

Manipulating the ensilage of wilted, unchopped grass through the use of additive treatments

J. McEniry^{1,2}, P. O’Kiely¹†, N.J.W. Clipson², P.D. Forristal³ and E.M. Doyle²

¹*Teagasc, Grange Beef Research Centre, Dunsany, Co. Meath*

²*UCD School of Biology and Environmental Science, University College Dublin, Belfield, Dublin 4*

³*Teagasc, Crops Research Centre, Oak Park, Co. Carlow*

Baled silage composition frequently differs from that of comparable conventional precision-chop silage. The lower final concentration of fermentation products in baled silage makes it more conducive to the activities of undesirable microorganisms. Silage additives can be used to encourage beneficial microbial activity and/or inhibit detrimental microbial activity. The experiment was organised in a 2 (chop treatments) × 6 (additive treatments) × 2 (stages of ensilage) factorial arrangement of treatments (n = 3 silos/treatment) to suggest additive treatments for use in baled silage production that would help create conditions more inhibitory to the activities of undesirable microorganisms and realise an outcome comparable to precision-chop silage. Chopping the herbage prior to ensiling, in the absence of an additive treatment, improved the silage fermentation. In the unchopped herbage, where the fermentation was poorer, the lactic acid bacterial inoculant resulted in an immediate increase (P < 0.001) in lactic acid concentration and a faster decline (P < 0.001) in pH with a subsequent reduction in butyric acid (P < 0.001) and ammonia-N (P < 0.01) concentrations. When sucrose was added in addition to the lactic acid bacterial inoculant, the combined treatment had a more pronounced effect on pH, butyric acid and ammonia-N values at the end of ensilage. The formic acid based additive and the antimicrobial mixture restricted the activities of undesirable microorganisms resulting in reduced concentrations of butyric acid (P < 0.001) and ammonia-N (P < 0.01). These additives offer a potential to create conditions in baled silage more inhibitory to the activities of undesirable microorganisms.

Keywords: additive; baled silage; precision-chop silage

†Corresponding author: padraig.okiely@teagasc.ie

Introduction

The two most commonly employed methods of conserving forage in Ireland are conventional precision-chop silage (0.60 of national silage area) stored in horizontal silos and individual bales of silage wrapped in polythene stretch-film (0.32 of silage area; O'Kiely *et al.*, 2000). Although the principles for achieving a successful preservation are the same for both systems, baled silage composition frequently differs from that of comparable precision-chop silage (Fychan, Fraser and Jones, 2002; Ohlsson, 1998). In the absence of the herbage being chopped and bruised during harvesting, the onset of fermentation in baled silage as evidenced by the increase in lactic acid concentration and decline in pH is slower than for precision-chop silage. Correspondingly, the overall concentration of fermentation products is lower and ammonia-N concentration higher, making the environment in the bale more conducive to the activities of undesirable microorganisms (e.g. Clostridia, yeast; McEniry *et al.*, unpublished data). The implications of a slower onset of fermentation in baled silage are further accentuated by wilting, which is an integral part of most baled silage production, and results in a more restricted fermentation compared to unwilted or lightly wilted precision-chop silages (McEniry *et al.*, 2006). In addition, the challenge from spoilage microorganisms such as moulds can be relatively high with baled silage (O'Brien *et al.*, 2005), implying that the extent of anaerobiosis achieved and/or maintained during storage on farms is less with baled than with precision-chop silage.

Silage quality is modified by microbial activity during production, storage and feedout (Lindgren, Bromander and Pettersson, 1988). Ensilage fermentation is not a fully controlled process under farm conditions, and hence most com-

ponents of a planned ensilage system contribute to stacking the probabilities in favour of a lactic acid dominant fermentation (O'Kiely and Muck, 1998). The application of silage additives during ensiling is sometimes used to encourage beneficial microbial activity and/or inhibit detrimental microbial activity (Whittenbury, 1968). Additives have been categorised based on their mode of action during ensilage and include stimulators of fermentation (e.g. sugar, lactic acid bacteria inoculants), selective inhibitors of fermentation (e.g. formic acid), inhibitors of aerobic deterioration (e.g. benzoic or propionic acid) and nutrients and absorbents, with some additive treatments falling into multiple categories (Kung, Stokes and Lin, 2003; O'Kiely and Muck, 1998; McDonald, Henderson and Heron, 1991). As a result of the differences in the rate and extent of fermentation, depending on the harvesting and storage system employed, the nature and intensity of the effects of additive treatments could differ for baled and precision-chop silages. Whereas most published experiments evaluating silage additives involved precision-chop silage, some workers have reported the benefit of inoculants and formic acid based additive treatments in improving the fermentation of baled silage (Haigh, Chapple and Powell, 1996; Jonsson *et al.*, 1990; Ohlsson, 1998; Keller, Nonn and Jeroch, 1998). However, few experiments have been conducted simultaneously comparing the responses to additives for baled and precision-chop silages.

The objective of this study was to quantify the effects of additive treatments, with contrasting modes of action, on the fermentation of wilted, unchopped herbage (as a model for baled silage) relative to that of precision-chopped herbage, under controlled conditions in laboratory silos.

