Biological and chemical additives maintain feed value of grass silage during air exposure

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Introduction Aerobic instability of grass silage has become a major concern to farmers, which negatively affects its hygienic quality due to fungal development and potential mycotoxin formation as well as dry matter (DM) and nutrient losses, thereby reducing animal feed intake and performance and farm profitability. Silage additives have been used to mitigate aerobic deterioration. However, as their mode of action differs, this study aimed at comparing different products on fermentation, aerobic stability and organic matter digestibility (OMD).

Materials and Methods Grass was harvested on July 18, 2016 on a dairy farm near Skara, Sweden, wilted over night to 42% DM, and chopped by a stationary laboratory chopper set at 20 mm theoretical particle length. Herbage was manually treated by spraying with the following additives, applied at 10 ml/kg fresh forage: *Lactobacillus buchneri* CNCM-I 4323, IR: 100,000 cfu/g (LB); *Lactobacillus buchneri* CNCM-I 4323, IR: 100,000 cfu/g + *Pediococcus acidilactici* DSM 11673, IR: 67,000 cfu/g (LBPA); or a chemical blend (SNHEPS) composed of sodium nitrite (195 g/L), hexamethylene tetramine (71 g/L) and potassium sorbate (106 g/L), applied at 2.5 L/t. All additives were kindly provided by KONSIL Europe GmbH, Germany. Untreated grass received tap water at 10 ml/kg. Subsequently, herbage was filled in 1.6-L glass jars, which were equipped with a hole (6 mm diameter) in the body and the lid. These holes were closed by rubber stoppers that were removed for 24 hours on day 28 and day 49 of fermentation enabling air to penetrate to stimulate fungal development. Silages were stored for 56 days at about 22 °C. Standard analytical procedures for silage evaluation were used. The *in vitro* OMD was determined and metabolizable energy (ME) content was calculated according to Lindgren (1979, 1983, 1988). Aerobic stability (ASTA) was evaluated by the temperature method over 276 hours (11.5 days) of aeration, as described by Honig (1990). All data were subjected to statistical analysis by the mixed model procedure of SAS 9.4. Significance was declared at $P<0.05$, and Tukey´s test was employed for pairwise comparisons between least-square means.

Results and Discussion Our results substantiate previous findings by Nadeau et al. (2012) that the use of the chemical additive resulted in the most efficient fermentation process as reflected by the lowest DM losses, highest water-soluble carbohydrate concentration and largely restricted formation of ethanol as well as proteolysis as indicated by lthe owest ammonia-N content (Table 1). Combining the homofermentative *P. acidilactici* with the heterofermentative *L. buchneri* in LBPA counteracted the increase in DM losses and proteolysis and the decrease in WSC that were observed in silages solely treated with LB. Yeast counts were lowest when *L. buchneri*-containing additives were used, resulting in aerobically stable silages over the entire period of aeration. However, the effects of SNHEPS on these parameters did not differ from those of untreated silage. This can be explained by the fact that one of the three replicate silages showed low ASTA of 50 hours, which may have been caused by too low of an application rate, or inhomogenous additive distribution.

Table 1 Fermentation characteristics, yeast count and aerobic stability of grass silage (data presented as LSmeans in % DM unless stated otherwise, n=3)

Parameter	CON[1]	LB[2]	LBPA[3]	SNHEPS[4]	SEM	*P*-value
DM loss (%)	5.0^{c}	6.6^{a}	5.3^{b}	4.1^{d}	0.03	<0.001
WSC[5]	7.41^{b}	1.79^{d}	3.08^{c}	9.12^{a}	0.717	<0.001
NH_3-N (% total N)	7.4^{b}	8.2^{a}	6.8^{c}	5.4^{d}	0.05	<0.001
pH	4.16^{c}	4.31^{a}	4.07^{d}	4.24^{b}	0.005	<0.001
Lactic acid	4.34^{b}	3.05^{d}	4.67^{a}	3.78^{c}	0.047	<0.001
Acetic acid	1.10^{b}	2.10^{a}	1.42^{ab}	1.10^{b}	0.143	<0.01
Ethanol	0.59^{ab}	0.86^{a}	0.24^{b}	0.11^{b}	0.114	<0.01
1,2-propanediol	0.41^{b}	1.43^{a}	0.99^{a}	0.32^{b}	0.112	<0.01
Yeast (log cfu/g)	6.4^{a}	2.2^{c}	3.3^{bc}	4.3^{ab}	0.57	<0.01
ASTA[6] (hours)	44^{b}	276^{a}	276^{a}	201^{ab}	37.8	<0.05
pH after ASTA[6] test	6.43^{a}	4.34^{b}	4.10^{b}	4.92^{ab}	0.363	<0.05

[1]untreated; [2]*L. buchneri* CNCM I-4323; [3]*L. buchneri* CNCM I-4323 + *P. acidilactici* DSM 11673; [4]liquid blend of sodium nitrite, hexamethylene tetramine, potassium sorbate; [5]water-soluble carbohydrates; [6]aerobic stability; LSmeans in rows with unlike superscripts differ at *P*<0.05 (Tukey´s test).

Both *in vitro* OMD and ME content of the silage at silo opening were high, and no differences between treatments were detected (Table 2). Aeration of untreated silage largely reduced *in vitro* OMD and ME, whereas these parameters remained unaffected when additives were applied. Assuming that feed value declines only when silages heat-up, these changes caused a reduction per day of heating of 0.71 digestibility units and 0.12 MJ ME/kg DM.

Table 2 *In vitro* organic matter digestibility (OMD) and metabolizable energy (ME) of grass silage before and after aeration as affected by additive use (data given as LSmeans, n=3)

Parameter	CON[1]	LB[2]	LBPA[3]	SNHEPS[4]	SEM	*P* - value*
in vitro OMD (%)						<0.001
before aeration	85.0^{Ax}	82.6^{Ax}	84.3^{Ax}	84.3^{Ax}	0.81	
after aeration	78.1^{By}	85.2^{Ax}	84.9^{Ax}	85.8^{Ax}		
ME[5] (MJ/kg DM)						<0.001
before aeration	10.8^{Ax}	10.4^{Ax}	10.7^{Ax}	10.7^{Ax}	0.13	
after aeration	9.6^{By}	10.8^{Ax}	10.7^{Ax}	10.8^{Ax}		

[1]untreated; [2]*L. buchneri* CNCM I-4323; [3]*L. buchneri* CNCM I-4323 + *P. acidilactici* DSM 11673; [4]liquid blend of sodium nitrite, hexamethylene tetramine, potassium sorbate; [5]calculated from *in vitro* OMD; [A,B]LSmeans in rows and [x,y]LSmeans in columns with unlike superscripts differ significantly at *P*<0.05; *denotes significance level of the interaction aeration x additive treatment.

Conclusions The use of additives with proven record to prevent the detrimental effects of air during feed-out on silage stability is strongly encouraged to maintain the feed value from silo opening until intake by animals.