AN EXAMINATION OF THE EFFICACY OF AN INOCULANT ADDITIVE ON IMPROVING THE FERMENTATION AND AEROBIC STABILITY OF GRASS SILAGE

Report

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FEED INNOVATION SERVICES

FINAL REPORT

December 2005

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AUTHENTICATION

I declare this report represents a fair and accurate record of the results obtained from the study carried out under my supervision.

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Date: 8/12/05

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Introduction

Silage additives are classified, in the UK, under the UK Forage Additive Approval Sceme (FAAS), which provides reliable information to help the farmer choose the most appropriate product to meet his requirements (see J. Weddell, SAC Technical Note T559, April 2004 for the types of additive and the categories under which they have been approved). In order to qualify for inclusion on the FAAS list, five separate studies have to be carried out using the additive in question.

Aim

The aim of the proposed experiment was to examine the silo characteristics of a bacterial inoculant additive. The categories to be investigated are:

- Is fermentation improved?
- Are ensiling losses reduced?
- Is aerobic stability improved?

Materials and method

Location

The experiment was carried out at SAC's Dairy Research Centre at Crichton Royal Farm, Dumfries.

Preparation of material

At the harvest of second-cut grass silage, a proportion of fresh untreated grass was set aside. The forage harvester used to cut the grass had no additive going through its system. To make quite sure that there was no residual additive, three trailer loads of cut grass were discarded before the load of grass to be used for the experiment. The fourth

load of grass was tipped onto a clean concrete surface, where the experimental treatments and samples were prepared.

The experiment additive was mixed manually, at the correct rate according to the manufacturer's instructions. The treatment additive was mixed into a separate section of the cut grass, the different sections of grass being clearly identified and well separated to avoid contamination. Different mixing equipment was used for the treatment and for the control. Amounts of grass (each about 2kg), either treated with the test inoculant (T), or untreated (U), were packed tightly into 5-litre plastic containers by hand, and the containers sealed with a screw-top lid, to simulate ensilage conditions.

The packed containers were clearly identified with the date the silage was made, the treatment code (T or U), which replicate (1, 2 or 3), and the day of the time course (see Table 1) when it was to be opened for sampling.

Sampling
A time course was run, where material was removed from containers over a series of days and frozen, halting the ensiling process in each case, to investigate the rate of acidification of grass with and without the additive. The timescale was, counting from the day of ensiling: 2 days, 4 days, 6 days, 2 weeks, 6 weeks. Three replicates of each of T and U were used on each of these occasions, apart from the 6 weeks stage, which had 6 replicate samples of each. The extra samples from week 6 (replicates 4, 5 and 6 from each of Treated and Untreated material) were used in the aerobic stability study. There were therefore 36 samples in total at the end of the time course.

Analysis
Each sample was divided in two. One half was analysed at SAC Analytical labs, Edinburgh by Near Infrared Spectroscopy (NIR) and pH by wet chemistry. NIR is the standard method of grass silage analysis. The samples opened on Day 42 of the time course were, in addition, analysed for Ammonia N by wet chemistry and were screened for total moulds and yeasts. The remaining half of each sample was analysed by High Performance Liquid Chromatography (HPLC) at SAC Aberdeen. The HPLC was necessary to measure critical compounds such as lactic acid and volatile fatty acids in the silage samples.

Table 1 contains a summary of the sample analysis requirements.

Table 1. Analysis required for experimental samples (Untreated and Treated)

Time course of samples	NIR	pH by wet chemistry	Ammonia by wet chemistry	Yeast & mould count	HPLC
DAY 2	YES	YES	-	-	YES
DAY 4	YES	YES	-	-	YES
DAY 6	YES	YES	-	-	YES
DAY 14	YES	YES	-	-	YES
DAY 42	YES	YES	YES	YES	YES

All samples were stored frozen, in carefully labelled plastic bags, until the end of the time course. They were then split and sent for the various analyses, as described above.

Aerobic stability

For the investigation of aerobic stability, the extra Week 6 samples (replicates 4, 5 and 6 each of T and U) were used. Each sample was removed from the tightly packed plastic container into a clean plastic bucket, of 15 litres' capacity, and teased out to allow air to enter the silage. A temperature probe was inserted into the silage, attached to a data logger, and the silage temperature was recorded every 1 hour for seven continuous days. Each bucket was placed in a larger plastic bin and surrounded by polystyrene beads to provide thermal insulation. The contents of each bin were covered with a sheet of polystyrene. All of the bins, containing the plastic buckets with the silage samples, were kept in a shed, away from drafts. The surrounding ambient air temperature was also measured at the same time.

