THE EFFECT OF DIFFERENT COMBINATIONS OF LACTIC ACID BACTERIA ON ESTER CONCENTRATIONS IN GRASS AND WHOLE-PLANT MAIZE SILAGE

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Abstract

Alcohol esters such as ethyl lactate and ethyl acetate are discussed as having a negative effect on silage palatability and intake. This study evaluated ester formation in grass and whole-plant maize silages treated with combinations of homo- and heterofermentative lactic acid bacteria. Concentrations of ethyl lactate and ethyl acetate were determined and compared with values estimated using a commonly applied ethanol-based prediction equation. Additive treatments influenced ester profiles, demonstrating treatment effects on fermentation pathways. However, under optimal ensiling conditions with limited air exposure and inoculation with heterofermentative lactic acid bacteria, ethanol concentration alone did not reliably predict ethyl lactate and ethyl acetate concentrations, and estimated values were generally higher than analytically determined concentrations. The results indicate that direct chemical determination of alcohol esters is necessary for accurate evaluation of silage fermentation characteristics, especially when silage quality or palatability is of concern.

Keywords: silage, alcohol esters, ethyl lactate, ethyl acetate, ethanol, lactic acid bacteria, fermentation quality, silage additives, VOCs

INTRODUCTION

Silages with elevated ethanol concentrations are frequently reported to exhibit reduced palatability. Weiss (2016) demonstrated a strong positive correlation between ethanol concentration and ethyl ester levels in silages, specifically ethyl lactate and ethyl acetate. These volatile organic compounds (VOCs) are often discussed as potential contributors to diminished feed intake and adverse effects on animal health. Owing to the labour-intensive and costly nature of VOC analyses, Weiss *et al.* (2017) proposed an estimation equation based on ethanol concentration.

The objective of the present study was to quantify alcohol esters in grass and maize silages treated with various silage additives and to evaluate their relationship to ethanol concentration.

MATERIAL AND METHODS

In experiment I, grass was ensiled in round bales at a dry matter (DM) content of 407 g/kg. The herbage originated from a field in northwestern Germany. During baling, grass was treated with three commercial silage inoculants. Nine bales were treated with a mixture containing only homofermentative lactic acid bacteria (HF; *L. plantarum*, *Enterococcus lactis*, and *Lactococcus lactis*). Eight bales were treated with an additive containing a mixture of two strains of homo- and heterofermentative lactic acid bacteria (LLLB; *Lactococcus lactis* and *L. buchneri*). Seven bales were treated with an additive containing a mixture of three strains of homo- and heterofermentative lactic acid bacteria (LBLDLP; *L. buchneri*, *L. diolivorans*, and *L. plantarum*). Bales were sampled after 100 days of ensiling.

In experiment II, whole-plant maize was ensiled in 1.7-liter volume mini-silos at a DM content of 339 g/kg. Five mini-silos per treatment were prepared. Control treatment (CON) was not inoculated. LLLB treatment was inoculated with a mixture of *Lactococcus lactis* and *L. buchneri*. LBLDLR treatment was inoculated with a mixture of *L. buchneri, L. diolivorans*, and *L. rhamnosus*. LBLP treatment was inoculated with a mixture of *L. buchneri* and *L. plantarum*. Mini-silos were stored for 109 days at room temperature before analysis.

Silages were analysed for proximate analysis, forage hygiene, and fermentation products, including ethyl lactate,

Tab. 1: Effect of treatment on the silage fermentation characteristics

	Grass silage			Whole-plant maize silage			
	HF	LLLB	LBLDLP	CON	LLLB	LBLDLR	LBLP
DM g/kg	388	407	371	347	342	344	347
pH	4.32[c]	4.64[a]	4.43[b]	3.76[d]	3.98[a]	3.84[a]	3.80[c]
Lactic acid g/kg DM	68.3[a]	36.6[b]	38.0[b]	46.6[a]	29.6[d]	33.7[c]	42.0[b]
Acetic acid g/kg DM	7.0[c]	26.7[b]	39.4[a]	17.4[b]	30.5[a]	31.8[a]	18.5[b]
Propionic acid g/kg DM	nd[c]	0.3[b]	3.4[a]	nd[c]	1.3[a]	0.4[b]	0.4[b]
Ethanol g/kg DM	5.7[b]	10.5[a]	9.8[a]	9.8[c]	12.8[b]	9.0[c]	14.9[a]
Propanol g/kg DM	0.4[c]	2.0[b]	18.0[a]	1.1[c]	8.7[b]	11.8[a]	1.3[c]
1,2-propanediol g/kg DM	1.5[c]	18.7[a]	8.1[b]	3.9[c]	5.2[b]	nd[d]	8.1[a]
Methanol mg/kg DM	367[a]	274[b]	242[b]	163[b]	164[b]	229[a]	171[b]
Ethyl acetate mg/kg DM	nd[b]	68[a]	83[a]	44[b]	133[a]	70[b]	54[b]
Propyl acetate mg/kg DM	nd[b]	20[b]	232[a]	nd[c]	103[b]	146[a]	nd[c]
Ethyl lactate mg/kg DM	72[a]	24[b]	54[a]	123[b]	102[c]	78[d]	152[a]

