

**End of Project Report**

**Reducing The Cost Of Beef Production By  
Increasing Silage Intake**

Grange Research Centre

Project No. 4622

Beef Production Series No. 51

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## **Reducing The Cost of Beef Production by Increasing Silage Intake**

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## 1. INTRODUCTION

Silage is an integral part of most milk and meat production farming systems in northern and north-western Europe. Besides silage being an important feedstuff for cattle, especially when they are housed indoors, silage production is often integrated into farming systems in a manner that also makes an essential positive contribution to both effective grassland management and internal parasite control with grazing animals. Furthermore, optimal recycling of nutrients collected from housed livestock within individual farms can often be best achieved by spreading the manures on the grassland used for producing silage.

Grass silage is potentially an expensive feedstuff. Other options such as alternative forages to ensile, concentrate feedstuffs and extending the grass-grazing season must be considered. Options such as these could require fundamental changes to the farming system, which may or may not be worthwhile. However, from a business perspective, we must periodically question how optimal are our current milk and beef production farming systems, the role of grass silage within these systems and the profitability of the technologies we currently employ for producing and feeding grass silage.

Grass silage must support the predictable, consistent and profitable production of quality animal produce within environmentally sustainable farming systems. This can be quite a challenge for a crop that is so strongly influenced by the prevailing variable weather conditions, and the many interactions of the latter with farm management practices. Research and scientific progress must therefore continue to provide improved technologies if grass silage is to fulfil the above requirements.

Yield, quality (including effects on intake, feed conversion efficiency, growth, meat quality, etc.), conservation losses, inputs and eligibility for EU financial supports determine the cost of providing cattle with silage, and this can have a major impact on the cost of producing milk or beef. Consequently, there has been an emphasis in the research reported here to add new information to the existing framework of knowledge on these cost determining factors so as to improve our ability to fulfil the role for silage stated above.

## 2. CHARACTERISTICS OF SILAGE ON IRISH FARMS – A SURVEY

Grass silage continues to be a hugely important feedstuff for the Irish cattle industry. It is the main forage available during the winter, and is an integral part of the successful operation of current grassland-based beef and dairy systems. Its integration into overall grassland management helps farmers manipulate the supply and condition the quality (i.e. digestibility) of grazed grass so as to meet the ongoing nutritional requirements of grazing cattle. It simultaneously helps reduce the challenge to grazing cattle from internal parasites. With an annualised cost of making, storing and feeding silage of over €400 million per year, it is important to know the characteristics and trends of this aspect of farming.

**Survey.** The Farm Management Survey is carried out each year by Teagasc. The data presented in this part of the report are based on a survey conducted during the autumn-winter period of 1999 by the National Farm Survey unit in Teagasc. The sample of 1028 farms are considered representative of a population of 123,400 farms throughout Ireland. These farms conform to the standard European Union definition of being bigger than two economic size units (ESU<sup>s</sup>). Other concurrent information from the Farm Management Survey which relates to baled silage (and to other silage-making machinery systems) is reported in Beef Production Series No. 50.

**Scale of silage.** Although first-cut silage accounts for over 70% of the total area harvested for silage (Table 1), this ranges from an average of 66% for dairy farms to 79 and 83% for beef and sheep farms, respectively. These values indicate the greater role of second or subsequent silage cuts on dairy farms compared to other farms. Correspondingly, individual dairy farms harvest larger total areas of silage (18.8ha) than beef (8.1ha) or sheep (5.8ha) farms.

First cut silage accounts for a higher proportion of all silage on smaller sized farms compared to farms with a larger area, indicating the greater role for second or subsequent cuts on larger sized farms.

**Table 1.** Scale and characteristics of silage-making within different farming enterprises on Irish farms

	Dairying	Dairying/ cattle	Cattle rearing	Cattle fattening	Mainly sheep	Tillage	All systems
Total no. of farms (farmers)	21600	15400	28100	34300	17800	6200	123400
No. of farms making silage	21500	15100	25100	28300	13500	3200	106700
- % farms making silage	99	98	89	83	76	52	86
Area of all farms (ha)	787500	706400	675900	908300	653800	367000	4098800
Area of farms making silage (ha)	784300	698000	631500	804000	505800	201100	3624700
Area of silage (ha)							
- first cut	262930	187374	138851	199838	65722	28086	882760
- total	411291	277013	171833	260138	78713	40267	1239255
Average area of silage (ha/farm)							
- first cut	12.3	12.4	5.5	7.0	4.9	8.7	8.3
- total	19.2	18.3	6.9	9.2	5.8	12.5	11.6
Ryegrass dominant swards for silage							
- % area	43	43	20	33	26	36	36

**Silage additives.** Although silage additives can generate considerable media coverage, their use by farmers is less widespread. The survey indicates that virtually no additives are used in making baled silage in Ireland (less than 0.5% of the area of baled silage was treated with an additive). Consequently, where additives are used, it is with single-, double- and precision-chop harvester systems (i.e. conventional silage systems). However, almost three-quarters of such conventional silage is currently made without any additive being applied (Table 2). The mean proportion not treated with additive is larger on beef (86%) and sheep (80%) farms compared to dairy (68%) farms. It would be expected that in individual years weather conditions prevailing around silage harvesting time could impact on the extent to which silage additives are used.

The data indicate that within the silage additive market, acid additives, molasses and biological additives account for 30, 12 and 58% of the area of silage treated with an additive. Farming enterprise has an important influence on the type of additive used, with dairy, beef and sheep farms that use additives preferring biological additives, acid additives and molasses, respectively. Similarly, farm size had an important impact on silage additive use. For the farm size categories <20ha, 20 to <100ha and equal to or above 100ha, the proportion of the silage area ensiled without additive was 89, 85 and 70% respectively. The corresponding proportions of biological silage additives were 2, 8 and 16% of the silage area. The use of other additives was less clearly related to farm size.