This information should help to identify additive treatments to be considered for use in baled silage production that would facilitate the creation of conditions that are more inhibitory to the activities of undesirable microorganisms and are comparable to that of precision-chop silage. Previous studies have confirmed that unchopped and precision-chop grass ensiled in the laboratory silos used in the present experiment are suitable models for studying the ensilage of conventional baled (McEniry *et al.*, unpublished data) and precision-chop (O'Kiely and Wilson, 1991) silages, respectively.

Materials and methods

Experimental design

The experiment was organised in a 2 (chop treatments) \times 6 (additive treatments) \times 2 (stages of ensilage) factorial arrangement of treatments. Herbage was wilted for 24 h and representative samples were ensiled, unchopped or precision-chopped, in laboratory silos with the addition of one of six additive treatments. Each chop \times additive treatment was replicated six times, involving 72 laboratory silos. Half of these silos were opened and sampled after 2 days ensilage with the remaining silos opened after 110 days. Silage fermentation variables were assessed at both sampling times, while indices of nutritive value and silage aerobic stability were assessed after 110 days ensilage.

Harvest and ensiling

An homogenous plot of *Lolium perenne* (cv. Fennema) was mown (Pottinger, Nova 310T conditioner mower) on the 21 July 2004 and wilted in the field for 24 h with frequent tedding (Krone rotary tedder, KW550/4 \times 7). There was no rainfall during wilting or harvesting.

After the wilting period (day = 0), six representative herbage samples were taken prior to the chop treatment and ensiling for subsequent chemical and microbiological analyses.

The wilted herbage was then representatively sampled and used to fill 72 laboratory silos (height = 0.75 m, internal diameter = 0.152 m, internal volume = 13.6 L; O'Kiely and Wilson, 1991), and the herbage samples for 36 of these silos were precision-chopped (Pottinger, Mex VI) immediately prior to ensiling. The chopping knife number and feed roller speeds were chosen, according to the manufacturers instructions, to give a theoretical chop length of 19 mm. The remaining 36 silos were filled using samples of unchopped herbage.

Prior to filling the laboratory silos, six randomly selected samples (each 5 kg) of both unchopped and chopped herbage were assigned to each additive treatment. The following additive treatments (and application rates) were applied to the herbage: (1) no additive (control treatment), (2) lactic acid bacterial inoculant (LAB; Bio-Sil[®], *Lactobacillus plantarum* DSM 8862 and *Lb. plantarum* DSM 8866; Dr. Pieper technologie- und Produktentwicklung, GmbH), 1 g/t (3×10^5 colony forming units/g herbage), (3) sucrose, 5 kg/t, (4) lactic acid bacteria inoculant plus sucrose (both prepared and applied as above), (5) formic acid based additive (Add SafeR[®], 70 g ammonia and 640 g formic acid per 1 kg additive; Trouw Nutrition, UK Ltd.), 3 L/t and (6) antimicrobial mixture (AMM; KofaSil[®], 80 g hexamethylene tetra-amine, 120 g sodium nitrite, 150 g sodium benzoate, 50 g sodium propionate and 600 g water per kg additive; Addcon Agrar, GmbH), 3 L/t. Each of the six treatments had 10 ml of liquid applied per kg of grass, which necessitated adding 0, 5, 7 or 10 ml distilled

water per 1 kg grass depending on the level of additive already applied. Aseptic techniques were used to prevent cross contamination between treatments.

A constant weight (4 kg) of wilted herbage (additive applied was additional) was then ensiled, the silos were then packed manually and sealed immediately by a screw-on top with a rubber seal. Compaction was achieved in the silos by the inclusion of a 10.5 kg weight (diameter = 0.1 m) directly on the herbage to exert a continual vertical pressure (5.83 kPa). The laboratory silos were stored at a room temperature of 15 °C prior to silo opening after 2 or 110 days ensilage.

Silage sampling

Three silos from each treatment combination were sampled after 2 days of ensilage and the 3 remaining silos were sampled at day 10. Silage from each laboratory silo was weighed and, after thorough aseptic mixing, one sample per silo was taken. All samples were stored at 4 °C prior to microbiological analyses (grass samples only) and at -18 °C for chemical analyses.

Chemical analyses

Grass and silage samples were dried at 98 °C and 85 °C, respectively, for 16 h in an oven with forced air circulation to estimate DM concentration. The latter was corrected for the loss of volatiles according to Porter and Murray (2001). Samples dried at 40 °C for 48 h were milled through a 1 mm screen prior to analysis for *in vitro* dry matter digestibility (DMD), ash, buffering capacity (BC) and water soluble carbohydrate (WSC) concentration on day 0 and 110, for neutral detergent fibre (NDF) and acid detergent fibre (ADF) on day 110 only and for total nitrogen on all three sampling days. Aqueous extracts were used for the determination of pH (day 0, 2 and 110), and fermentation

products (lactic acid, acetic acid, propionic acid, butyric acid and ethanol) and ammonia-N on day 2 and 110, as described previously (McEniry *et al.*, 2006).

