

Development of an optimal system for sampling the outer horizon of silage bales for fungal propagules

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Introduction

Fungal contamination of baled grass silage can lead to potential health challenges to consumers, farmers and livestock, as well as to wastage of feedstuffs, with a concomitant increase in feeding costs. It is important to be able to accurately quantify the number of fungal propagules in baled silage in order to establish its hygienic quality. The aim was to identify optimal sampling intensity and positions when estimating the numbers of fungal propagules in baled silage.

Materials and Methods

In February 2003, ten bales were chosen from a collection of 40 bales which were made at Oak Park the previous summer and stored on their curved side. Only visibly mould-free bales where the plastic wrap was undamaged were sampled. Bales were sampled in eight positions using a sharpened cylindrical steel corer (length, 22 cm; inner diameter, 3.5 cm and thickness, 1 mm) powered by an electrical drill. Sampling points were at 1400, 1600, 2000 and 2200 h clock positions on the bale barrel, *ca* 40 cm from each end. At each sampling position, a sample weighing approximately 60 g was cored. Cores were taken to a depth of 15 to 25 cm - this variation was due to different silage densities at different positions within and between bales. The corer was disinfected between samples with 99% industrial methylated spirits. Samples were transferred from the corer to clean plastic bags, the air expelled and the bags sealed. Samples were stored at 1 to 4°C until submitted for microbiological analyses the following day. Dry matter (DM) and pH values were obtained for each bale. Mould and yeast counts were determined using the spread plate method described by Auerbach et al., (1998). Briefly, each sample was divided into two 30 g subsamples. A stock suspension was prepared from a 30 g silage sample and 270 ml quarter strength Ringer's solution containing 0.05% Tween 80. After approximately 30 min. at room temp., the suspension was homogenised in a stomacher (Lab-Blender 400) for 2 min. and serially diluted (1:9). Malt yeast extract sucrose agar (MYSA) was used as the enumeration

medium as recommended by Skaar and Stenwig (1996). Petri dishes containing MYSA were inoculated singly by transferring 0.1ml of each dilution onto the surface of the medium. The plates were incubated for five days at 25°C. The mould and yeast colony forming units (cfu) were enumerated separately. The mould and yeast count refers to the number of cfu/g wet weight of sample. Statistical analyses were completed using SAS, Version 6.12. A nested design was used, with 'position' nested within 'bale' and 'subsample' nested within 'position'. There were 10 bales, 8 positions within each bale and 2 subsamples within each position. Because of the large variation in the data, the natural log (ln) counts were analysed. In the case of zero counts, a small constant (0.1) was added to the data in order to allow calculation of the natural log.

Results and Discussion

The baled silage had 416 (s.d. 67.0) g DM/kg and a pH of 5.2 (s.d. 0.23). Both mould and yeast cfu varied greatly between bales and within bales (Table 1). Yeast cfu were higher than for mould and showed more variability between (s.e. 3.32) than within the bales (s.e. 1.23). The variation of yeast numbers between and within bales was significant ($P < 0.05$). Because there were many mould cfu close to zero, the data could not be statistically analysed as described above. There was no one position in the outer horizon of these bales that would be representative of the bale as a whole for either mould or yeast propagule numbers.

Conclusions

Because of the very considerable variation both within and between bales in the numbers of yeast in individual core samples of silage, it was not possible to devise a practicable sampling location or frequency strategy to accurately quantify the number of fungal propagules in baled silage using counts obtained on individual samples. It is proposed instead that to get a reasonable estimation of fungal propagule numbers in the outer horizon of a bale, a number of subsamples should be taken per bale at pre-determined points and composited to provide one homogenous sample per bale.

References

- Auerbach, H. , Oldenburg, E. and Weissbach, F. (1998). *Journal of Science, Food and Agriculture*. 76: 565-572
 Skaar, I. and Stenwig, H. (1996). *Applied and Environmental Microbiology*. 62:3614-3619.

Table 1. Mould and yeast colony forming units (cfu) in baled grass silage.

Bale no.	Yeast (ln cfu/g)				Mould (ln cfu/g)			
	Mean	s.d.	Min.	Max.	Mean	s.d.	Min.	Max.
1	<0.1	1.40	0	2.0	<0.1	0.88	0	1.2
2	5.6	3.45	0	8.6	<0.1	0.88	0	1.2
3	<0.1	2.81	0	4.6	<0.1	0.88	0	1.2
4	5.3	1.53	2.6	7.6	<0.1	1.43	0	1.2
5	7.2	1.30	4.6	8.8	0	0	0	0
6	6.1	4.67	0	13.0	2.1	5.93	0	14.6
7	7.9	2.73	2.3	11.1	1.2	5.25	0	13.2
8	6.7	3.99	1.9	13.9	1.5	4.55	0	12.0
9	3.3	4.96	0	11.3	2.1	4.47	0	12.4
10	5.6	3.94	0	10.4	0.8	4.68	0	11.2
s.e.m.	1.17							