**Table 2.** Additive type used for making silage in the main farming enterprises

	<b>Dairying</b>	<b>Beef</b>	<b>Sheep</b>	<b>All systems</b>
% silage area treated with additive				
- none	77	92	92	84
- formic	3	1	0	2
- sulphuric	1	2	0	1
- molasses	4	4	6	4
- biological	14	1	2	8
- other	1	0	0	1
% conventional <sup>1</sup> silage area treated with additive				
- none	68	86	80	73
- formic	6	4	0	5
- sulphuric	2	6	0	3
- molasses	2	3	20	3
- biological	21	2	0	15
- other	0	0	0	0

<sup>1</sup>Single-, double - and precision-chop combined

**Ryegrass.** Although ryegrass is recognised as offering distinct potential yield, nutritive value and ensilability advantages in grass silage production, only 36% of the area harvested for silage are swards dominated by ryegrass (Table 1). This proportion varies to only a relatively modest degree with farm enterprise. Farms that make conventional silage only tend to have a greater proportion of their silage land (44%) under ryegrass dominant swards whereas the ryegrass proportion is less than half of this on farms making only baled silage. Larger sized farms tended to have a greater proportion of their silage land under ryegrass dominant swards (56% of the silage area on farms  $\geq$  100ha) compared to the situation on smaller sized farms (26% and 37% for farm sizes <20ha and 20 to <100ha, respectively).

**Trends.** Economic and social influences together with technological and market opportunities combine to maintain a state of change in our ruminant production systems. This in turn is reflected in changes in the role of silage and in the associated technologies and practices underpinning it.

**Table 3.** Trends in silage making, harvester type and additive use

	<b>1991-92</b>	<b>1996</b>	<b>1999</b>
Average total silage area (ha/farm)	11.0	11.3	11.6
- % first cut	-	72	71
Silage additive (% silage area)			
- none	58	73	84
- formic acid	4	3	2
- sulphuric acid	10	3	1
- molasses	20	8	4
- biological	8	12	8

**Table 4.** Trends in the proportion (%) of farms within the main farming enterprises that make silage

	<b>1991/92</b>	<b>1996</b>	<b>1999</b>
Dairying	91	97	99
Beef	52	78	86
Sheep	50	71	76
<b>All systems</b>	<b>65</b>	<b>82</b>	<b>86</b>

The survey indicates the total national area harvested for grass silage appears to have increased marginally between 1996 and 1999 from 1.18 to 1.24 million ha. On individual farms the average area harvested for all silage has also increased marginally (Table 3). Whereas virtually all dairy farmers have been making grass silage throughout the past decade, the proportion of beef and sheep farmers making silage, although lower, has been increasing steadily (Table 4). Simultaneously, the number of farms with dairying as the main enterprise has decreased while the numbers in beef has increased slightly.

Since 1996, first cut silage appears to have stabilised at around 70% of the total area of silage (Table 3). The proportion of silage made without an additive being applied has increased steadily during the past decade. Possible explanations for this trend include the expansion in baled silage based on wilted grass, a perceived lesser need for additives due to improved grass production and silage making practices, a conscious effort by farmers to cut input costs where appropriate (in some cases this is also associated with harvesting higher yields at a more advanced growth stage, where ensiling conditions are likely to be easier) and a reluctance by contractors to carry the additional costs of additives related to time, labour, insurance, applicators and machine maintenance and depreciation. Furthermore, despite the considerable investment in marketing the merits of individual silage additive brands, the technologies underpinning silage additives appear to have progressed relatively little during the past decade. Within the additive market, acid and sugar based additives have progressively decreased in significance since 1991/92. During the same time biological additives initially increased in importance but appear to have subsequently declined between 1996 and 1999.

Silage making practices will continue to change in responses to market forces. The substantial replacement of hay by baled silage that was evident in some years may be partially reversed if the costs of making baled silage continue to rise. This may be facilitated by EU farm extensification schemes and by the greater mechanisation feasible with big (round) bale hay now compared to heretofore.

**Acknowledgements.** The considerable input of the staff of the National Farm Survey in collecting and collating the data summarised in this paper is acknowledged.



### 3. IMPROVING GRASS YIELD, ESTIMATED NUTRITIVE VALUE AND ENSILABILITY

A series of experiments are reported in this section which studied factors that could influence grass yield, estimates of nutritive value and/or ensilability.

#### (a) Nitrogen fertiliser – form, rate and timing

##### *Experiment 1. Grass ensilability indices as affected by the form and rate of inorganic nitrogen fertiliser and the duration to harvesting*

Crops of grass in Ireland vary considerably in the ease with which they undergo a lactic acid dominant fermentation during ensilage. Factors such as prevailing weather conditions, grass species and cultivar, and the supply pattern and amount of nitrogen available to the crop interact through their effects on fermentable substrate, buffering capacity, microflora, temperature etc. to influence the direction of fermentation and therefore the final standard of preservation. In the present experiment, the influence of the rate and form of nitrogen fertiliser on indices of grass ensilability between April and June, was investigated.

**Materials and Methods.** Nine adjacent field plots (each 5 m x 3 m) were marked out in each of 4 replicate blocks in a *Lolium perenne* sward, and calcium ammonium nitrate (CAN; 27.5% N) or urea (46% N) applied at 0, 50, 100, 150 or 200 kg N/ha. Nitrogen fertiliser, as well as 35 kg P and 150 kg K/ha, were spread on 30 March, and the grass was sampled from each plot after 30, 37, 43, 51, 58 and 65 days. Samples were analysed for dry matter (DM), crude protein, nitrate and water soluble carbohydrate (WSC) concentrations, and buffering capacity.

**Results and Discussion.** There was no interaction between form of nitrogen and any of the variables examined. Increasing rates of application of N fertiliser (Table 5) linearly reduced grass dry matter (DM) concentration. Whereas grass DM concentration might be expected to increase as time progressed and the crop matured physiologically, changeable weather conditions (particularly rainfall) interfered with this, resulting in a variable pattern over time (Table 6). Crude protein concentration increased linearly with an increase in the rate of application of CAN and urea. This effect was most marked between 30 and 43 days after application and decreased rapidly thereafter (Table 2). The concentration of water soluble carbohydrates (WSC), whether expressed on a DM or aqueous phase basis, decreased as increasing rates of N were applied. Although neither rate nor form of N applied interacted significantly with the application to sampling interval, the effect of rate of N applied appeared most pronounced after 43 days. Buffering capacity of grass increased linearly with increasing rates of N application, and decreased with time after spreading, with no interaction between application rate and spreading to harvesting interval.