Microbiological analyses

Grass samples were processed for microbial enumeration within 3 h of sample collection. Lactic acid bacteria, yeast, Enterobacteria, and spores of Clostridia and Bacilli were all enumerated on media as described by McEniry *et al.* (2006). The colony forming units (cfu) on each plate were enumerated and the number of microorganisms/g silage expressed as log₁₀.

Silage aerobic stability: After sampling on day 110, approximately 2 kg of the remaining silage from each silo was assessed for aerobic stability and deterioration as described by O'Kiely and Marron (2003). Briefly, each silage was placed in a polythene-lined polystyrene (2.5 cm thick) box (59 cm × 39 cm × 22 cm) with a polystyrene lid loosely fitted on top. Thermocouples were placed in the middle of the silage in each box and the temperature was automatically recorded hourly for 192 h. Containers of water stored near the silage acted as reference temperatures to which all silage temperatures were compared. The following indices of aerobic stability were used: (1) interval until the temperature rose more than 2 °C above the reference temperature, (2) maximum temperature rise (°C), (3) time interval to maximum temperature, and (4) accumulated temperature rise (°C) in the first 120 and 192 h of aerobiosis.

Fresh weight loss

Silage fresh weight loss was calculated as the difference in the herbage fresh weight ensiled and removed from the laboratory silos (expressed as a proportion of the

fresh weight ensiled). For wilted herbage (no effluent production) in properly sealed silos this provides a useful index of total losses during ensilage (Lingvall, personal communication).

Statistical analyses

Mean (s.d.) values were calculated for grass composition variables. Appropriate silage data were analysed by analysis of variance for a $2 \times 6 \times 2$ factorial arrangement of treatments within a completely randomised design using Proc GLM of SAS (SAS, 2000). Variables measured on day 110 only were analysed by two-way analysis of variance for a 2×6 factorial arrangement of treatments using the same procedure.

Results

Mean values for grass composition after the 24 h wilting period are shown in Table 1.

Chop treatment

Lactic acid concentration and the proportion of lactic acid in fermentation products were higher ($P < 0.001$), while butyric acid, ethanol and ammonia-N concentrations

were lower ($P < 0.001$) in the precision-chop silage compared to the unchopped silage (Table 2). However, chopping had no effect ($P > 0.05$) on silage DM, pH, acetic acid, propionic acid or the total concentration of fermentation products. Silage fresh weight loss, on average, was slightly higher ($P < 0.001$) for the unchopped compared to the chopped herbage (9 versus 7 g/kg) but the difference was small (Table 2).

Silage buffering capacity was higher ($P < 0.001$), while WSC concentration was lower ($P < 0.001$) in the precision-chop silage after 110 days ensilage (Table 3). Ash ($P < 0.05$), NDF ($P < 0.001$) and ADF ($P < 0.05$) concentrations were all slightly lower for the precision-chop silage (measured on day 110 only) but differences were modest in scale. Chopping had no effect on DMD or CP concentrations.

On average, the precision-chop silage was less stable on exposure to air after 110 days ensilage than the unchopped herbage (Table 4), with a shorter ($P < 0.001$) time to onset of heating (i.e. temperature rise > 2 °C), a higher ($P < 0.001$) maximum temperature rise and higher accumulated temperatures to 120 ($P < 0.01$) and 192 ($P < 0.001$) h.

Table 1. The mean (s.d.) chemical (g/kg DM, unless otherwise stated, except for pH) and microbiological composition (\log_{10} cfu/g) of herbage after a 24 h wilt and prior to ensiling

Variable	Mean
Dry matter (g/kg)	246 (3.4)
Dry matter digestibility (g/kg)	798 (11.8)
Ash	105 (3.4)
Crude protein	154 (4.4)
pH	6.06 (0.078)
Buffering capacity (mEq/kg DM)	471 (12.1)
Water soluble carbohydrate	170 (10.2)
Lactic acid bacteria	5.0 (0.49)
Enterobacteria	4.5 (0.45)
Clostridia	2.0 (0.35)
Bacilli	2.8 (0.25)
Yeast	2.6 (0.14)

Table 2. Treatment means and significance of treatment effects for silage pH, dry matter concentration, ammonia-N concentration, fermentation products and fresh weight loss (g/kg DM unless otherwise stated)