Statistical analysis

The results were analysed using Genstat 7 (Lawes Agricultural Research Station, Rothamstead, 2004), with Treatment defined as treatment and Block defined as replicate.

Results

Proximate analyses

The results from the NIR and wet chemistry analyses are contained in Table 2. Significantly different results are in bold type. Intake factor has not been reported for samples where fermentation was not complete. Differences in ME ($P<0.05$) were measured on Day 2 and Day 42 (Untreated was 11.0 MJ $kgDM^{-1}$ and Treated was 10.8 MJ $kgDM^{-1}$, in each case. Protein degradability (deg) was higher for Untreated than Treated silage on Day 2, 6, 14 and 42 of the fermentation. NDF was lower in Untreated than in Treated silage on Day 4, 6, 14 and 42. The difference in sugar content was significantly different on Day 2 (28.7 g $kgDM^{-1}$ for Untreated and 3.7 g $kgDM^{-1}$ for Treated material). Thereafter no sugar was present in the Treated silage.

There was no difference between Untreated and Treated for dry matter, crude protein, pH (either measured by NIR or by wet chemistry), Ammonia or intake factors. Crude protein and pH values dropped over the course of the fermentation for all samples.

Table 2. NIR and wet chemistry analysis results for Untreated and Treated samples

	U/T	Day 2	sed	P	Day 4	sed	P	Day 6	sed	P	Day 14	sed	P	Day 42	sed	P
DM	U	348.7	5.67	0.084	357.3	10.33	NS	366.0	5.03	0.086	360.0	6.49	NS	346.3	7.54	NS
	T	330.3			346.0			350.0			347.7			341.0		
ME	U	11.0	0.02	**0.015**	11.2	0.07	NS	11.1	0.12	NS	11.0	0.03	0.057	11.0	0.03	**0.038**
	T	10.8			11.0			10.8			10.9			10.8		
CP	U	188.7	2.65	NS	182.3	2.73	NS	179.3	2.96	NS	177.3	0.88	NS	175.0	1.67	NS
	T	193.7			183.0			183.7			176.7			177.3		
Deg	U	0.78	0.003	**0.02**	0.80	0.013	NS	0.80	0.003	**0.010**	0.80	0.006	**0.035**	0.79	0.003	**0.038**
	T	0.76			0.77			0.77			0.77			0.77		
PH Wet	U	5.03	0.067	NS	4.80	0.153	NS	4.50	0.058	NS	4.27	0.033	NS	4.10	0	-
	T	4.87			4.60			4.40			4.20			4.10		
PH NIR	U	5.00	0.033	NS	4.87	0.116	NS	4.83	0.00	-	4.50	0.067	NS	4.33	0.058	NS
	T	5.03			4.87			4.83			4.47			4.33		
NH4	U	74.7	2.08	NS	68.0	2.08	0.062	68.3	3.79	NS	90.7	3.84	NS	94.0	5.04	NS
	T	80.7			76.0			69.3			81.3			89.7		
NDF	U	499.7	5.61	NS	497.0	2.33	**0.025**	497.0	0.88	**0.026**	488.7	2.03	**0.015**	485.0	1.76	**0.009**
	T	505.0			511.3			502.3			505.0			503.7		
Sug	U	28.7	3.61	**0.02**	10.7	5.04	NS	13.7	4.48	0.093	7.0	4.36	NS	0	0	-
	T	3.7			0			0			0			0		
IFC	U	n/a			n/a			n/a			n/a			123.3	1.73	NS
	T	n/a			n/a			n/a			n/a			122.3		
IFS	U	n/a			n/a			n/a			n/a			122.0	4.0	NS
	T	n/a			n/a			n/a			n/a			118.0		

Table 3. HPLC analysis results for Untreated and Treated samples (g kg DM^{-1})