**Conclusion.** Increasing rates of application of CAN or urea to grass grown for first-cut silage similarly reduced grass DM and WSC concentrations and increased buffering capacity, thereby rendering the crop more difficult to preserve. Some of these effects did not decrease as much as expected over time, possibly because of the effects of changeable weather conditions.

**Table 5.** Main effects of rate and form of N fertiliser on grass ensilability indices.

	Rate (R) of N (kg/ha)					Form (F) of N			R x F SEM <sup>6</sup>
	50	100	150	200	SEM	CAN	Urea	SEM <sup>6</sup>	
Dry matter <sup>1</sup>	183	173	159	152	2.0***	167	166	1.4	2.9
Crude protein <sup>2</sup>	139	156	183	195	3.1***	166	171	2.2	4.4
WSC - DM <sup>2</sup>	107	100	93	88	3.6**	100	94	2.5	5.1
WSC - L <sup>3</sup>	24	21	18	16	1.0***	20	19	0.7	1.4
Refractometer <sup>4</sup>	8.0	7.6	6.9	6.7	0.17***	7.4	7.2	0.12	0.24
Buffer capacity <sup>5</sup>	232	258	278	280	4.7***	263	260	3.3	6.7

<sup>1</sup>g/kg, <sup>2</sup>g/kg DM, <sup>3</sup>g/kg juice, <sup>4</sup>refraction index, <sup>5</sup>mEq/kg DM, <sup>6</sup>not significant at P<0.05

**Table 6.** Main effects of interval between N application (March 30) and measurement of grass ensilability indices.

	Application to measurement interval (I) (days)						IxR	IxF	
	30	37	43	51	58	65			SEM
Dry matter	154	163	151	178	189	166	2.2***	4.5	3.2
Crude protein	203	194	187	166	132	127	2.9***	6.2**	4.4
WSC - DM	102	110	100	76	95	99	4.8***	9.4	6.7
WSC - L	19	22	18	16	22	20	1.1***	2.2	1.5
Refractometer	7.8	8.3	7.2	8.0	7.0	5.5	0.15***	0.32	0.23
Buffer.capacity	303	298	300	262	215	193	5.3***	10.7	7.6

### (b) Nitrogen fertiliser – split application, form and timing

Inorganic nitrogen (N) fertiliser is applied to grassland managed for silage production to ensure that economically viable yields are available for harvesting at a time when the feed value of the grass is still adequate. However, it can make the grass more difficult to preserve as silage by reducing dry matter (DM) and water soluble carbohydrate (WSC) concentrations and increasing buffering capacity. The effects of N fertiliser on either yield or ensilability depend on the weather conditions prevailing around the time of fertiliser spreading and between then and harvesting. Strategies for reducing the potential scale of any negative effects of weather include the judicious early spreading and split application of N fertiliser. The two experiments reported were conducted in consecutive years, and aimed to determine if there was a benefit to grass yield, estimated nutritive value or ensilability of (1) splitting the application of inorganic N fertiliser spread on grassland managed for first-cut silage, (2) altering the application date, and (3) altering the form of N fertiliser used.

#### *Experiment 2: Split application of nitrogen fertiliser on grassland managed for first-cut silage*

**Materials and methods.** Six replicate blocks of plots (10 m x 2.5 m plots) were marked out in a randomised complete block design in a *Lolium perenne* dominant sward. Urea (460 g N/kg) or calcium ammonium nitrate (CAN; 275 g N/kg) were manually applied at a rate equivalent to 120 kg N/ha. All of the N was spread on 18 February or 19 March (Early), or as a 2:1 or 1:2 split across these dates. Similar treatments were imposed using the dates 4 March and 1 April (Late). An additional treatment received no N fertiliser. All plots were harvested to a stubble height of 5 cm on 4 June. Grass yields, extent of lodging, ensilability and nutritive value were quantified.

**Results and discussion.** The mean response to 120 kg inorganic N/ha was to increase grass DM yields from 4.28 to 5.42 t/ha (Table 7). The mean DM yields where all the N fertiliser was applied on 18 February, 4 March, 19 March or 1 April were 5.22, 5.36, 5.43 and 5.70 t/ha. Thus, under the soil, sward, meteorological and management conditions prevailing, later application of the complete complement of N fertiliser was associated with a higher DM yield compared to earlier application. In general, splitting the application of N fertiliser gave similar DM yields (5.42 t/ha) to the mean of applying all the N in a single application (5.43 t/ha), and the effects of the proportions of the split were not significant. Urea (5.53 t DM/ha) was associated with a higher ( $P < 0.05$ ) yield than CAN (5.31 t DM/ha).

The application of N fertiliser in general resulted in a reduction in grass DM concentration (233 to 211 g/kg), with no main effect of the form in which fertiliser was applied. Grass DM concentration was higher when N fertiliser was applied by early March (217 g/kg) rather than after mid-March (204 g/kg), with split applications giving intermediate values. Grass crude protein concentrations were generally low, and increased from 104 to 125 g/kg DM in response to N fertiliser. There was no significant effect of form of N fertiliser, time of application or split application. Grass *in vitro* DM digestibility (DMD) was reduced from 752 to 727 g/kg when N fertiliser was applied, and there was no significant main effect of form of N fertiliser, time of application or split application. Values for organic matter digestibility (OMD) were similar to the DMD values. Ash and nitrate concentrations were generally low and were not significantly affected by treatments. Grass WSC concentration was

reduced by N fertiliser application (28 versus 23 g/l). Mean WSC concentrations where all the N fertiliser was applied on 18 February, 4 March, 19 March or 1 April were 23, 24, 20 and 21 g/l. Neither fertiliser type nor split application of fertiliser changed the grass WSC values significantly. Although grass buffering capacity increased from 335 to 374 mEq/kg DM in response to the application of N fertiliser, there was not a significant effect of fertiliser form, timing or splitting on buffering capacity. Inorganic N fertiliser increased the proportion of the crop that lodged (0.03 to 0.55), whereas neither the form of fertiliser nor split application effected lodging. However, lodging became more severe as the date of applying all of the N fertiliser became later (0.47, 0.49, 0.55 and 0.71).

