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Different experimental designs in testing of silage additives

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Introduction

Quality of silage fermentation and consequent aerobic stability of silages is still a common problem of many types of silage. Experimental testing of silage additives is commonly conducted under routine ensiling condition with properly consolidated forages and airtight silos. Consequently, it is not surprising that results of these trials do often not display a potential of a product in the agricultural practice. Punctures and other damages of silo cover as well as uneven forage consolidation in a silo are common. These defects make ensiling conditions more difficult and challenge a silage additive to fulfil its purpose. It has been observed that silos which were not tight under the fermentation process are more prone to be aerobically unstable (Jonsson & Pahlow, 1984). Based on this observation, a German system for evaluation effects of silage additives (DLG, 2009) applies a design where silage additives are tested under difficult ensiling condition by two times of air ingress into a mini-silo for 8-12 hours combined with a very low packing density. This condition, however, does not properly reflect silo un-tightness. It is more common that a silo is exposed to a weak but constant air ingress. This condition is more closely reflected by a design with a 2-hours weekly air ingress used by Pauly and Hjelm (2015) in testing efficiency of silage additives on conservation of crimped maize.

Therefore, the objective of this study was to compare the impact of ensiling challenged by weekly aeration in a silage additive test to improve forage conservation.

Materials and Methods

A mixture of timothy (15%, head visible), perennial ryegrass (30%, vegetative stage), meadow fescue (16%, head visible), and red clover (vegetative stage, 39%) was harvested with a scythe on 10th of June 2015. The crop was directly chopped in a stationary cutter to approx. 2 cm particle length. After chopping, the forages were mixed with a suspension of *Clostridium tyrobutyricum* spores at a rate of 1 x 10^5 per g fresh matter (FM) and partitioned into two fractions. One fraction was left untreated and served as a control. Remaining fraction was treated with the additive Safesil (20% sodium benzoate, 10% potassium sorbate, and 5% sodium nitrite) at a rate of 3 L/t (FM). The silage additive was applied by hand with a spray bottle on the forage spread out on a sheet of plastic film and mixed thoroughly. Sub-samples (5 kg FM) from each fraction were then ensiled in 6 mini-silos each (1.7 L volume with a fermentation lock in the lid) and ensiled under two ensiling conditions. Half the mini-silos were tightly sealed with a fermentation lock during the entire storage time (DLG design for testing efficiency of silage additives WR1, DLG, 2009). Silos in the challenged ensiling condition were packed at the same density as the tight silos, but had lids fitted with rubber stoppers (≈ 6 mm), when removed, allowed air ingress into the silos. The rubber stoppers were removed for two hours every week during the storage period.

Each treatment consisted of 3 replicates and the silos were stored for 98 days at a room temperature of 21˚C. At the end of storage, silages samples were extracted and analysed for dry matter, volatile fatty acids, lactic acid, ethanol, pH, water soluble carbohydrates, lactic acid bacteria, clostridia spores, yeasts and tested for aerobic stability.

Results and Discussion

The chemical and microbiological composition of the forage, prior to ensiling, is presented in Table 1. Chemical composition of fresh forage represented a common composition found in first cut grass crops in Sweden except for the low nitrate content. Calculated fermentation coefficient of 38 characterizes the forage as slightly above the limit for a difficult crop to ensile (Weissbach et al., 1974).

Table 1 Chemical and microbiological compositions of fresh forage (n=2).

Analyses	Unit	Clover-grass
DM	%	19.9
Ash	%	9.5
CP	%	11.6
WSC	%	15.7
NDF	%	44.8
Nitrate-N	mg/kg DM	2.1
ME	MJ/kg DM	11.1
Ammonia-N	% total N	1.2
Buffering capacity	g LA/100 g DM	7.1
LAB-homofermentative	log cfu/g FM	6.2
LAB-heterofermentative	log cfu/g FM	3.9
Clostridia spores	log cfu/g FM	3.8
pH of fresh forage		5.8
Fermentation coefficient		38

DM-dry matter; FM-fresh matter; CP-crude protein; WSC-water-soluble carbohydrates; NDF-neutral detergent fiber; ME-metabolisable energy.

Results from chemical and microbiological analyses of the silages are presented in Tables 2 and 3. As expected, low DM content of the crop caused extensive fermentation, signified by low pH, high levels of fermentation products and depletion of WSC.

Results also show major differences between control and additive treatments. Additive treated silages were found to have a lower silage pH and a higher concentration of lactic acid. Butyric acid concentrations were below the detection limit in all additive treated silages and significantly less in comparison with controls. As butyric acid is considered to be a major product of clostridia, it is not surprising that control silages were found to have significantly higher counts of clostridia spores than additive treated ones.

