Novel results on the formation of volatile organic compounds (VOC) in silages

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Abstract. The incidence of volatile organic compounds, including ethyl esters of lactic and acetic acids, in grass silages was evaluated based on a total of 620 samples from 10 different lab-scale ensiling experiments. These substances were detected after cold-water extraction by gas-chromatography. The correlations between ethanol and esters concentrations varied greatly depending on the trial. It was shown that ethanol concentration and pH of the silages affected ester formation. Low silage pH stimulated ester accumulation. Up to pH of 4.3, a stronger relationship between ethanol and esters contents was found than in grass silages having higher pH values. By allocating the silages to different ethanol classes it was shown that at up to 10 g/kg DM, the correlation between pH and total ester content was very weak (r_S =-0.22). At >10 g ethanol per kg DM, there was a strong negative relationship between the two tested parameters (r_S =-0.82). Based on all available data (n=1148) from different types of silages, a generally valid model is proposed to predict total ester concentrations (y) as a function of ethanol content (x): y=114x (R^2=0.76). It is recommended to use silage additives with proven record of reducing ethanol in silages, thereby minimizing the production of ethyl esters and excluding the potential negative effects of volatiles on feed intake.

Keywords: Grass silage, ethanol, ethyl acetate, ethyl lactate, volatile organic compounds (VOC).

Introduction

Based on empirical observations from commercial farms that well preserved, but odd-smelling maize silages may cause problems regarding feed intake and milk yield by dairy cows, volatile organic compounds (VOC) were analyzed in recent studies (Weiss *et al.*, 2009a, 2009b). According to Gerlach *et al.* (2012), feed intake by goats was negatively correlated with the concentration of the ester ethyl lactate. Strong correlations were found in maize silages between ensiling conditions, type of silage additive and ethanol content and the concentrations of the ethyl esters – ethyl acetate (EA) and ethyl lactate (EL). Ester and ethanol levels were highest in silages stored under strict anaerobic conditions. Elevated levels of ethanol and the corresponding esters EA and EL were not only detected in maize silages, but also in silages from whole-crop cereals and sorghum (Weiss and Auerbach 2012a, b). Regardless of silage type, silage additive and ensiling conditions, in the most cases there was found a strong correlation between ethanol and ester concentrations, thereby confirming the pure chemical nature of the reaction of ester formation.

Extensive literature search yielded one study only by Krizsan *et al.* (2007), who detected variable concentrations of esters in grass silage, but the mean content never exceeded 30 mg/kg DM. Therefore, the aim of this study was to determine the incidence of VOC in grass silages, particularly ethanol and the ethyl esters of lactic and acetic acids. Additionally, a simple model for all silage types was developed to estimate total ester concentration solely based on ethanol content.

Material and methods

Data sets from ten laboratory ensiling experiments with grasses were used (Table 1). The botanical composition

Table 1. Characterization of the data set from grass silages (lab-scale ensiling experiments, n= 620).

Trial	Silage DM (g/kg)	n	Storage length (days)	Silage additive type used in experiment
1	211 - 438	213	252 - 266	biological, chemical, molasses (Lengyel *et al.* 2012)
2	191 - 464	209	252 - 266	biological, chemical, molasses (Lengyel, unpublished data)
3	230 - 318	49	81	biological, chemical (Nadeau, unpublished data)
4	318 - 383	12	91	biological (Nadeau, unpublished data)
5	223 - 299	45	90	biological, chemical (Kalzendorf, unpublished data)
6	274 - 357	17	142	biological (Nadeau, unpublished data)
7	283 - 373	12	270	chemical (Nadeau, unpublished data)
8	202 - 219	21	131	biological, chemical (Kalzendorf, unpublished data)
9	223 - 240	21	121	biological, chemical (Kalzendorf, unpublished data)
10	243 - 268	21	139	biological, chemical (Kalzendorf, unpublished data)

Table 2. Fermentation products, pH and ester concentrations in grass silages (n= 620).