Treatment combination ¹			Variable ²									
Chop	Additive	Stage of ensilage	DM	pH	LA	AA	PA	BA	Eth	L/FP	NH ₃ -N	FWL
UC	Control	2	239	4.57	40	22	1.2	0.2	9.3	0.56	47	4
UC	LAB	2	244	4.33	72	15	0.3	0.2	8.5	0.75	38	4
UC	Sucrose	2	245	4.53	44	18	0.5	0.1	8.0	0.63	40	3
UC	LAB + Suc.	2	246	4.43	62	11	0.4	0.3	6.0	0.78	34	4
UC	Formic acid	2	252	4.70	34	11	0.4	0.3	5.0	0.68	55	2
UC	AMM	2	257	4.57	46	15	0.7	0.1	4.2	0.70	43	3
PC	Control	2	246	4.73	46	12	0.4	0.2	5.1	0.74	44	5
PC	LAB	2	249	4.56	50	10	0.3	0.1	4.9	0.77	38	8
PC	Sucrose	2	251	4.70	44	14	0.3	0.1	5.0	0.70	40	4
PC	LAB + Suc.	2	250	4.56	43	8	0.3	0.2	3.6	0.78	33	5
PC	Formic acid	2	249	4.80	34	9	0.4	0.2	2.9	0.74	66	2
PC	AMM	2	249	4.87	39	12	0.9	0.1	2.5	0.73	45	4
UC	Control	110	237	4.23	101	8	0.7	19.9	24.5	0.66	122	20
UC	LAB	110	247	4.13	113	8	0.5	14.7	19.8	0.73	97	17
UC	Sucrose	110	242	4.30	96	11	0.7	15.5	21.2	0.66	103	19
UC	LAB + Suc.	110	243	4.07	114	9	0.6	8.8	18.3	0.77	70	15
UC	Formic acid	110	247	4.30	102	12	0.7	5.4	12.8	0.77	102	13
UC	AMM	110	240	4.07	107	21	1.0	3.2	9.0	0.77	92	9
PC	Control	110	244	3.90	148	15	0.6	0.6	13.1	0.83	77	11
PC	LAB	110	244	3.90	136	14	0.4	0.7	14.8	0.82	62	10
PC	Sucrose	110	246	3.90	138	19	0.5	0.3	15.2	0.80	68	9
PC	LAB + Suc.	110	247	3.90	130	15	0.1	0.3	12.5	0.83	62	8
PC	Formic acid	110	246	3.90	116	15	0.4	0.7	10.9	0.81	88	10
PC	AMM	110	243	4.03	127	25	1.1	0.6	9.8	0.78	83	8
		s.e. ³	3.8	0.046	5.2	4.0	0.24	1.28	2.32	0.047	4.2	1.1

Significance (*F*-test) for:

Chop					***			***	***	***	***	***
Additive				***	***		*	***	***	*	***	***
Stage of ensilage		**	***	***				***	***	**	***	***
Chop × additive				*	***			***			**	
Chop × stage of ensilage			***	***	**			***			***	***
Additive × stage of ensilage			***					***			**	**
Chop × additive × stage of ensilage ¹								***			*	*

¹ UC = unchopped, PC = precision-chopped; Control = no additive; LAB = lactic acid bacteria inoculant; LAB + Suc. = lactic acid bacteria inoculant + sucrose; AMM = antimicrobial mixture.

² DM = dry matter (g/kg), LA = lactic acid, AA = acetic acid, BA = butyric acid, PA = propionic acid, Eth = Ethanol, L/FP = proportion of lactic acid in fermentation products (g/g), NH₃-N = ammonia-N (g/kg N), FWL = fresh weight loss (g/kg), NS = not significant, * = $P < 0.05$, ** = $P < 0.01$, *** = $P < 0.001$.

³ s.e. relates to 3 factor interaction.

Additive treatment

For the main effect of additive, lactic acid concentration was highest ($P < 0.001$) for the LAB inoculant (Table 2). Correspondingly, the proportion of lactic acid in fermentation products was lowest ($P < 0.05$) for the control (no additive)

and sucrose treatments. On average, silage pH was lower ($P < 0.001$) for the LAB and the LAB + sucrose additive treatments than for the other additive treatments. Ammonia-N concentration was lowest ($P < 0.001$) for the LAB + sucrose treatment and highest for the formic acid

Table 3. Treatment means and significance of treatment effects for silage buffering capacity and chemical composition (g/kg DM, unless otherwise stated) after 110 days ensilage

Treatment ¹		Variable ²						
Chop	Additive	BC	DMD	NDF	ADF	Ash	CP	WSC
UC	Control	892	760	496	296	113	160	18
UC	LAB	918	773	479	293	111	158	31
UC	Sucrose	878	771	485	284	113	155	15
UC	LAB + Sucrose	902	774	469	285	108	151	38
UC	Formic acid	830	786	465	289	107	155	21
UC	AMM	842	781	467	289	110	157	15
PC	Control	988	765	464	283	110	153	12
PC	LAB	978	776	463	283	109	155	13
PC	Sucrose	982	779	462	281	106	151	16
PC	LAB + Sucrose	928	776	457	278	107	154	16
PC	Formic acid	863	785	455	282	107	151	14
PC	AMM	891	769	456	280	108	156	11
	s.e.	14.3	7.2	4.6	7.1	2.1	2.9	2.9

Significance (F-test) for:

Chop treatment	***		***	*	*		***
Additive type	***		**				***
Chop × additive							**

¹ See footnote for Table 2.

² BC = buffering capacity (mEq/kg DM), DMD = dry matter digestibility (g/kg), NDF = neutral detergent fibre, ADF = acid detergent fibre, CP = crude protein, WSC = water soluble carbohydrates.

