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Effect of Wilting and Silage Additives on Silage Quality of Lucerne, Red Clover and Legume-Grass-Mixtures

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Abstract

Lucerne (*Medicago sativa*), red clover (*Trifolium pratense*) and the mixtures with grass are regarded difficult to ferment based on low contents of water-soluble carbohydrates (WSC) and nitrate as well as high buffering capacity (BC). The objective of this laboratory ensiling trial was to investigate the effects of 24, 48 and 72 hours wilting on dry matter (DM) as well as content of yeasts and lactic acid bacteria (LAB). Furthermore, the effects of chemical and biological additives on silage quality were investigated regarding the concentration of lactic acid, acetic acid and butyric acid, ethanol and ethyl esters depending on the level of wilting in ensiling material. With extended wilting period, the contents of WSC decreased, whereas the contents of yeasts and LAB increased. A lower range of DM is associated with a higher risk of butyric acid formation, which requires the addition of chemical additives against clostridia activity. The results demonstrate the occurrence of volatile organic compounds (VOC), especially ethanol and ethyl esters, in legume silages. The DM content and silage additives affect the concentrations of ethanol, acids and esters. Elevated levels of ethanol and esters occur in silages with low DM. For the reduction of ethanol and ester formation, chemical additives against yeasts and bacteria are necessary.

Keywords: Legumes; Silage quality; Volatile organic compounds; Esters

Introduction

Legumes are crops difficult to ensile due to low contents of water-soluble carbohydrates (WSC), buffering capacity (BC) and nitrate. However, they play an important role in supplying nitrogen in rotational food production systems and may have a high nutritive value, especially high contents of crude protein. The objective of this study was to examine the silage management factors such as wilting and silage additives on legume and legume/grass/mixture silage quality in combination with the incidence of VOC. Weiß & Auerbach [1] postulated that esters can be frequently found in maize, whole crop wheat and sorghum silages and also in grass silages and that ester concentrations are strongly correlated with the ethanol concentration and silage pH.

Materials and Methods

Lucerne, red clover and the mixtures with grass were harvested on 1st of June 2015 near Oldenburg, Germany, chopped to a theoretical particle length of 4cm and wilted for 24, 48 and 72 hours. Fresh and wilted ensiling material (EM), with and without any additives, were filled into 1.5 L glass jars and stored for 90 days under anaerobic conditions at 20 °C. All treatments were done in triplicates. Each crop received the ensiling treatments of no additive (Control), different commercial products of lactic acid bacteria (LAB 1, LAB 2) with inoculation rates of 105cfu g^{-1} FM, and commercial chemical products with a mixture of nitrite, hexamine and/or benzoate (Salt). Herbage (EM) with DM contents of <400g kg^{-1} was treated with products of aim-of-acting (WR) 1a, 1b or 1c according to DLG (2011), EM with DM >400g kg^{-1} with products of WR 2. The contents of DM, LAB, yeasts, WSC and nitrate were analysed according to standard methods (LUFA). Silage analyses were conducted in aqueous extracts of frozen silages as described by Weiss & Auerbach [1]. Lactic acid (LA) was analysed by HPLC. Fermentation acids, alcohols and esters were determined by GC. Statistical analyses were performed with SAS 9.3. For treatment comparisons, the analysis of variance was used.

Results and Discussion

Table 1: Effects of silage additives on contents of ethanol and esters in silages of legume depending on ensiling material, characterized by the parameters of ensilability, FC, WSC.