	U/T	Day 2	sed	P	Day 4	sed	P	Day 6	sed	P	Day 14	sed	P	Day 42	sed	P
Acetic	U	6.5	0.46	0.083	9.1	0.47	NS	9.7	0.43	NS	11.5	0.93	NS	18.3	2.01	NS
acid	T	8.0			9.3			10.2			13.1			16.3		
Ethanol	U	0	0	-	0	0.015	NS	0	0	-	0.045	0.0031	**0.011**	0.55	0.262	NS
	T	0			0.03			0			0.074			0.38		
Lactic	U	24.9	1.67	NS	38.0	2.85	NS	44.9	2.83	NS	61.2	5.85	NS	79.9	6.37	NS
Acid	T	26.8			34.4			41.7			61.9			74.4		
Propionic	U	0.6	0.15		0.6	0.19	NS	0.7	0.02	NS	0.8	0.06	0.083	1.9	0.31	NS
Acid	T	0.7			1.0			1.1			1.0			1.9		
1,2 propane	U	3.1	0.68	NS	3.9	1.69	NS	6.0	1.84	NS	5.1	2.17	NS	8.0	2.35	NS
diol	T	3.3			4.1			3.9			5.3			6.7		

The result of lactic acid, from day 42 Untreated sample, replicate 2, was not included in the analysis as it was an outlier, so was treated as a missing plot. No difference was observed between Untreated and Treated samples for acetic acid, lactic acid, propionic acid or 1,2-propane diol. There was a significant difference in ethanol concentration on Day 14 of fermentation ($p<0.05$), where the contents were 0.045 and 0.074 g kg DM^{-1}, for Untreated and Treated, respectively.

Yeast and mould counts

Fungal counts were relatively low and yeast was only detected on one sample as shown below in Tables 4 and 5 (Treated sample, replicate 2).

Table 4. PDA (growth on potato dextrose agar at 25°C). Number of organisms per gram fresh weight of material

Sample	Fungal count / gram silage	Yeast count /gram silage
T Day 42 R1	300	<100
T Day 42 R2	100	500
T Day 42 R3	<100	<100
U Day 42 R1	<100	<100
U Day 42 R2	<100	<100
U Day 42 R3	100	<100

Table 5. MEA (growth on malt extract agar at 37°C). Number of organisms per gram fresh weight of material

	Fungal count / gram silage	Yeast count /gram silage
T Day 42 R1	400	<100
T Day 42 R2	100	<100
T Day 42 R3	<100	<100
U Day 42 R1	<100	<100
U Day 42 R2	100	<100
U Day 42 R3	200	<100

Aerobic stability

Results from the aerobic stability tests are contained in Tables 6 and 7, and Figures 1 and 2.

Table 6. Temperature of samples (^{o}C) over 7 days, for replicates 4, 5 and 6 of both Untreated and Treated samples from Day 42, and ambient air temperature over the same time.

	ambient	Ur4	Ur5	Ur6	Tr4	Tr5	Tr6
mean day1	17.1	17.1	17.4	16.8	18.0	16.8	16.6
mean day2	16.9	17.0	17.2	16.7	18.0	16.3	16.3
mean day3	16.3	17.0	17.2	16.5	17.6	15.7	16.3
mean day4	16.0	16.0	17.2	15.7	17.0	15.7	16.3
mean day5	15.6	16.0	17.0	15.7	17.0	15.6	16.0
mean day6	16.0	16.0	17.0	15.7	17.0	15.4	15.5
mean day7	16.3	16.0	17.2	15.7	17.0	15.7	15.6