Table 4. Treatment means and significance of treatment effects for silage aerobic stability after 110 days ensilage

Treatment ¹		Variable				
Chop	Additive	Time to temperature rise >2 °C (h)	Maximum temperature rise (°C)	Time to maximum temperature (h)	Accumulated temperature rise to 120 h (°C)	Accumulated temperature rise to 192 h (°C)
UC	Control	192	0.6	118	1.4	2.2
UC	LAB	120	2.2	104	5.1	9.4
UC	Sucrose	192	0.9	12	2.7	4.3
UC	LAB + Sucrose	91	5.5	127	8.4	17.0
UC	Formic acid	166	1.5	137	3.1	6.5
UC	AMM	192	1.0	69	3.6	5.6
PC	Control	93	10.8	103	14.8	32.4
PC	LAB	45	10.9	66	23.2	61.6
PC	Sucrose	192	1.5	121	3.7	6.0
PC	LAB + Sucrose	38	17.6	53	3.1	69.4
PC	Formic acid	147	2.1	124	4.7	8.8
PC	AMM	192	0.9	127	3.1	4.7
	s.e.	24.9	2.44	29.4	5.55	8.00

Significance (F-test) for:

Chop	**	***		**	***
Additive	***	**		**	***
Chop × additive ^s				*	**

¹ See footnote for Table 2.

based additive. Ethanol ($P < 0.001$) and butyric acid ($P < 0.001$) concentrations were lowest for the AMM treatment, with the highest concentrations of these products observed in the control treatment. Propionic acid concentrations were low (< 1 g/kg DM) in all silages. Additive treatment had an effect ($P < 0.001$) on average silage fresh weight loss but the magnitude was relatively modest, with mean values for the main effect of additive treatment falling in the range of 6–10 g/kg, but with the AMM and formic acid treatments at the lower end of this range.

For variables measured on day 110, the main effect of additive indicated that the NDF concentration was slightly higher ($P < 0.01$) in the control treatment compared to other additives, while additive treatment had no effect on silage DMD, ash, CP and ADF concentrations (Table 3). Silage buffering capacity was similarly lower ($P < 0.001$) for the AMM and formic acid based additive treatments.

On average, the LAB + sucrose treated silage was the least stable on exposure to air after 110 days ensilage (Table 4), followed by the LAB only treatment. This was evidenced by a faster ($P < 0.001$) time to onset of heating, a higher ($P < 0.01$) maximum temperature rise and higher accumulated temperatures to 120 ($P < 0.01$) and 192 ($P < 0.001$) h.

Stage of ensilage

Lactic acid, butyric acid, ethanol and ammonia-N concentrations were all higher ($P < 0.001$), while pH was lower ($P < 0.001$) after 110 compared to 2 days ensilage (Table 2). There was a greater decrease in pH from day 0 to day 2 of ensilage, than from day 2 to day 110. Acetic and propionic acid concentrations did not differ ($P > 0.05$) after 2 compared to 110 days of ensilage. Just over half of the concentration of ammonia-N on day 110 was

present at day 2 (0.51), while proportionately 0.38 of lactic acid was present. The proportion of lactic acid in the fermentation products was higher ($P < 0.01$) after prolonged storage (110 d). Herbage fresh weight loss was higher ($P < 0.001$) after 110 days (12 versus 4 g/kg) than 2 days ensilage (Table 2).

Chop × additive interactions

When averaged across stage of ensilage, pH was lower ($P < 0.05$) for the control and sucrose additive treatments in the presence of chopping, while pH was higher ($P < 0.05$) in the chopped herbage after the addition of the AMM additive (Table 2). The lactic acid concentration was higher ($P < 0.001$) with chopping for all additive treatments, except for the LAB and LAB + sucrose treatments. The largest increase in lactic acid concentration due to chopping occurred for the control treatment.

Butyric acid concentration was low (< 1 g/kg DM) in all chopped herbage but an increase was observed due to the absence of chopping, with the concentration being highest ($P < 0.001$; 10 g/kg DM) in the control treatment, followed by the LAB and sucrose only additive treatments (~ 8 g/kg DM). Ammonia-N concentration was lower ($P < 0.01$) with chopping for each additive treatment, with the largest decrease in ammonia-N concentration due to chopping occurring for the control silage. There was no significant interaction between chop and additive treatment for DM, acetic acid, propionic acid, ethanol and fresh weight loss.

For the precision-chop silage after 110 days ensilage, the WSC concentration was similar (range 12 to 16 g/kg DM) irrespective of additive treatments (Table 3). However, in the unchopped silage, the WSC concentration was higher ($P < 0.01$) for the LAB and the LAB + sucrose treatments

(31 and 38 g/kg DM, respectively). The interaction of chop and additive treatment was not significant for silage DMD, NDF, ADF, CP, ash or buffering capacity.

Precision chopping resulted in an increased ($P < 0.05$) accumulated temperature to 120 h for the control and the LAB treated silages, while having little effect on the aerobic stability of the other silages (Table 4). In the former silages and in the LAB + sucrose treated silage, chopping resulted in a large increase ($P < 0.01$) in accumulated temperature to 192 h.