**Conclusions.** Nitrogen fertiliser improved grass DM yields, but disimproved digestibility and ensilability indices. Under the prevailing soil, sward, meteorological and management conditions, split application of N fertiliser for first cut silage did not improve grass DM yields, estimated nutritive value or ensilability. Later application favoured higher yields and there was a small effect of the form of fertiliser.

**Table 7.** Grass yield, physical state and chemical composition.

Fertiliser (F)	None						CAN						Urea						Significance	SEM		
	Early			Late			Early			Late			Early			Late						
	120+0	80+40	40+80	0+120	120+0	80+40	40+80	0+120	120+0	80+40	40+80	0+120	120+0	80+40	40+80	0+120	F <sup>1</sup>	A <sup>1</sup>			X <sup>2</sup>	
Application split (A)	120+0	80+40	40+80	0+120	120+0	80+40	40+80	0+120	120+0	80+40	40+80	0+120	120+0	80+40	40+80	0+120	F <sup>1</sup>	A <sup>1</sup>	X <sup>2</sup>			
Yield (t DM/ha)	4.28	5.15	5.14	5.10	5.26	5.51	5.01	5.74	5.28	5.47	5.48	5.60	5.21	5.79	5.78	5.65	*	NS	****	0.069	0.196	
Dry matter (g/kg)	233	215	214	207	205	216	208	209	221	220	208	203	216	220	208	199	NS	NS	***	*	2.13	6.0
Crude protein(g/kg DM)	104	128	126	128	122	121	124	125	119	122	127	125	122	120	129	137	NS	NS	NS	*	1.6	4.4
Ash (g/kg DM)	67	61	57	59	60	59	58	60	61	56	57	59	58	55	58	59	NS	NS	NS	NS	0.7	2.0
DMD (g/kg)	752	732	719	730	713	727	731	734	743	729	734	719	714	724	721	732	NS	NS	NS	*	2.7	7.5
OMD (g/kg)	751	730	717	727	711	724	729	733	740	728	733	717	712	723	718	730	NS	NS	NS	NS	2.8	7.9
WSC (g/kg DM)	93	82	80	89	76	87	92	76	86	81	83	83	87	99	80	85	NS	NS	NS	NS	2.0	5.6
(g/l)	28	22	22	23	20	24	24	20	25	23	22	21	24	28	21	21	NS	NS	*	*	0.6	1.7
NO <sub>3</sub> (g/l)	0	125	141	129	71	12	163	88	159	143	53	100	148	43	37	89	NS	NS	NS	NS	19.5	53.5
Buffer capacity (mEq/kg DM)	335	387	372	381	374	369	373	384	370	368	377	372	364	363	375	387	NS	NS	NS	NS	3.5	9.7
Physical state																						
- proportion of crop lodged	.03	.41	.47	.70	.68	.51	.50	.60	.38	.53	.48	.83	.58	.47	.52	.73	NS	NS	***	****	0.032	0.088

<sup>1</sup>Main effects of treatment from ANOVA of 2 x 2 x 4 factorial design (i.e. 16 treatments, with "no N" treatment omitted); <sup>2</sup>Treatment effect where all (n=17) treatments included in one-

way ANOVA; DM = dry matter; DMD = in vitro DM digestibility; OMD = in vitro organic matter digestibility; WSC = water soluble carbohydrates.

Early = 18 February + 19 March; Late = 4 March + 1 April.

**Experiment 3: Split application of nitrogen fertiliser on grassland managed for first-cut silage**

**Materials and Methods.** Six replicate blocks of plots (10 m x 2.5 m plots) were marked out in a randomised complete block design in a *Lolium perenne* dominant sward. Urea (460 g N/kg) or calcium ammonium nitrate (CAN: 275 g N/kg) were manually applied at a rate equivalent to 120 kg N/ha. All of the N was spread on 4 March or 1 April (Early) or as a 2:1 or 1:2 split across these dates. Similar treatments were imposed using the dates 18 March and 15 April (Late). An additional treatment received no N fertiliser. Each plot received 375 g 0-7-30 (g N-P-K/kg) on 11 March, and was harvested to a stubble height of 5 cm on 25 May. Grass yields, extent of lodging, ensilability and nutritive value were quantified. Data were subjected to analysis of variance for randomised complete block (17 treatments) and for 2x2x4 factorial with replicate blocks (zero N treatment omitted) designs.

**Results and Discussion.** The mean response to 120 kg inorganic N was to significantly increase ( $P < 0.01$ ) herbage DM yield from 2.57 to 5.91 t/ha, crude protein concentration from 99 to 145 g/kg DM, ash concentrations from 70 to 83 g/kg DM and buffering capacity from 307 to 429 m Eq/kg DM, while simultaneously reducing ( $P < 0.01$ ) the concentration of DM from 246 to 181 g/kg and WSC from 46 to 24 g/l (140 to 107g/kg DM), and the DM digestibility (DMD) from 839 to 798 g/kg. Although the effects of CAN and urea on DM, ash, DMD, organic matter digestibility (OMD), buffering capacity and WSC did not differ significantly ( $P > 0.05$ ), CAN was associated with a higher ( $P < 0.001$ ) DM yield (6.19 t/ha) than urea (5.63 t/ha). The mean DM yields where all the N fertiliser was applied on 4 March, 18 March, 1 April or 15 April were 6.36, 6.03, 5.56 and 4.92 (s.e. 0.160) t/ha, respectively, indicating (Table 8) that earlier application improved the yield response to N. In general, splitting the application of N fertiliser improved ( $P < 0.01$ ) DM yields from 5.72 to 6.10 (s.e. 0.080) t/ha, and the effects of the proportions of the split were not significant ( $P > 0.05$ ). The mean DMD values when all the N was applied on the above 4 dates were 786, 789, 805 and 816 (s.e. 4.5) g/kg, the buffering capacities were 396, 417, 450 and 453 (s.e. 8.6) mEq/kg DM and the WSC concentrations 27, 23, 23 and 22 (s.e. 1.0) g/l. Splitting the application of N fertiliser did not alter DMD, buffering capacity or WSC concentration ( $P > 0.05$ ) and the effects of the proportions of the split were not significant ( $p > 0.05$ ).