Trial	pH (range)	Lactic acid (g/kg DM)	Acetic acid (g/kg DM)	Ethanol (g/kg DM)	Total esters* (mg/kg DM	Correlation** (r_s)	*P* value
1	3.7 - 6.7	0 - 99.5	1.5 - 62.8	0.7 - 39.6	0 - 3540	0.35	<0.001
2	3.6 - 5.8	0 - 89.8	2.0 - 46.7	0 - 35.3	0 - 3995	0.37	<0.001
3	4.0 - 4.5	60.6 - 117.5	11.1 - 36.5	2.2 - 18.7	0 - 359	0.91	<0.001
4	3.8 - 4.2	42.7 - 81.8	13.2 - 35.4	6.7 - 12.0	216 - 455	0.52	ns
5	3.8 - 4.5	32.3 - 89.2	14.2 - 76.7	1.6 - 13.1	73 - 378	0.64	<0.001
6	4.2 - 4.9	30.0 - 116.7	19.7 - 49.3	2.4 - 7.8	0		-
7	4.3 - 4.7	36.6 - 86.5	7.5 - 13.3	2.1 - 19.9	0 - 161	0.65	<0.05
8	3.8 - 4.2	42.6 - 105.1	8.4 - 19.9	0.9 - 15.1	0 - 378	0.84	<0.001
9	3.9 - 4.3	49.9 - 110.6	1.6 - 13.9	1.0 - 14.1	0 - 189	0.85	<0.001
10	4.0 - 4.7	24.0 - 76.2	14.0 - 31.5	3.9 - 12.3	62 - 272	0.85	<0.001

*sum of ethyl acetate and ethyl lactate, ** correlation between ethanol and total ester concentrations, r_S Spearman rank correlation coefficient, ns not significant

of the silages in trial 7 was 26% red clover and 74% grass. Storage temperature was set at 15°C in trials 1 and 2, whereas in all other studies incubation was carried out at 20-25°C. Aqueous extracts of frozen silages were prepared by blending the samples (50 g) with 200 ml to 400 ml of distilled water, depend-ing on the dry matter content of ensiling material. One ml of toluol was added before storage in the refrigerator overnight. Extracts were subsequently filtered through a folded filter and there-after through a microfilter (0.45 µm). Determination of the pH was done potentio-metrically by using a calibrated electrode. Lactic acid was analyzed by HPLC (RI detector), acetic acid and ethanol were determined by GC (flame ionisation detector, FFAP column) as described by Weiss (2001). The esters EA and EL were measured according to Weiss and Sommer (2012) by GC (flame ionisation detector, Optima Wax column). Correlation analyses between grass silage indices were performed by using PROC CORR of SAS. On account of skewed data distribution and zero values, the Spearman rank correlation coeffici-ent (r_S) was employed, and significance declared at P<0.05. The general prediction model across all silage types was deducted by regressing total ester concentrate-ion against the mean ethanol content of each ethanol class (n=11).

Results and discussion

Grass silages contained high ethanol and ester con-centrations, particularly in those from trials 1 and 2 (Table 2). This may be attributed to the lower storage temperature which promotes ester formation. Weiss *et al.* (2009a) observed that maize silages stored at 20°C had higher ester contents than were detected at 35°C. The correlation coefficients presented in table 2 varied widely between 0.35 and 0.85, depending on the trial.

The pH of the silages had a pronounced effect on ester levels (Table 3). Strong relationships (r_s>0.50) were mostly observed when the pH of the silages did not exceed the value of 4.25. This is in line with observations by Hangx *et al.* (2001) who found ester reactions could be stimulated by low pH in the environment.

The allocation of the grass silages to different ethanol classes was done as described by Weiss and Auerbach (2012a) and showed clear effects of ethanol content on the relationship between pH and total ester concentration (Table 4). Within each ethanol class, a great variation in ester concentration was observed. The detected ester levels in grass silages were extremely high compared with those reported by Weiss and Auerbach (2012b) for maize silages.