Chop × stage of ensilage interactions

When averaged across additive treatments, silage pH was lower ($P < 0.001$), while lactic acid ($P < 0.001$) and acetic acid ($P < 0.01$) concentrations were higher after 2 days ensilage in the unchopped compared to the chopped herbage (Table 2). The opposite was the case for each variable after 110 days ensilage.

Butyric acid ($P < 0.001$) was low (< 1 g/kg DM) for all treatments except for the unchopped silage after 110 days storage (11 g/kg DM). Ammonia-N concentration was similar on day 2 for both chop treatments but was higher ($P < 0.001$) in the unchopped herbage after 110 days storage. Silage fresh weight loss increased from day 2 to 110, but was higher ($P < 0.001$) for the unchopped compared to the precision-chopped herbage after 110 days ensilage. There was no significant interaction between chop and stage of ensilage for DM, propionic acid, ethanol and the proportion of lactic acid in fermentation products.

Additive × stage of ensilage interactions

When averaged across chop treatments, silage pH and ammonia-N concentration were lowest ($P < 0.001$) for the LAB and the LAB + sucrose additive treatments after 2 days ensilage (Table 2). However,

the silage pH was similar for all treatments at the end of the storage period (110 days). Ammonia-N concentration increased during ensilage for all treatments (from day 2 to day 110), but with the lowest ($P < 0.01$) concentration observed for the LAB + sucrose treatment after 110 days. Ammonia-N concentration was highest for the formic acid based treatment on day 2 and similarly higher for the formic acid based and control treatments after 110 days ensilage.

Butyric acid concentration was low in all silages at day 2, but increased during ensilage and was highest ($P < 0.001$) for the control treatment after 110 days ensilage. Butyric acid concentration after 110 days ensilage was lowest for the AMM additive, followed by the formic acid based treatment. The interaction of stage of ensilage and additive treatment was not significant for lactic acid, acetic acid, propionic acid or ethanol concentrations. Fresh weight loss was similar for all treatments on day 2 and increased between days 2 and 110. Fresh weight loss was lowest ($P < 0.01$) for AMM treatment after 110 days ensilage, followed by the LAB + sucrose and formic acid based additive treatments.

Chop × additive × stage of ensilage interactions

Butyric acid concentration was low (< 1.0 g/kg DM) in all silages after 2 days ensilage and low in the precision-chopped silage after 110 days storage (Table 2). However, the concentration increased ($P < 0.001$) in the unchopped herbage from day 2 to 110. Butyric acid concentration in the unchopped herbage after 110 days ensilage was < 5.0 g/kg DM for the AMM treatment only, while the concentration was > 10 g/kg DM for the control, sucrose and LAB additive treatments. The ammonia-N concentration was higher ($P < 0.05$) for each additive treatment on

day 110 in the unchopped compared to the chopped herbage. The LAB + sucrose additive treatment inhibited the increase in ammonia-N concentration most in the unchopped herbage.

Herbage fresh weight loss was greater after 110 days ensilage for both chop treatments, but was higher ($P < 0.05$) for the unchopped compared to the chopped herbage (range 9–20 versus 8–11 g/kg; Table 2). There were no further significant interactions for any of the other variables measured.

Discussion

Grass composition

Wilting resulted in a herbage DM concentration at ensiling of 246 g/kg and a corresponding pH of 6.06. The ash concentration was slightly above normal (105 g/kg), perhaps indicating some level of soil contamination during wilting, with the latter possibly being responsible for the Clostridia and Bacilli spores recorded. Numbers of lactic acid bacteria and Enterobacteria on the wilted herbage were normal for grass under Irish conditions (Moran *et al.*, 1990; McEniry *et al.*, 2007).

Silage fermentation

Chopping the herbage prior to ensiling, in the absence of additive application, improved silage fermentation by increasing lactic acid concentration and its contribution to fermentation products, and by reducing the concentrations of butyric acid, ethanol and ammonia-N. The more extensive fermentation in the chopped herbage was further reflected in the lower concentration of residual WSC and higher buffering capacity at the end of the storage period. Although these effects were not evident on day 2, their strongly evident presence after 110 days of ensilage was

in accord with Seale *et al.* (1981), Pauly, Hansson and Tham (1999) and McEniry *et al.* (2007). Chopping and other herbage disrupting mechanical treatments serve to improve silage fermentation by liberation of plant cell juices and also assist in bringing about anaerobic conditions more rapidly (Seale *et al.*, 1981; Greenhill, 1964). A more rapid onset of fermentation in the chopped herbage, as reported by Seale *et al.* (1981), was not observed in this experiment where variables were measured at only one time point (day 2) in the early stages of ensilage. The largest effect of chopping on fermentation products at this stage was on lactic and acetic acid concentrations. Although pH was lower (4.57 versus 4.72) and the concentration of lactic plus acetic acid higher (62 versus 58 g/kg DM) for the unchopped compared to the chopped herbage ensiled without additive treatment, the differences observed were modest. Overall, the profile of fermentation products measured does not explain the higher pH in chopped herbage. Differential metabolism of unquantified constituents such as plant organic acids could have contributed to the changes. However, a markedly more homofermentative lactic acid dominant fermentation was evident in the chopped herbage after 2 days ensilage, creating conditions early in ensilage that were more inhibitory to the activities of undesirable microorganisms.