**Conclusions.** Nitrogen fertiliser improved herbage DM yield and crude protein concentration, but disimproved indices of digestibility and ensilability. Under the prevailing conditions, split application of N fertiliser increased DM yields by proportionately 0.07, but did not alter indices of digestibility or ensilability. Earlier applications of N favoured higher yields and better ensilabilities but lower digestibilities. CAN improved DM yield compared to urea, but both N sources had similar effects on digestibility and ensilability.

**Table 8.** Timing and splitting of N fertiliser application and their effects on herbage yield, nutritive value and ensilability.

Time of application (T)	Early				Late				s.e.			Significance		
	120 + 0	80 + 40	40 + 80	0 + 120	120 + 0	80 + 40	40 + 80	0 + 120	0 + 80	80 + 120	TxA	T	A	TxA
Application split (A)	120 + 0	80 + 40	40 + 80	0 + 120	120 + 0	80 + 40	40 + 80	0 + 120	0 + 80	80 + 120	TxA	T	A	TxA
Yield (t DM/ha)	6.36	6.35	6.18	5.56	6.03	6.16	5.72	4.92	4.92	4.92	0.160	***	***	NS
Dry matter (g/kg)	196	183	180	178	180	179	176	174	174	174	3.2	**	**	NS
Crude protein (g/kgDM)	130	133	149	158	143	147	144	160	160	160	3.9	*	***	*
Ash (g/kg DM)	77	82	85	86	83	83	85	86	86	86	1.3	NS	***	NS
DMD (g/kg)	786	787	798	805	789	803	802	816	816	816	4.5	**	***	NS
OMD (g/kg)	778	777	790	797	780	794	792	807	807	807	4.8	*	***	NS
WSC (g/l)	27	24	23	23	23	24	22	22	22	22	1.0	NS	*	NS
(g/kgDM)	110	106	104	106	107	111	107	105	105	105	4.6	NS	NS	NS
Buffering capacity (mEq/kg DM)	396	411	436	450	417	432	437	453	453	453	8.6	NS	***	NS

### (c) Phosphorous fertiliser – rate of application

#### *Experiment 4: Silage conservation characteristics of grass that received a range of rates of phosphorus fertiliser*

This research was conducted in collaboration with Dr. Hubert Tunney and co-workers from Teagasc, Johnstown Castle, Co. Wexford

Phosphorous (P) loss to water is the major contributor to the growing problem of eutrophication in Irish lakes. Preliminary calculations indicated that the loss of P/ha in an intensive grassland system may exceed by a significant margin the quantity inland lakes could accept each year, if eutrophication is to be avoided. Whereas certain minimum quantities of P are necessary to support good grass growth rates, there is no benefit of excessively high rates. Grassland from which silage is harvested often has considerable quantities of slurry applied, in addition to inorganic fertiliser P, frequently leading to high P concentrations in soil. There is very little information whether such grass crops, in turn, can be difficult to preserve properly when ensiled. The present experiment determined if a range of rates of P applied to grassland over several years affected its conservation characteristics.

**Materials and Methods.** Grass was collected from plots (15 m x 4 m) that had received 0, 20, 30, 40 and 50 kg P/ha, each October for the previous seven years. Samples were used from 4 of the 5 replicates of each treatment, at each of three sites (Oak Park, Co. Carlow - October 3; Clonroche, Co. Wexford - October 4; Johnstown Castle, Co. Wexford - October 5). In each case, samples were precision-chopped the following day and ensiled (6 kg grass/silo) in laboratory silos for 100 days.

**Results.** Grasses were dry, and crude protein concentrations were relatively high, as is normal with autumn grass. The moderate digestibilities reflect the presence of stemmy vegetation, with some dead material at the base of the crop. Grass buffering capacities were high, reflecting, at least in part, the high crude protein values. The mean grass composition varied between sites.

Averaged across sites (Table 9), as the annual rate of P application increased, grass WSC concentrations increased, while buffering capacity, nitrate concentrations and *in vitro* DMD were not altered. The increasing P rates did not alter silage crude protein concentration or aerobic stability, but reduced lactic acid concentration, and increased pH and silage DM recovery. No silage effluent was produced, due to the high DM concentrations of the grasses. Extracted grass juice dry matter concentration was estimated following oven drying or the use of a refractometer. The correlation ( $R^2$ ) between both sets of values was 0.99, with a linear regression equation of  $y = 0.832x + 8.35$  for  $y =$  oven DM (g/kg) and  $x =$  refractometer DM (g/kg). Phosphorous fertiliser application resulted in elevated concentrations of P in silage juice, but the dose response was weak. The ratio of P:N in the extracted silage juice was approximately 1:5, compared to 1:10 in the grass DM.

**Overall**, however, phosphorous fertiliser application did not make grass more difficult to preserve, it appeared to restrict fermentation and in-silo losses and increase the P concentration in the aqueous extract of silage. The P in the extracted silage juice averaged 730 mg/l, or 20000 times higher than the level of 0.035 mg/l considered limiting for eutrophication. Silage effluent could be an important source of P loss to water if not managed properly.

