants solely containing LAB_{ho} and combination products (LAB_{ho+he}) of differing LAB_{ho}:LAB_{he} ratio on fermentation, aerobic stability (ASTA) and volatile organic compounds (VOC) production.

Materials and Methods A first cut, *Lolium perenne*-dominated natural grassland (31% DM) from a dairy farm near Bautzen, Germany, was chopped on May 10, 2016 by a Class Jaguar set at a theoretical particle length of about 30 mm. The herbage was treated with one of the following additives, applied at 10 ml/kg fresh forage, to give an incoulation rate (IR) of: *L. buchneri* DSM 13573, IR: 100,000 cfu/g + *L. plantarum* DSM 3677, IR: 50,000 cfu/g + *L. plantarum* DSM 3677, IR: 50,000 cfu/g (LBLP); *Lactobacillus buchneri* CNCM-I 4323, IR: 100,000 cfu/g + *P. acidilactici* DSM 11673, IR: 67,000 cfu/g (LBPA); *L. plantarum* DSM 16627, IR: 100,000 cfu/g + *L. paracasei* NCIMB 30151, IR: 50,000 cfu/g (LPLP); *Lc. lactis* NCIMB 30160+*P. acidilactici* DSM 16243+*L. paracasei* DSM 16245, total IR: 125,000 cfu/g (LLPL). Untreated grass received tap water at 10 ml/kg. After packing the material into 1.6-L glass jars, which were equipped with a hole (6 mm diameter) in the body and the lid closed by rubber stoppers, the jars were stored for 62 days at about 22 °C. The rubber stoppers were removed for 24 hours on days 28 and 55 of fermentation to allow air ingress. Standard methods for silage quality analysis were used. Fermentation pattern and ethyl esters were determined according to Weiss et al. (2016), and aerobic stability (ASTA) was evaluated over 336 hours (14 days) of aeration (Honig, 1990). Data were submitted to statistical analysis by procedures MIXED and REG of SAS 9.4. Significance was declared at $P<0.05$, and the Tukey´s test was employed for discrimination between least-square means.

Results and Discussion All inoculants improved fermentation efficiency as reflected by lower DM losses when compared to CON (Table 1). There was a shift to more homolactic fermentation at the expense of acetic acid for pure LAB_{ho} treatments, which explains higher fungal counts and lower ASTA compared with CON and LBPA (Auerbach et al., 2013). However, also the LAB_{ho+he} additive LBLP showed these effects, which may be attributed to the ratio between LAB_{ho} and LAB_{he} in the product. Apparently, the two *L. plantarum* strains outperformed *L. buchneri*. Our data substantiate previous observations by Auerbach et al. (2013) that aerobic stability strongly depends on acetic acid concentration ($y=10.65e^{0.1702x}$, $R^2=0.96$, $P<0.001$), yeast count ($y=819.4x^{-1.912}$, $R^2=0.98$, $P<0.001$) and water-soluble carbohydrate content ($y=739.1x^{-1.243}$, $R^2=0.94$, $P<0.001$) upon silo opening.

Table 1 Fermentation characteristics, yeast count, aerobic stability and ethyl esters in grass silage (data presented as LSmeans in % DM unless stated otherwise, n=3)

Parameter	CON[1]	LBLP[2]	LBPA[3]	LPLP[4]	LLPL[5]	SEM	*P*
DM loss (%)	8.6^{d}	5.4^{a}	7.3^{c}	5.3^{a}	5.8^{b}	0.06	***
WSC[6]	3.06^{a}	11.75^{c}	2.48^{a}	17.18^{d}	9.06^{b}	0.163	***
NH_3-N (% total N)	11.5^{e}	6.8^{a}	9.5^{d}	7.4^{b}	7.8^{c}	0.08	***
pH	4.00^{c}	3.79^{a}	3.95^{b}	3.94^{b}	3.77^{a}	0.005	***
Lactic acid	6.68^{a}	7.61^{ab}	7.27^{a}	8.52^{b}	7.60^{ab}	0.220	**
Acetic acid	2.33^{c}	1.04^{b}	2.79^{d}	0.68^{a}	1.16^{b}	0.075	***
Ethanol	1.07^{c}	0.51^{a}	0.69^{b}	0.64^{ab}	0.51^{a}	0.034	***
1,2-propanediol	1.31^{b}	0.07^{a}	1.12^{b}	0^{a}	0.11^{a}	0.042	***
Yeast (log cfu/g)	2.2^{a}	5.2^{b}	1.7^{a}	6.2^{c}	4.9^{b}	0.13	***
Moulds (log cfu/g)	2.0^{a}	4.7^{b}	1.7^{a}	4.5^{b}	2.9^{ab}	0.40	***
ASTA[7] (hours)	125^{b}	33^{a}	319^{c}	24^{a}	45^{a}	8.5	***
Esters[8] (mg/kg DM)	189^{b}	114^{a}	129^{a}	90^{a}	99^{a}	10.3	***