Table 3. Relationship between ethanol and ester contents in grass silages (n= 620) as affected by pH.

pH Class	n	Total esters*	Ethanol	Correlation**	
		(mg/kg DM)	(g/kg DM)	r_s	*P* value
>3.50 - 3.75	19	482-3995	0 - 35	0.6	<0.01
>3.75 - 4.00	126	0 -1856	1 - 40	0.72	<0.001
>4.00 - 4.25	176	0 - 920	1 - 25	0.55	<0.001
>4.25 - 4.50	131	0 - 762	1 - 18	0.21	<0.05
>4.50 - 4.75	81	0 - 550	1 - 24	0.26	<0.05
>4.75 - 5.00	42	0 - 384	0 - 38	0.49	<0.001
>5.00 - 5.25	26	0 - 255	1 -37	0.49	<0.05
>5.25 - 5.50	10	63 - 211	4 - 28	-0.35	ns
>5.50	9	0 - 171	3 - 24	0.25	ns

*sum of ethyl acetate and ethyl lactate, ** correlation between ethanol and total ester concentrations, r_s Spearman rank correlation coefficient, ns not significant

As shown in Figure 1a, the correlation between total ester content and pH in grass silages was very weak (r_S=-0.22; *P*<0.001) up to an ethanol content of 10 g/kg DM, whereas a very strong negative relationship was found (r_S=-0.82; *P*<0.001) at higher ethanol levels (Fig 1b). The least correlation existed if the silage pH exceeded the threshold value of 4.3.

In summary it can be stated that grass silages may contain ethyl esters. However, the relationship between ethanol and ethyl esters in grass silages seems to be not as close as that for maize silages. This can be explained by the fact that the intensity of ester reactions is affected by the pH of the silage and grass silages often have pH values above 4.0. As a consequence, the correlation coefficients decrease with increasing pH.

Based on a total of 1148 data sets from grass silages shown in this paper as well as from silages from whole-crop maize (n=380), whole-crop wheat (n=34), sorghum (n=84), high- moisture corn (n=30) (Weiss and Auerbach, 2012), a regression model was used to

Table 4. Relationship between pH and ester content in grass silages (n= 620) as affected by ethanol.

Ethanol class (g/kg DM)	n	Total esters* (mg/kg DM)	pH range	Correlation** r_S	*P* value
≤ 5	257	0 - 1180	3.7 - 5.8	-0.12	ns
> 5 - 10	181	0 - 1856	3.8 - 6.7	-0.46	<0.001
> 10 - 15	100	0 - 1147	3.7 - 5.7	-0.66	<0.001
> 15 - 20	39	87 - 3116	3.7 - 5.7	-0.88	<0.001
> 20 - 25	21	0 - 3540	3.6 - 6.1	-0.93	<0.001
> 25 - 30	12	63 - 3589	3.7 - 5.3	-0.83	<0.001
> 30 - 35	5	274 - 2054	3.8 - 4.8	-0.60	ns
> 35 - 40	5	182 - 3995	3.7 - 5.2	-0.90	<0.05

*sum of ethyl acetate and ethyl lactate; ** correlation between pH and total ester concentration; r_s Spearman rank correlation coefficient; ns not significant