HF = *L. plantarum*, *Enterococcus lactis*, and *Lactococcus lactis*; LLLB = *Lactococcus lactis* and *L. buchneri*; LBLDLP = *L. buchneri*, *L. diolivorans*, and *L. plantarum*; CON = untreated whole-plant maize silage, LBLDLR = *L. buchneri*, *L. diolivorans*, and *L. rhamnosus*; LBLP = *L. buchneri* and *L. plantarum*; DM = dry matter corrected for volatiles; nd = not detected, set as 0 for the statistical analysis. Values with different superscript letters within crop are significantly different ($P < 0.05$).

https://doi.org/10.11118/978-80-7701-093-1-0076

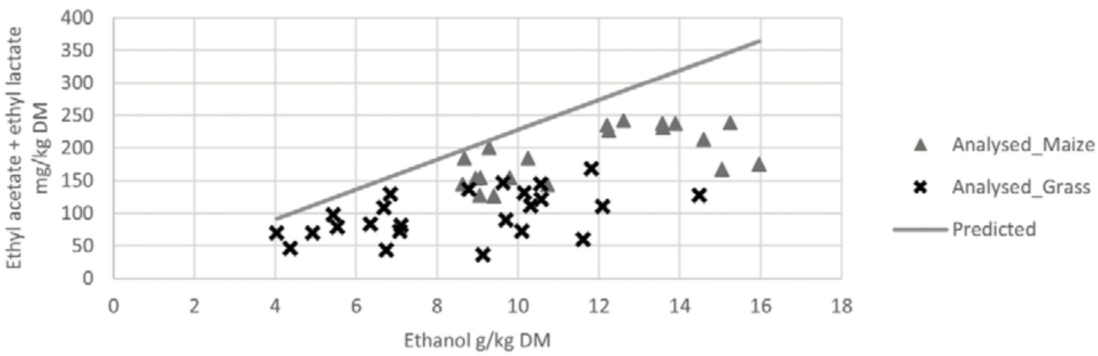

Fig. 1: Comparison between predicted as *Σ ethyl lactate + ethyl acetate (mg/kg DM) = ethanol (g/kg DM) × 114/5* (Weiss 2017) and analysed concentrations of ethyl acetate and ethyl lactate in grass and whole-plant maize silages

ethyl acetate, and propyl acetate. Predicted content of the sum of ethyl lactate and ethyl acetate was calculated with the equation of Weiss (2017). Data were analysed separately for each experiment as a completely randomized design using treatment as a fixed factor.

RESULTS AND DISCUSSION

The products LLLB and LBLDLP significantly increased acetic acid and ethanol in grass silage compared with HF treatment (Table 1). In experiment II, LBLP silage had the highest ethanol concentration, followed by LLLB.

The increased concentrations of acetate and ethanol in silages treated with products containing *L. buchneri* are consistent with the proposed pathway for anaerobic degradation of lactic acid into 1,2-propanediol, acetic acid, and trace amounts of ethanol (Oude Elfering *et al*., 2001). The predicted concentrations of ethyl acetate and ethyl lactate were higher than the chemically analysed values in all samples (Figure 1).

The weak correlation between ethyl esters and ethanol content in grass silages can be explained by the high pH (>4.25) observed in our study (Weiss 2017). Weiss (2022) reported that the regression lines for ethyl acetate and ethyl lactate with respect to ethanol content may exhibit different slopes depending on the practical silo type and sampling time. In our experiments, silages were prepared under optimal conditions: immediate wrapping of bales after grass baling and airtight sealing of mini-silos within 3 hours after maize harvest. The silages were not exposed to air before sampling. The counts of yeasts and moulds in silages were below the detection level of 500 cfu/g in all treatments. Weiss *et al*., (2022) confirmed that yeasts can produce ethyl acetate. Yeasts are also associated with increased ethanol content in silages and increased dry matter loss (McDonald *et al*, 1991). In our study, we used products containing *L. buchneri*. On one side, these bacteria convert lactic acid into acetic acid and trace amounts of ethanol. On another side, acetic acid produced by *L. buchneri* inhibits yeasts. The dry matter loss was below 5 % in all treatments (data not shown). It can explain the weaker correlation between ethanol content and the sum of ethyl esters in our study.

CONCLUSION

Previous studies demonstrated a strong correlation between ethanol content and ethyl esters, especially in conditions where high ethanol content was associated with greater dry matter losses in the presence of yeasts in silage. Our study demonstrated that under optimal ensiling conditions with minimal air exposure and silage inoculation with heterofermentative lactic acid bacteria, which reduces yeast counts, ethanol concentration alone cannot reliably predict ethyl lactate and ethyl acetate levels. Consequently, in situations where silage quality or palatability is of concern, direct chemical determination of alcoholic esters is recommended to ensure accurate evaluation.

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