**Table 9. Effects of fertiliser P inputs on the silage conservation characteristics of grass**

	kg P/ha/year					SEM	Sig
	0	20	30	40	50		
<b>Grass composition</b>							
Dry matter (g/kg)	214	213	221	218	220	1.8	*
DMD (g/kg)	680	676	679	680	685	4.5	NS
pH	6.47	6.22	6.03	6.18	6.22	0.062	**
WSC (g/kg DM)	60	63	63	74	70	3.0	*
(g/l)	16	17	19	21	20	0.9	*
Buffer. cap (mEq/kg DM)	534	518	514	512	518	5.5	NS
NO <sub>3</sub> (mg/l)	313	307	455	452	381	54.4	NS
<b>Silage composition</b>							
Dry matter (g/kg)	203	208	214	208	213	1.0	***
Crude protein (g/kg DM)	193	191	190	193	191	1.2	NS
NH <sub>3</sub> -N (g/kg N)	81	72	81	81	86	1.1	***
pH	3.99	4.03	4.10	4.09	4.07	0.014	***
Lactic acid (g/kg DM)	125	108	105	96	108	1.4	***
Acetic acid (g/kg DM)	35	30	32	37	31	1.2	**
Propionic acid (g/kg DM)	1.6	0.9	1.7	1.9	1.7	0.09	***
Butyric acid (g/kg DM)	0.09	0.06	0.15	0.11	0.07	0.057	NS
Ethanol (g/kg DM)	12	12	13	15	12	0.4	***
<b>Silage losses</b>							
DM recovery (g/kg)	937	967	957	945	956	4.5	**
Juice oven DM (g/kg)	91	93	96	94	94	0.6	***
Juice refractometer DM (g/kg)	99	102	104	104	102	0.8	**
Juice N (g/kg)	4.0	4.1	4.0	3.5	3.4	0.11	**
Juice P (g/kg)	0.62	0.77	0.76	0.70	0.68	0.023	**
<b>Aerobic stability<sup>1</sup></b>							
Days to pH rise	3.1	1.0	2.5	3.0	3.0	0.50	
Days to pH max.	7.5	7.5	7.5	8.0	8.0	0.37	
Max. pH rise	4.9	4.9	4.8	4.4	4.8	0.17	
Days to °C rise	2.5	2.0	2.0	2.0	2.0	0.22	
Days to °C max	6.5	5.0	6.0	5.0	7.5	0.55	
Max. °C rise	37	35	33	28	31	2.5	
Acc. °C rise to day 5	57	90	72	70	46	5.5	*

<sup>1</sup>Samples from Johnstown Castle only**(d) Radio-frequency electromagnetic-field treatment*****Experiment 5: Quantitative and qualitative effects of radio frequency electromagnetic field-treated water on newly sown *Lolium perenne****

A prototype device emitting long-wave radiation, and when immersed in water inducing effects matching those derived from magnetic or electromagnetic stimuli, has been developed (Morse *et al.*, 1997). It is thought that electromagnetic modifications temporarily imprinted in the water result in a change in the hydrogen bonding structure and thus in some of the characteristics of the water. This experiment aimed to quantify the effects of applying such activated water to soil on the germination and growth of grass, to describe these effects in both quantitative and qualitative terms, and to identify an optimum application rate or regime.



**Materials and Methods.** Forty plastic gardening pots (23 cm diameter on top; 10 l capacity) wrapped in aluminium foil were filled to within 5 cm of the top with a weighed amount of a mixture of soil, “seed and potting compost” and fertiliser. On 15 May, 0.92 g of the tetraploid mid-season cultivar of *Lolium perenne*, Twins, was evenly spread on top of the seed bed in each pot, and then covered by a 0.5 cm layer of the soil/peat moss/fertiliser mixture. Pots were randomly allocated to the following treatments:

- 1) Untreated water applied at 165 ml per pot at the start, and weekly thereafter.
- 2) Activated water applied at 2 ml/pot plus untreated water at 163 ml/pot, at the start. Untreated water applied at 165 ml per plot at weekly intervals thereafter.
- 3) Activated water applied at 165 ml/pot at the start. Untreated water applied at 165 ml per pot at weekly intervals thereafter.
- 4) Activated water applied at 165 ml per pot at the start, and weekly thereafter.

Pots were positioned at least 0.5 m apart. They were stored outdoors on short grass in a relatively sheltered site. Water was applied to the soil surface in each pot. Two heavy-gauge, black plastic 300 l capacity troughs positioned 5 m apart, and more than 5 m from the pots, were used for storing the water applied to the pots. Both were filled with fresh (potable) water each week, with one being assigned to the radio frequency electromagnetic field treatment. The radio frequency signal was delivered with a helical resonator source (Morse *et al.*, 1997) which was used according to the suppliers instructions. It was immersed in the appropriate water trough for at least one hour before water was withdrawn. Twenty-two days after sowing the seed, an estimate was made of the rate of germination plus establishment of grass seed in each pot. Mean plant height above soil level was measured on a series of occasions. On 10 July, after 56 days growth, mean plant height and tiller density per pot was measured, grass in each pot was cut at the height of the top rim of the pot, and weighed, and sub-samples of grass were subjected to chemical analysis. Pots were then re-positioned in the storage area previously used. The treatments originally imposed were continued for a further 18 days (until 28 July), after which time herbage was again cut, weighed and had its DM concentration determined.

**Results.** The mean (sd) weight of soil in each pot was 7.67 (0.295) kg. Germination was gradual and appeared similar for each treatment. For the final four days before the primary growth was harvested on July 10, the grass in each of the 10 replicates of Treatment 4 appeared to lose turgor and wilted visibly. The results are summarised in Table 10.

**Conclusions.** Activated water did not appear to affect germination of grass seed. Treatment 4 increased primary growth dry matter (DM) yield, DM concentration and water soluble carbohydrate concentration (on aqueous phase basis only). It did not affect herbage *in vitro* DM digestibility. Some of the activated water treatments decreased the concentrations of K, Na, Ca and Mg.