The much greater reduction, due to chopping, in NDF than ADF concentration after 110 days ensilage is indicative of a more extensive hydrolysis (mainly acid mediated) of hemicellulose than cellulose (McDonald *et al.*, 1991). This would have contributed further to the substrate available for fermentation and facilitated the higher concentration of fermentation products.

After 110 days of ensilage, there was evidence of a more extensive secondary

fermentation in the unchopped relative to the precision-chopped control silage, as indicated by higher concentrations of butyric acid and ammonia-N (Haigh and Parker, 1985). These indices reflect mainly saccharolytic (and to a lesser extent proteolytic) clostridial, as well as possibly some enterobacterial, activity (Pahlow *et al.*, 2003). This in turn would indicate a greater need for effective additive treatments with the unchopped herbage.

In the chopped herbage, where a lactic acid dominant fermentation already prevailed, the addition of the homofermentative LAB additive had relatively little impact after 2 or 110 days ensilage. In contrast, the application of this additive treatment to the unchopped herbage, where the fermentation was poorer, resulted in a rapid (i.e., by day 2) increase in the concentration of lactic acid and in its contribution to fermentation products and a correspondingly faster decline in pH. Similar results were observed by Rooke and Kafilzadeh (1994) and Lindgren *et al.* (1988). During the subsequent 108 days of ensilage there followed a reduction in the concentrations of butyric acid, ethanol and ammonia-N, relative to the control treatment, indicating the partial inhibition of the activities of undesirable microorganisms such as Clostridia and Enterobacteria, in accord with Kung *et al.* (2003), Winters *et al.* (1998) and Rooke *et al.* (1988). These effects of the added lactic acid bacteria with the unchopped herbage are indicative of the dominance of the added homofermentative strains during ensilage and in turn the creation of more inhibitory conditions earlier in the storage period (Winters *et al.*, 1998; Chamberlain, 1988).

For the unchopped and, in particular, the chopped herbage, adding sucrose made little improvement to the fermentation variables measured. This suggests that the

supply of fermentable substrate from the herbage to the indigenous epiphytic microbial population did not limit their ability to produce adequate lactic acid. However, it is also possible that the added sucrose was equally accessible to both the indigenous lactic acid bacteria and to undesirable microorganisms, such as Enterobacteria, at the start of ensilage. After 110 days ensilage the residual WSC concentration of the silage where sucrose was applied was similar to the control treatment indicating that the added sucrose was utilised during ensilage. Consequently, adding sucrose (20.3 g/kg) DM to herbage with a WSC concentration of 170 g/kg DM and with a DM and buffering capacity of 246 g/kg and 471 m. Eq/kg DM, respectively, had little beneficial effect on butyric acid and ammonia-N concentrations, in accord with Nishino and Uchida (1999).

Whereas the LAB + sucrose additive treatment applied to the chopped herbage had little effect on the final outcome of a fermentation already dominated by lactic acid, there was a clear improvement of fermentation in the unchopped herbage. The latter was evidenced by a 10% increase in lactic acid concentration and a two-fold increase in residual WSC concentration. These changes, together with the reduction in the concentrations of butyric acid and ammonia-N, were probably indicative of the inhibition of enterobacterial and clostridial activity. This combined additive treatment tended to have a more pronounced effect on these variables than the individual additives applied singly, in accord with Ohyama, Masaki and Morichi (1973) and Lindgren *et al.* (1988). The greater beneficial effect of the LAB + sucrose, compared to the LAB only additive treatment, with the unchopped herbage was not apparent on day 2 of ensilage suggesting that the positive effects occurred later in the storage period. This

suggests that fermentable substrate supply in the latter stages of ensilage constrained the dominance of the primary fermentation, and permitted a secondary fermentation under conditions where heterofermentative lactic acid bacteria dominated.

The formic acid based additive treatment caused a restriction of the fermentation in the chopped herbage relative to the control treatment in accord with Rooke *et al.* (1988) and Mayne (1990). This was particularly evident in the reduced concentration of lactic acid and of total fermentation products. The similar pH to the control treatment, together with the lower buffering capacity indicates that less fermentation acids were needed to decrease herbage pH when the formic acid based additive was used. The increase in ammonia-N concentration was indicative of the direct contribution of ammonia-N from the additive (potentially a maximum of 0.36 of the ammonia-N in the silage treated with formic acid-based silage additive could have come directly from the additive) and thus overestimates the impact of proteolysis during ensiling (Randby, 2000). The addition of the formic acid based additive also resulted in an apparent increase in acid hydrolysis of hemicellulose. Whereas in the absence of additive application the chopping of herbage immediately prior to ensiling reduced undesirable fermentation products, one effect of the formic acid based additive was to produce silages from both chop treatments that exhibited more consistent and desirable fermentation characteristics. Thus, in the unchopped herbage, the formic acid based additive appeared to result in greater inhibition of enterobacterial and clostridial activity, as evidenced by reduced concentrations of ethanol, butyric acid and ammonia-N (corrected for ammonia provided directly by additive), in accord with Haigh (1988). The absence of an increase in yeast activ-

ity and in turn of ethanol concentration, as reported by some authors (Rooke *et al.*, 1988), would suggest that the rate (3 L/t) of this additive applied in this experiment was capable of inhibiting yeast activity. Some of these latter effects could have also been due to the inhibitory effects of added ammonia (Kung *et al.*, 2003; McDonald *et al.*, 1991).