Parameters of Ensilability				Ethanol in gkg^{-1} DM					
	DM[2])	FC[3])	WSC[4])	Control	LAB1	LAB2	Salt	SEM	P[5])
Lucerne (Lu)									
24h	232	28	39	10.8^{a}	8.8^{ab}	8.2^{b}	3.2^{c}	0.5	<0.001
48h	266	27	0	9.0^{a}	8.6^{a}	8.4^{a}	1.8^{b}	0.3	<0.001
72h[6])	471	47	2	4.8^{a}	4.7^{a}	4.8^{a}	1.3^{b}	0.03…0.2	<0.001
Red clover (Rc)									
24h	179	32	89	10.7^{a}	9.9^{a}	9.8^{a}	1.6^{b}	0.5	<0.001
48h	209	24	26	11.1^{a}	10.9^{a}	11.0^{a}	2.0^{b}	0.2	<0.001
72h[6])	333	35	11	7.5^{a}	7.7^{a}	7.3^{ab}	1.8^{b}	0.1…0.9	0.047
Mix of Lu/Grass									
24h	245	36	75	8.9^{a}	9.2^{a}	8.4^{a}	2.0^{b}	0.3	<0.001
48h	316	36	33	7.4^{a}	7.6^{a}	7.3^{a}	2.6^{b}	0.9	0.002
72h[6])	416	46	33	5.1^{b}	6.2^{a}	5.5^{b}	1.5^{c}	0.2	<0.001
Mix of RC/Grass									
24 h	211	39	107	8.9^{a}	8.7^{a}	8.6^{a}	1.6^{b}	0.3	<0.001
48 h	284	35	51	10.9^{a}	10.9^{a}	10.8^{a}	1.3^{b}	0.3	<0.001
72h[6])	440	49	30	6.3^{a}	6.8^{a}	5.4^{a}	1.0^{b}	0.3	<0.001
Ester total (Ethyl acetate + Ethyl lactate) in mgkg-1DM									
Lucerne (Lu)									
24h	232	28	39	142^{a}	129^{b}	126^{b}	0^{c}	2.7	<0.001
48h	266	27	0	130^{ab}	129^{ab}	n.a.[7])	0^{b}	6.5…64.4	0.003
72h[6])	471	47	2	155^{a}	126^{a}	100^{b}	67^{b}	1.5…16.9	0.017
Red clover (Rc)									
24h	179	32	89	182^{a}	190^{a}	189^{a}	0^{b}	5.8	<0.001
48h	209	24	26	166^{a}	171^{a}	168^{a}	0^{b}	2.8	<0.001
72h[6])	333	35	11	148^{a}	177^{a}	143^{a}	0^{b}	27.6	0.008
Mix of Lu/Grass									
24h	245	36	75	189^{a}	137^{b}	119^{b}	83^{c}	7.7	<0.001
48h	316	36	33	124^{a}	126^{a}	116^{b}	0^{c}	1.3	<0.001
72 h[6])	416	46	33	130^{ab}	222^{a}	184^{a}	0^{b}	39.5	0.019
Mix of RC/Grass									
24h	211	39	107	191^{ab}	202^{a}	185^{b}	0^{c}	2.5	<0.001
48h	284	35	51	197^{a}	206^{a}	205^{a}	0^{b}	4	<0.001
72h[6])	440	49	30	171^{a}	156^{a}	175^{a}	0^{b}	5.4	<0.001

1)n=3; means within rows are significantly different if they have no letters in common (P<0.05, Tukey`s test).2)Dry matter in g kg-1FM 3) Fermentation coefficient FC=DM(%)+8WSC/BC4)Water soluble carbohydrates in g kg-1 DM. 5)P-Values of global F-test.6)silage additives according DLG aim-of-acting 2, otherwise DLG 1b (LAB1) or 1b, c (LAB 2).7) not analysed.

The legumes used in the trial were estimated as difficult (fermentation coefficient FC<35) or moderate fermentable (FC 35-45) according to DLG (2011). Three EM with FC >45, all wilted for 72h, had DM contents over 400g kg^{-1} and low levels of WSC. Although all EM were nitrate-free and thus with a higher risk for poor fermentation, butyric acid (BA) was only analysed in lucerne after 48 h of wilting and DM of 266g kg^{-1} (data not shown). BA could be suppressed only with silage salt (WR 1a, DLG (2011)). Polyphenol oxidases (PPO), which occur in red clover [2], are able to inhibited proteolysis. On the other hand high levels of DM and therefore higheraw-values in combination with higher acidification rate can reduce clostridia activity [3]. The epiphytic content in our study was high (LAB from 6.9 to 8.6 log $cfug^{-1}$ FM) and increased with wilting period. Lactic acid contents

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ranged between 49.8 and 104.5, acetic acid between 11.8 and 26.8g kg^{-1} DM (data not shown). Laboratory ensiling trials are characterised by clean experimental conditions that means free of clostridia spores. However, yeast counts (from 6.8 to 8.4 log $cfug^{-1}$ FM) were high and increased during wilting period. In accordance to the fact that under anaerobic conditions yeasts are responsible for ethanol formation, the ethanol content in silages without additives was between 4.8 and 10.9g kg^{-1} DM (Table 1) with a strong negative correlation to DM content (R^2=0.81) and positive correlation to ester content (R^2=0.65). The total esters ranged between 124 and 197mg kg^{-1} DM in untreated silages and consisted of only ethyl lactate. These ester contents are comparable with contents in grass silage considering the pH level between 4.0 and 6.3. As shown in Table 1 the contents of ethanol were mainly not affected by silage additives with LAB, the same applies for the contents of esters. The additive salts containing benzoate, nitrite and hexamine strongly reduced the ethanol and ester contents. According to Woolford [4] these substances are able to inhibited yeasts and possibly hetero fermentative LAB which also produce ethanol [5].

Conclusion

Ensiling within the lower DM range of legumes is associated with a higher risk of BA formation in lucerne. Elevated levels of ethanol, particularly in the lower range of DM, and esters occur in legume silages. For the reduction of ethanol and ester formation, chemical additives against yeasts and bacteria are necessary.

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