Possibilities to Avoid Growth of Clostridia and/or Fungi in Wilted Silage by Use of Organic and Inorganic Salts

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POSSIBILITIES TO AVOID GROWTH OF CLOSTRIDIA AND/OR FUNGI IN WILTED SILAGE BY USE OF ORGANIC AND INORGANIC SALTS

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Abstract

The hygienic quality of silage will be of great importance in the future as poor quality not only influences the animal production but also the animal health and the food quality. This study examined the impact of mixtures of sodium benzoate (NaB), sodium nitrite (NaN), hexamine (HMTA), sodium propionate (NaP), sodium bisulphite, and propionic acid on low and high wilted clover/grass. The silage (crop wilted to 300 or 600 g DM kg^{-1} of fresh weight) consisted of about 50% red clover (*Trifolium pratense*) and 50% timothy (*Phleum pratense*) and the study covered 7 additive treatments. The forage was un-chopped and ensiled in two types of silos: 25 litre stainless laboratory silos, stored during 100 days and 1,7 litre glass silos in 14 days. In both studies samples were taken for chemical and microbial analyses. High contents of nitrite could influence animal health and were of specific interest. Residual nitrite contents were higher in silage after 14 days than 120 days storage but both concentrations were negligible. The additives highly restricted growth of yeast in all treatments but the control silage. As a consequence of low growth of yeast in the silage, the production of ethanol was significantly lower compared to the control silage. Even the count of clostridia spores was significantly restricted in treated silages and so was the reduction in DM - losses except for treatment E. The conclusion is that sodium nitrite in combination with hexamine

effectively prevented clostridia growth as well as sodium benzoate restricted yeast growth. The nitrate / nitrite concentration were reduced already after 14 days.

Keywords: wilted silage, clostridia, fungi, nitrite, losses

Introduction

Silage today is an important feed not only for ruminants but also for horses. Costs of bale silage are highly related to the DM content (Lingvall at al., 1995). Low DM silage is sensible to clostridia growth and when used for horses *Clostridium botulinum* is a specific problem. High DM silage therefore is to prefer but as this silage is sensible for mould growth, it is important to find a combined additive to secure the hygienic quality. The composition of such an additive should demonstrate a sufficient antimicrobial property regarding growth of both unwanted bacteria and fungi, and also a stimulating effect on lactic acid bacteria. In high DM silage the pH value will be restricted as a result of low acidification. This relation linkages to the efficiency of some additive to improve fermentation or reduce negative bacteria. Woolford (1975) and Thylin (2000) referred that Sodium benzoate was more effective at lower pH values. Furthermore, Kwella et al (1993) confirmed positive effects of Sodium Nitrite and Hexamine on silage preservation. Based on work of Woolford (1975) the effects of different mixtures and doses of sodium benzoate, sodium nitrite, hexamine, sodium propionate, sodium bisulphite and propionic acid on the final residual concentrations, clostridia and fungi growth and hygienic quality of the silage were examined. Sodium propionate and propionic acid were examined because they have been demonstrated to inhibit aerobic deterioration of silage. Sodium bisulphite was used to support lactic acid-type fermentation by suppressing the growth of butyric acid-producing micro-organisms. Sodium nitrite possesses general antimicrobial properties but in rates of 50 mM it restricts the growth

of all the bacteria and moulds (Woolford, 1975). In addition, Spoelstra (1985) reported the positive effect of nitrate on reduction of clostridia growth but the inhibition is caused by nitrite and nitric oxide rather than by nitrate itself (McDonald & Henderson, 1991). The use of hexamine and sodium nitrite in combination with sodium benzoate and sodium propionate may considerably improve the hygienic quality and storage stability of silage as well as necessity of using effective additives when silage is made from unchopped and wilted grass (Lättemäe & Lingvall, 1996, Lingvall & Lättemäe, 1999).

Material and Methods

A mixture of red clover (*Trifolium pratense*) and timothy (*Phleum pratense*) 50 / 50 was harvested in the beginning of September 1999. Wilted (300 or 600 g DM kg^{-1} of fresh weight), un-chopped forage (Ash=88,5±0,43 g kg^{-1} DM; CP=171±1,39 g kg^{-1} DM; WSC=123±3,84 g kg^{-1} DM; NO_3-N=0,155 ±0,004 g kg^{-1} DM; ME=10,8±0,12 MJ,kg^{-1} DM), was mixed by hand with the additives presented in Table 1. Each additive was applied with a spray bottle into the grass and ensiled into 25 litre stainless silos or 1,7 litre glass silos. The 25 litre silos were opened 120 days after filling and the 1,7 litre silos were opened after 14 days. The low dry matter variant (30% DM) was used for seven treatments and the high dry matter (60% DM) for eight treatments. Each treatment had three silo replicates. The silos were filled with the low dry matter grass at a density of 150 kg DM/m^3 and the high dry matter grass at a density of 200 kg DM/m^3 as expected in a baler. All silos were stored at a constant temperature of 25 °C. Samples were taken aseptically. The following chemical analyses were done: dry matter, water soluble carbohydrates (spectrophotometer), pH (Metrohm 654 pH meter), nitrate, nitrite, volatile fatty acids, ethanol (HPLC) and ammonia-nitrogen (volatile N-fraction in silage juice distilled on Kjeltec Autosystem 1030). The number of yeast, moulds

and clostridia spores were determined by plate counts. The aerobic stability was determined from silage stored for 120 days.