**Table 10.** Grass yield, height, density and composition results.

	Treatment				SEM	Significance
	1	2	3	4		
<b><i>First growth (15 May to 10 July)</i></b>						
No. plants per pot						
- June 6	101	105	106	105	3.7	NS
Fresh yield (g/pot)	60.2	71.2	63.8	60.9	3.56	NS
Dry matter (DM) (g/kg)	230	232	233	281	4.4	***
DM yield (g/pot)	13.9	16.6	14.8	17.2	0.94	P=0.067
<i>in vitro</i> DMD <sup>2</sup> (g/kg)	846	844	843	842	5.4	NS
<i>in vitro</i> OMD <sup>3</sup> (g/kg)	841	837	838	834	5.5	NS
Ash (g/kg DM)	100	104	105	106	2.1	NS
Crude protein (g/kg DM)	128	133	132	134	3.0	NS
Nitrate (mg/l)	0	0	0	0	--	--
Buffering capacity (mEq/kg DM)	461	455	479	460	8.6	NS
WSC <sup>4</sup> (g/l)	52	56	52	67	2.4	***
(g/kg DM)	174	187	169	171	5.9	NS
K (mg/kg DM)	41.4	39.6	37.3	40.1	0.91	*
Na (mg/kg DM)	0.94	0.89	0.87	0.87	0.018	*
Mg (mg/kg DM)	0.52	0.55	0.44	0.49	0.023	*
Ca (mg/kg DM)	4.39	3.97	3.80	3.92	0.063	***
Mean grass height <sup>1</sup> (cm)						
- June 6	6.0	6.5	6.4	6.3	0.29	NS
- June 12	11.9	12.3	11.9	12.0	0.32	NS
- June 19	15.3	15.5	15.1	15.6	0.32	NS
- June 26	18.6	18.8	18.4	18.6	0.50	NS
- July 3	21.1	21.2	21.2	21.1	0.50	NS
- July 10	22.2	22.0	22.3	21.7	0.55	NS
<b><i>Second growth (10 July to 28 July)</i></b>						
No. plants per pot						
- July 28	104	105	105	104	4.1	NS
Fresh yield (g/pot)	20.1	21.6	21.2	24.8	1.58	NS
DM (g/kg)	312	312	311	309	3.8	NS
DM yield (g/pot)	6.2	6.7	6.6	7.7	0.46	NS
Mean grass height <sup>1</sup> (cm)						
- July 28	11.1	12.2	11.7	13.4	0.58	P=0.057
<b><i>First plus Second growth</i></b>						
DM yield (g/pot)	20.2	23.2	21.4	24.8	1.32	P=0.081

<sup>1</sup>Above soil level; <sup>2</sup>dry matter digestibility; <sup>3</sup>organic matter digestibility; <sup>4</sup>water soluble carbohydrates. NS = not significant; \* = P<0.05; \*\* = P<0.01; \*\*\* = P<0.001

### ***Experiment 6: Yield, digestibility and ensilability of grass from a sward subjected to a range of slurry treatments***

This research was conducted in collaboration with Dr. Owen Carton, Teagasc, Johnstown Castle, Co. Wexford.

Contrasting responses have been reported for plants to which radio frequency electro-magnetic field treated water has been applied. Experiment 1 above identified one treatment applied to newly sown grass seed which resulted in an increase in dry matter (DM) yield ( $P=0.067$ ), DM concentration ( $P<0.001$ ) and water soluble carbohydrate (WSC) concentration (g/l;  $P<0.001$ ). In contrast, Maher *et al.* (1999) conducted investigations with tomatoes, lettuce, impatiens and carrots grown in fertilised peat substrate under glasshouse conditions and obtained no crop response to treatment with such activated water. It has been suggested that the ability of plants to absorb nutrients from ionic solutions surrounding their roots may be increased where Vi-aqua-treated water is present. Were such the case, Vi-aqua treatment of cattle slurry applied to grassland managed for silage production might offer the opportunity to improve nutrient recovery, and in particular nitrogen (i.e. ammonium-N) recovery, from applied slurry, with consequent benefits to the growth, yield and possibly composition of the grass crop. This experiment was designed to (a) quantify the effects of Vi-aqua treatment of cattle slurry applied to silage ground on the yield, nutritive value and ensilability of the crop at harvest, (b) compare these effects over a series of rates of slurry application, and (c) relate these effects to those of inorganic nitrogen fertiliser.

**Materials and Methods.** A *Lolium perenne* dominant sward was mown to a stubble height of 5 cm on 1 June. Eleven plots (each 10 m x 3 m) were marked out in each of 6 replicate blocks, together with a 1 m wide fallow strip between every pair of plots. Plots received 26 kg P and 111 kg K/ha. The eleven treatments applied within each block on 5 June were negative control (no N fertiliser or slurry applied), cattle slurry at 15, 30 or 45 t/ha, Vi-aqua treated cattle slurry at 15, 30 or 45 t/ha and inorganic N fertiliser at 40, 80, 120 or 160 kg N/ha. Inorganic N fertiliser was applied as calcium ammonium nitrate (275 g N/kg). Agitated cattle slurry was stored in two plastic-lined tanks – one remained untreated while the other had the radio frequency signal delivered using a Vi-aqua helical resonator source submerged in the slurry. On 4 August, a Haldrup plot harvester was used to weigh and sample the crop.

**Results.** Application rates of inorganic N fertiliser equal to or greater than 80 kg N/ha increased ( $P<0.05$ ) grass DM yield and reduced ( $P<0.05$ ) DM concentration at harvesting (Table 11). All rates of applying inorganic N fertiliser reduced ( $P<0.05$ ) *in vitro* DM digestibility (DMD) and organic matter digestibility (OMD) but did not effect ( $P>0.05$ ) the concentrations of crude protein or WSC, or of most minerals. Untreated slurry applied at 30 or 45 t/ha increased ( $P<0.05$ ) DM yield, while the 45 t/ha rate reduced DM concentration and increased ash concentration ( $P<0.05$ ). Slurry did not significantly effect DMD, OMD, crude protein or WSC. It did increase the concentrations of P and K. Vi-aqua slurry applied at 45 t/ha increased ( $P<0.05$ ) DM yield, while the 30 and 45 t/ha rates reduced grass DM concentration and increased ash concentration ( $P<0.05$ ). Vi-aqua slurry did not affect DMD, OMD, crude protein, nitrates or WSC. Its effects on mineral concentrations were similar to those of untreated slurry.