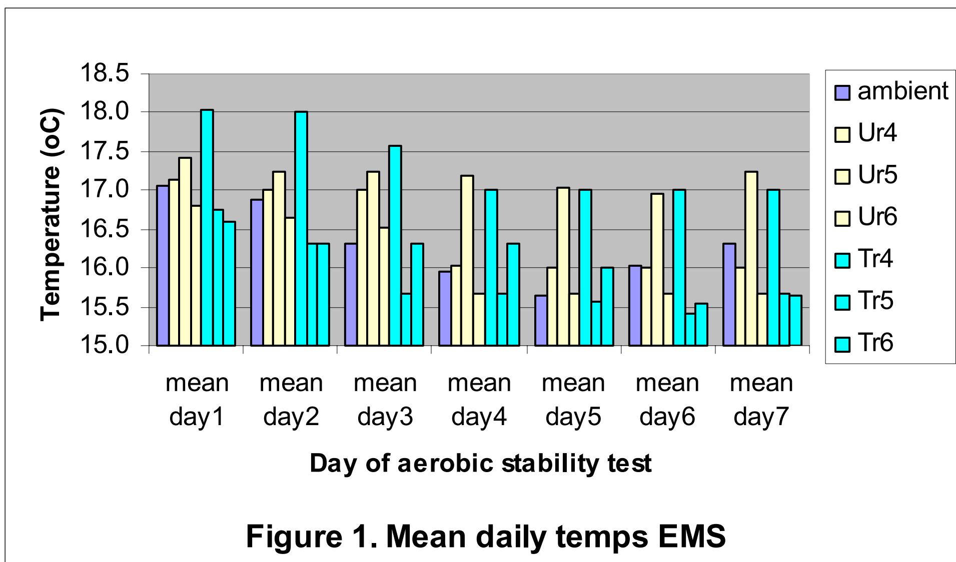

Figure 1. Mean daily temps EMS

Table 7. Average temperature (^{o}C) of Day 42 Untreated and Treated samples and ambient air temperature over the same time.

ambient	U	T
17.2	17.1	17.1
16.6	17.0	16.9
16.0	16.9	16.5
15.9	16.3	16.3
15.9	16.2	16.2
15.8	16.2	16.0
16.4	16.3	16.1

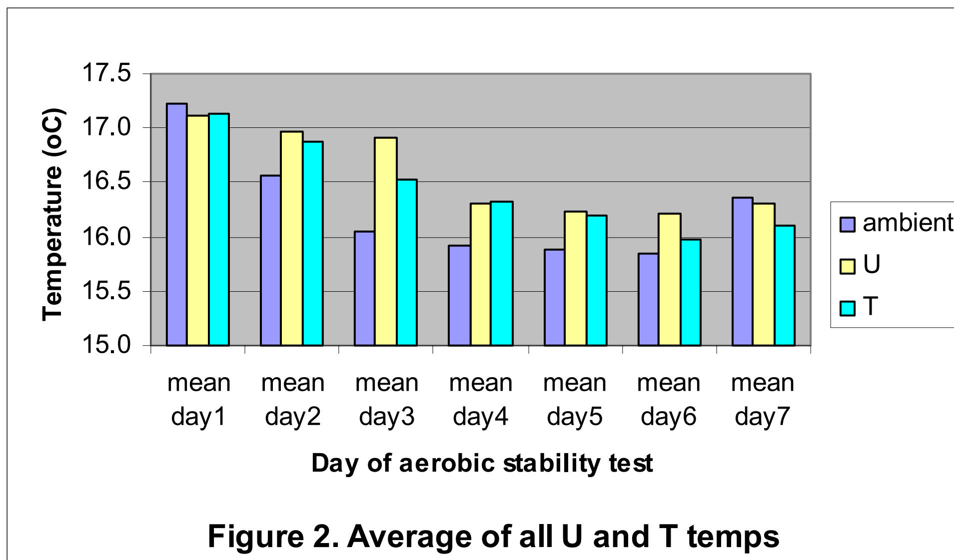

Figure 2. Average of all U and T temps

The temperature of the silage, once it was exposed to air decreased until Day 5, for both treated and untreated silage. The ambient temperature was higher on Day 7, which is reflected in the small rise in temperature of the samples. The temperature of the treated samples was lower than that of the untreated material.

Conclusions

- The main statistically significant differences were ME and sugar at first sampling and NDF and ME on some other occasions. The ME was lower for the treated material and the difference appeared immediately so it is unlikely to be due to the additive and more likely that the ME and NDF differences are because of a small sub-sampling difference. The grass was relatively dry with adequate sugar for its moisture content.

- The high dry matter of the grass might have made it aerobically unstable but it was not dry enough for this to happen. Enough acid was produced and residual sugar was so low that very little heat was produced.

- There is some indication of a faster fermentation for the Treatment, as pH tended to be lower for treated silage at each stage when measured by wet chemistry. Slightly lower ammonia levels as fermentation progressed may indicate a marginally more stable product in terms of protein.

Reference

LAWES AGRICULTURAL TRUST, 1990. Genstat 7 Release 1 Harpenden, Hertfordshire: Rothamsted Experimental Station.