Results and Discussion

Silages with the lower dry matter content were characterised by a relatively high pH value and a high content of water soluble carbohydrates (WSC), but concentrations of volatile fatty acids (VFA), nitrate-N, and ethanol were relatively low. The concentration of ammonia-N was on an acceptable level. Differences between treated silages (A_1, A_2, B, C, D, E, F) were small at 30 % DM in all parameters while large differences were present between the treated and the control silage (0). Higher values of ethanol, losses of sugar, and ammonia-N were found in treatment E. It was statistically confirmed that the content of ammonia-N in all treated silages at this DM level were decreased. All additives significantly reduced clostridia growth. As a consequence of rapid reduction in the yeast counts in silages (A_1, A_2, B, C, D, E, F) the contents of ethanol were very low. In silage where additives containing nitrite were applied, very little of the plant nitrate was degraded compared to treatments (0, E, F) where nitrite was not used.

High wilted silages had a higher pH value, a high concentration of residual WSC, and a restricted content of fatty acids - especially of lactic acid. In contrast to low wilted silages no influence of treatments on the ammonia-N concentration was detected. Concentrations of residual nitrite were negligible in all treatments of both DM variants. Almost in all treatments a reduction in DM losses were obtained. The level of nitrite remaining in silage opened after 14 days of fermentation was also negligible. In treatment A_2 where water was added, better distribution of additive was not demonstrated in comparison to treatment A_1. Generally, the results of the analysis of high wilted silages showed a very low rate of fermentation and therefore the differences between treatments were not large as seen in Table 2. The aerobic

stability of all treated silages was prolonged compared to the control silages. It is concluded that the sodium nitrite together with hexamine had a good effect in restricting clostridia growth. In spite of the high pH values of the silages caused by a high DM contents and a low acidification, the additives ensured good quality of silage. Residual nitrite and nitrate, which are toxic in higher concentration, were kept on very low levels.

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Table 1 - Chemical composition of used additives and their application rates

Treatment	Water (litre /tonne)	Na-Benzoate (g/tonne)	Na-nitrite (g/tonne)	HMTA* (g/tonne)	Na-propionate (g/tonne)	Propionic acid (g/tonne)	Na-bisulphite (g/tonne)
0	-	-	-	-	-	-	-
A_1	3,0	465	360	240	165	-	-
A_2 (+water)	3,0+1,5	465	360	240	165	-	-
B	4,5	698	540	360	248	-	-
C	6,0	930	720	480	330	-	-
D	3,0	375	360	240	-	-	194
E	2,0	418	-	-	-	-	216
F	4,5	672	-	-	425	1890	-

* HMTA – hexamine or hexamethylene-tetramine

Table 2 - Chemical and microbial composition of silage after 100 - 121 days. $LSD^{P<0.05}$ states the least significant difference between treatments at 5 % probability level.

	Treatment									
Analyses (low DM)	**0**	**A_1**	**A_2**	**B**	**C**	**D**	**E**	**F**	**Mean**	**$LSD_{0,05}$**
DM (g/kg)	309	306	312	322	nd	319	317	335	314	
pH	5,1	5,1	5,1	5,2	nd	5,1	5,0	5,0	5,1	0,13
WSC	70,7	86,0	85,7	96,3	nd	93,7	80,0	104,3	88,1	16,8
Lactic acid (g/kg DM)	21,3	17,0	17,4	15,8	nd	18,8	19,6	11,9	17,4	4,6
Acetic acid (g/kg DM)	7,3	6,4	6,5	6,6	nd	6,0	6,8	5,8	6,5	1,31
Butyric acid (g/kg DM)	1,10	1,10	1,10	1,00	nd	1,03	1,10	1,00	1,06	0,004
Nitrate-N (g/kg DM)	0,06	0,15	0,15	0,13	nd	0,13	0,08	0,10	0,11	0,02
Ammonia-N (g/kg TN)	76,3	54,0	55,6	51,4	nd	54,4	67,7	48,4	58,3	6,06
Clostridia(log CFU/g silage)	3,9	1,7	1,7	1,7	nd	1,7	2	2,3	2,1	1,2
Yeast (log CFU/g silage)	4,2	1,5	1,5	1,5	nd	1,5	2,2	2,0	2,1	0,7
Ethanol (g/kg DM)	8,7	3,4	3,3	3,1	nd	3,8	6,3	3,4	4,6	0,9
Losses of sugar (g/kg DM)	50	20	9	17	nd	20	42	2	22,7	12,6
Analyses (high DM)										
DM (g/kg)	602	603	593	602	606	614	594	615	602	
pH	5,4	5,4	5,4	5,4	5,4	5,4	5,4	5,4	5,4	0,05
WSC	83,7	98,7	100,3	102,0	106,3	102,7	95,3	108,0	99,6	8,5
Lactic acid (g/kg DM)	0,73	0,63	0,80	0,60	0,60	0,57	0,77	0,63	0,67	0,02
Acetic acid (g/kg DM)	1,26	1,30	1,23	1,20	1,27	1,17	1,30	1,30	1,25	0,17
Butyric acid (g/kg DM)	0,50	0,50	0,50	0,50	0,50	0,47	0,50	0,47	0,49	0,005
Nitrate-N (g/kg DM)	0,08	0,10	0,11	0,11	0,10	0,09	0,10	0,08	0,10	0,008
Ammonia-N (g/kg TN)	22,5	25,4	25,7	21,9	19,9	21,6	24,5	23,1	23,1	3,39
Clostridia(log CFU/g silage)	1,7	1,7	2,5	1,7	1,7	1,64	1,7	1,7	1,8	0,8
Yeast (log CFU/g silage)	2,6	1,5	1,5	1,5	1,5	1,5	2,8	1,5	1,9	0,9
Ethanol (g/kg DM)	7,97	3,20	3,63	1,17	1,17	2,77	5,23	1,17	3,29	4,28
Losses of sugar (g/kg DM)	13	11	8	1	4	5	9	2	6,6	13,2

nd – not determined