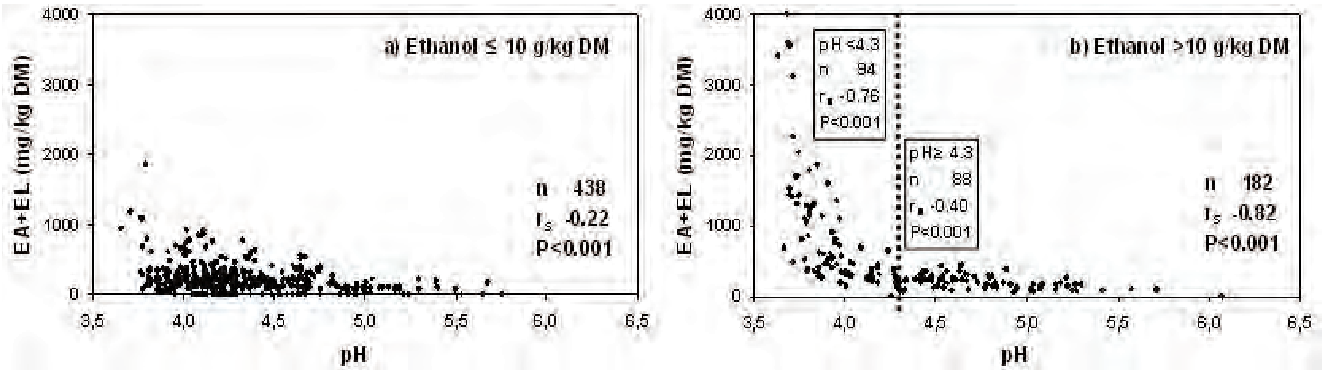

Figure 1. Total ester concentrations (ethyl acetate and ethyl lactate) as affected by ethanol class. Left: ≤10 g/kg DM and Right: >10 g/kg DM.

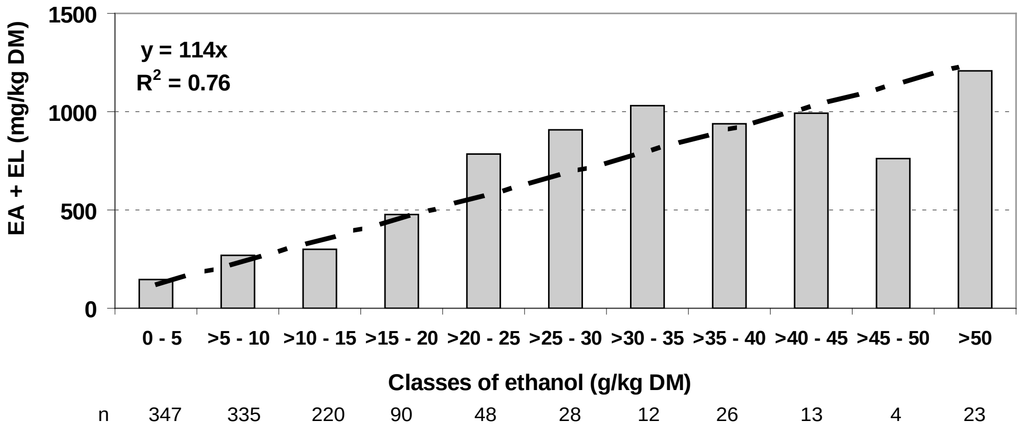

Figure 2. Mean total ester content (ethyl acetate and ethyl lactate) as affected by ethanol class in silages from whole-crop maize, whole-crop wheat, sorghum, high-moisture corn and grass.

describe the relationship between total ester and ethanol concentrations, which is valid for all silage types. As shown in Figure 2, each incremental increase in ethanol content by 5 g/kg DM resulted in increased total ester concentration by 114 mg/kg DM (R^2= 0.76). Therefore, the following equation can be applied to calculate ester concentration in silages based on their ethanol content: predicted total ester concentration [mg/kg DM] = ethanol concentration [g/kg DM] x 114/5. The use of this predictive model offers the possibility to avoid laborious and expensive chemical ester analyses.

With regard to the current body of evidence on VOC formation in silages and their potential negative impact on feed intake dairy cows and goat it can be stated that the reduction in ethanol production may lead to lower levels of ethyl esters. This is substantiated by data from ensiling experiments on the effects of different silage additives on ester formation in maize and sorghum silages. Only chemical products containing active ingredients with specific antifungal properties (sodium benzoate, potassium sorbate) consistently and significantly reduced ethyl ester concentrations (Auerbach and Weiss, 2012, Weiss and Auerbach, 2012b).

Conclusions

Ethyl esters of lactic and acetic acids can be frequently found in grass silages. Their concentrations are strongly correlated with the ethanol concentration and the silage pH. A simple model for all silage types is proposed to estimate the total ester content solely based on ethanol concentration.

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