Table 2 Chemical composition of silages after 98 days of storage (n=3)

Treatment	DM	pH	NH_3-N*	Lactic acid	Acetic acid	Butyric acid	2.3-butanediol	Ethanol	WSC
	%		% of TN	% of DM					
Control	18.1	4.5	10.9	9.3	2.6	1.7	2.9	2.0	0.7
Safesil	19.4	4.1	4.9	11.6	1.4	0.0	0.1	0.4	6.4
Control-Air	17.9	4.6	11.1	5.4	6.5	1.0	3.0	1.9	0.8
Safesil-Air	19.3	4.1	5.9	11.2	2.4	0.0	0.2	0.5	5.6
$LSD_{0.05}$		**0.06**	**0.38**	**0.88**	**0.69**	**0.19**	**0.44**	**0.27**	**0.25**
P-additive		0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001
P-air		0.01	0.001	0.001	0.001	0.001	0.4	0.6	0.003
P-add+air		0.02	0.01	0.001	0.001	0.001	0.8	0.1	0.001

* N.S. – Not significant. DM-dry matter; FM-fresh matter; TN-total nitrogen; WSC-water-soluble carbohydrates.

Table 3 Aerobic stability and microbiological composition of silages after 98 days of storage at an ambient temperature of 20.2˚C (n=3)

Treatment	Time (h) until temp. rise of 3˚C	Max-temp (˚C)	Max. temp-increase (˚C)	pH after stability	Yeasts	Clostr. spores	LAB Homoferm.	Heteroferm.	Weight loss
					log cfu/g				% DM
Control	216	20.5	0.0	4.5	<1.7	4.6	<4.7	7.4	14.7
Safesil	216	20.7	0.2	4.1	<1.7	1.7	<4.7	6.2	2.4
Control-Air	84	37.1	16.5	7.5	3.6	4.3	<4.7	7.9	15.1
Safesil-Air	216	20.7	0.1	4.1	<1.7	1.8	<4.7	4.7	4.2
$LSD_{0.05}$	**26.2**			**0.62**	**0.29**	**0.21**	**-**	**0.29**	**0.45**
P-additive	0.001			0.001	0.001	0.001	n.s.	0.001	0.001
P-air	0.001			0.001	0.001	0.2	n.s.	0.001	0.001
P-add+air	0.001			0.001	0.001	0.02	n.s.	0.001	0.001

* N.S. – Not significant. DM-dry matter; LAB-lactic acid bacteria.

The results confirm results from previous studies (Knicky & Spörndly, 2009, 2011) of the ability of the present additive composition to eliminate clostridia. A similar development, as for butyric acid, was observed for ammonia-N formation where control silages displayed higher ammonia-N levels than additive treated silages. Reduced formation of undesirable ensiling products such as butyric and acetic acid, ethanol and 2,3-butanediol were probably the reason for significantly lower silage losses in the additive treatments compared to controls (Fig. 1).

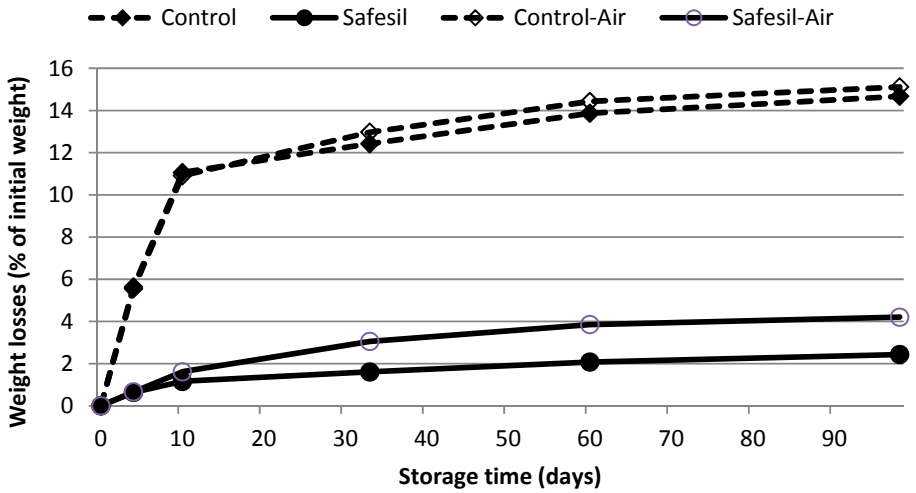

Figure 1 Weight losses of silages stored for 98 days (n=3).

The air ingress during ensiling mainly influenced silage quality parameters of the control treatments. Control aerated silos were found to have a higher pH, a lower concentration of lactic acid and butyric acid, but a higher concentration of acetic acid than control silages without aeration. Increased formation of acetic acid can be attributed to different LAB fermentation pathways. One likely pathway can be associated with *L. plantarum* that possesses the ability to oxidize lactate to acetate (McDonald et al., 1991). Although elevated acetic acid formation, control aerated silages were the only silages containing yeasts and were found to be less aerobically stable than control silages without aeration and other treatments as well. Air ingress affected fermentation parameters of the additive treated silages to a lesser extent. Aeration increased formation of acetic acid and ammonia-N and significantly decreased number of heterofermentative LAB in comparison with additive treated silages without aeration. These changes in fermentation patterns of aerated silages were reflected in increased silage losses in comparison with silages without aeration (Fig. 1).

Conclusions

Two hours weekly air ingress sufficiently challenged the ensiling condition by promoting the growth of undesirable yeasts. The application of the silage additive improved silage fermentation, reduced silage losses and maintained silages aerobically stable under both ensiling conditions.

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