Whereas the AMM treatment tended to reduce lactic acid and increase acetic acid concentrations in the chopped silage that had undergone a successful lactic acid dominant fermentation, it had a marked beneficial effect on inhibiting a secondary fermentation in the unchopped silage. However, it increased acetic acid concentration in both types of silage, with this effect occurring after the first 2 days of ensilage. In the unchopped herbage, where the challenge from undesirable microorganisms appeared greater, there were reduced concentrations of butyric acid, ethanol and ammonia-N indicating the restriction of clostridial and possibly yeast activity. Hexamethylene tetra-amine, the main component of this additive, liberates formaldehyde under acid conditions, and the latter restricts microbial activity (Woolford, 1975a) and proteolysis (Kung *et al.*, 2003), particularly under acid conditions. Sodium nitrite and sodium benzoate, the other components of this additive, have inhibitory effects on Clostridia and yeast, respectively (Kung *et al.*, 2003). Furthermore, the elevated concentration of acetic acid due to the AMM additive treatment could also have inhibited yeast activity (Woolford, 1978; Danner *et al.*, 2003).

Silage aerobic stability

The poorer aerobic stability of the chopped herbage disagrees with previous work by McEniry *et al.* (unpublished data), but could be explained by the effects of

a more restricted secondary fermentation (Woolford, 1978) compared to the unchopped silages.

Due to the poorer aerobic stability of the chopped silages, together with their better preservation, these silages provided the more sensitive test of the effects of the additives on silage aerobic stability. The more lactic acid dominant fermentations of the LAB and the LAB + sucrose treated silages may have permitted greater proliferation of yeast on exposure to air and thus resulted in the poorer silage aerobic stability observed (Crawshaw, Thorne and Llewelyn, 1980; Kung *et al.*, 2003). Filya *et al.* (2000) reported that high concentrations of lactic acid, associated with dominant homofermentative inoculant treatments, could serve as a substrate for lactate-assimilating yeast on exposure to air.

The reason for the beneficial effect of sucrose on silage aerobic stability was not evident. The beneficial effect of the formic acid based additive on aerobic stability was a combined effect of both formic acid (Crawshaw *et al.*, 1980) and ammonia (Britt and Huber, 1975) reducing the deterioration caused by aerobic bacteria, yeast and mould, and is in accord with O'Kiely *et al.* (2005). The antifungal properties of sodium propionate and sodium benzoate (Woolford, 1975b; Kung *et al.*, 2003), components of the AMM additive treatment, resulted in a silage exhibiting increased aerobic stability. In addition, the AMM treated silage had the highest concentration of acetic acid, which can also inhibit yeast and mould growth (Filya *et al.*, 2000).

Losses during ensilage

As no signs of excessive respiration (e.g. mould growth) were evident and no effluent was produced, fresh weight loss was largely reflective of the efficiency

and extent of fermentation. The scale of fresh weight loss was higher than that reported by McEniry *et al.* (2007) for the unchopped herbage, while lower values for the chopped herbage reflected its more dominant lactic acid fermentation. However, in all cases the results demonstrate the relatively small scale of losses that emanate from fermentation where respiration, effluent and physical losses are curtailed. Overall, the scale of effects of additives on in-silo losses, although statistically significant, was of little practical importance.

Conclusions

The generally poorer fermentation in the unchopped compared to the chopped silage suggests that when similar grass is ensiled, at similar DM concentrations, as conventional unchopped baled or precision-chop silage, that there is a greater requirement for the fermentation to be assisted with the baled silage system. This could be achieved by more extensive wilting (Marsh, 1979; Dawson *et al.*, 1999) and/or by evenly applying adequate effective additives. Under such conditions, contrasting additives such as the LAB + sucrose, the formic acid based and the AMM additives could assist fermentation in baled silage, giving preservation approaching that of precision-chop silages.

A negative aspect from the use of the LAB based additives was the reduced aerobic stability of the resultant silage. This highlights the importance of maintaining adequate anaerobic conditions in the inoculant treated silage during storage. Poorer aerobic stability at feedout should not generally pose a major problem with baled silage in farm practice, as the bales would be consumed on most farms within 1 to 3 days of initial exposure to air. However, if assistance is required to improve aerobic stability, the AMM and

the formic acid based additives were the most effective.

Further studies are required to establish whether similar responses for these additives can be obtained across a range of DM concentrations as with baled silage on Irish farms.

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