[1]untreated; [2]*L. buchneri* DSM 13573+*L. plantarum* DSM 3676+*L. plantrum* DSM 3677; [3]*L. buchneri* CNCM I-4323+*P. acidilactici* DSM 11673; [4]*L. plantarum* DSM 16627+*L. paracasei* NCIMB 30151; [5]*Lc. lactis* NCIMB 30160+*P. acidilactici* DSM 16243+*L. paracasei* DSM 16245; [6]water-soluble carbohydrates; [7]aerobic stability;[8]ethyl lactate+ethyl acetate; LSmeans in rows with unlike superscripts differ (Tukey´s test), ***P*<0.01, ****P*<0.001.

By reducing ethanol concentrations, the additives also restricted ethyl esters formation, which supports results by Weiss and Auerbach (2013). There was strong linear relationship between the concentrations of ethanol and ethyl esters (y=23.8+146.4x, R^2=0.66. $P<0.001$) (Figure 1).

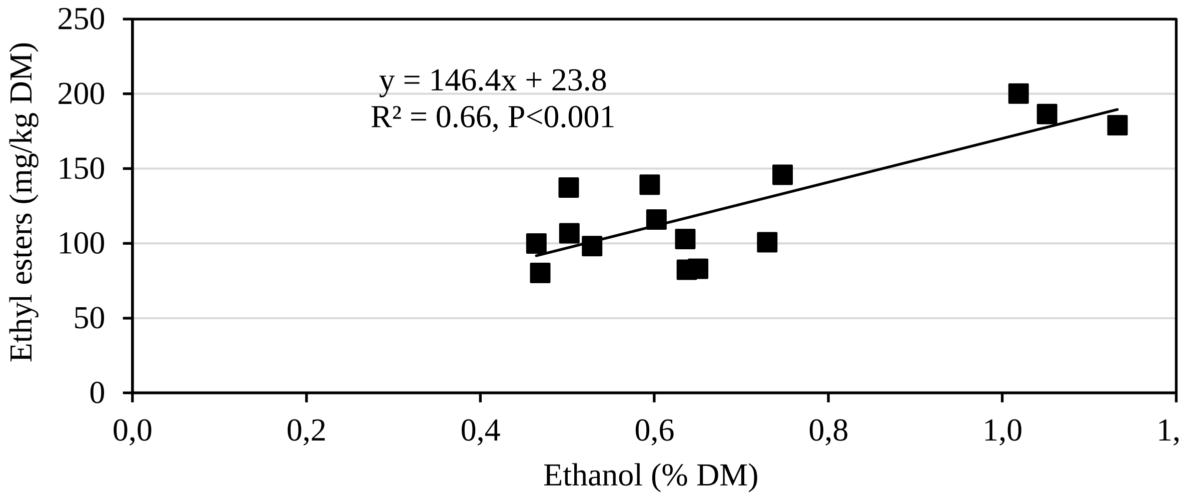

Figure 1 Relationship between the concentrations of ethanol and ethyl esters (n=15).

Conclusions The use of inoculants containing LAB_{ho} improved the fermentation process but only in combination with LAB_{he} did they have the protential to improve aerobic stability. The composition of LAB_{ho+he} products appears to be crucial, and warrants further investigations.