**Conclusions.** Compared to untreated slurry and inorganic N fertiliser applied at a series of incremental rates to silage ground, comparable rates of Vi-aqua treatment of cattle slurry did not enhance the yield, nutritive value or ensilability of grass harvested for ensiling.

**Table 11.** Grass yield and chemical composition.

Fertiliser/slurry	None		Untreated slurry (t/ha)		VI-AQUA slurry (t/ha)		Inorganic N (kg/ha)				SEM	Sig.	
	15	30	45	15	30	45	40	80	120	160			
Yield (t/ha)													
- fresh	13.43	16.34	18.34	20.84	16.14	17.17	21.31	14.86	20.02	20.60	22.66	1.167	***
- dry matter	3.04	3.35	3.92	4.01	3.38	3.50	4.05	3.19	3.89	4.18	4.69	0.228	***
Composition													
Dry matter (g/kg)	226	210	216	195	214	208	192	220	198	205	209	5.9	**
Ash (g/kg DM)	101	105	105	109	102	107	109	99	105	103	100	1.7	***
Crude protein (g/kg DM)	135	129	131	134	136	132	138	128	137	136	139	3.9	NS
<i>in vitro</i> DMD (g/kg)	744	744	729	727	724	730	736	719	719	716	701	7.8	*
<i>in vitro</i> OMD (g/kg)	732	733	713	711	721	717	720	706	703	698	686	8.7	*
WSC (g/l)	27.3	24.6	22.2	21.2	24.9	23.6	22.3	26.0	20.9	21.9	22.3	1.6	NS
(g/kg DM)	92	93	80	87	91	89	93	92	85	86	84	4.4	NS
NO <sub>3</sub> (mg/l)	1	1	2	8	15	4	1	1	32	9	35	10.7	NS
Buffering capacity (mEq/kg DM)	339	330	307	325	332	330	340	340	366	351	342	9.5	*
PH	5.80	5.85	5.77	5.77	5.73	5.82	5.73	5.88	5.63	5.75	5.68	0.079	NS
P (g/kg DM)	2.7	2.9	3.0	3.3	2.8	3.1	3.1	2.6	2.7	2.7	2.6	0.09	***
K (g/kg DM)	31	33	34	36	32	35	36	30	31	32	30	0.83	***
Na (g/kg DM)	1.7	1.9	1.4	1.7	1.5	1.9	2.1	1.9	2.3	2.1	2.3	0.14	***
Ca (g/kg DM)	9.7	9.4	8.0	8.7	8.9	9.3	9.2	9.4	9.9	9.5	8.9	0.33	**
Mg (g/kg DM)	2.1	2.0	1.8	2.1	2.1	2.0	2.1	2.2	2.3	2.3	2.3	0.08	*

## 4. MANIPULATING GRASS FERMENTATION

### (a) Influence of grass nitrate concentration on silage fermentation

Grass ensilability is influenced by a number of characteristics of the herbage including dry matter (DM) and available water soluble carbohydrate (WSC) concentrations, buffering capacity and the numbers and types of epiphytic lactic acid bacteria and undesirable bacteria and yeast (O'Kiely and Muck, 1998). The buffering capacity of grass is related to its concentration of protein, organic acids and anions such as sulphates, phosphates and nitrates (McDonald et al., 1991; Muck et al., 1991). Although nitrate can make some contribution to buffering capacity, it is normally converted via nitrite to ammonia under the influence of the strongly reducing conditions prevailing in silage, and Weissbach (1996) has suggested that the nitrite component can have a strongly anti-clostridial effect and thus help prevent a deleterious secondary fermentation during ensilage. This contrasts with the commonly articulated view that high concentrations of nitrate in grass are correlated with poor ensilability and thus to silage being susceptible to a clostridial fermentation. The latter may be inadvertently due to other effects of nitrogen fertiliser application which besides increasing grass nitrate concentration, also increases buffering capacity and reduces the concentrations of DM and WSC. These two experiments tested the hypothesis that elevated concentrations of nitrate in grass at ensiling would not promote a clostridial fermentation.

This research was conducted in collaboration with Dr. Evelyn Doyle and co-workers from the Department of Industrial Microbiology at University College Dublin.

#### *Experiment 7: Grass ensilability in response to incremental concentrations of nitrate*

**Materials and Methods.** A crop of *Lolium perenne* that had not received nitrogenous fertiliser was finely-chopped and ensiled in laboratory silos (6 kg grass per silo; 4 silos per treatment) in early October. Potassium nitrate was carefully mixed with grass immediately prior to ensiling at 0 (none), 0.65 (low), 1.3 (medium) and 1.95 (high) g/kg grass. The laboratory silos were stored at approximately 15°C for over 100 days.

**Results.** The mean (s.d.) composition of the grass was DM 228 (2.5) g/kg, *in vitro* DM digestibility 667 (14.9) g/kg, ash 119 (2.2) g/kg DM, crude protein 122 (2.5) g/kg DM, buffering capacity 328

(9.9) mEq/kg DM and K 16.8 (0.38) g/kg DM. The achieved rates of addition of nitrate ( $\text{NO}_3^-$ ) were 0, 520, 1030 and 1550 mg/l grass aqueous phase. No effluent was produced in any of the silos. Silages made without  $\text{KNO}_3$  addition underwent a lactic acid dominant fermentation and were well preserved (Table 12). The application of  $\text{KNO}_3$  resulted in an increase ( $P < 0.05$ ) in silage DM concentration and DM recovery rate, and a decrease ( $P < 0.05$ ) in ammonia-N concentration and buffering capacity. The concentration of K, but not of nitrate, increased with increasing rates of addition of  $\text{KNO}_3$ , presumably indicating that much of the added nitrate was converted to another product/products. The medium and high rates of addition of  $\text{KNO}_3$  reduced ( $P < 0.001$ ) lactic acid concentration, but did not affect ( $P > 0.05$ ) the concentrations of acetic acid, propionic acid, butyric acid, ethanol or WSC.

### **Conclusions.**

- A negligible clostridial challenge to silage fermentation occurred - the evidence indicated that saccharolytic clostridia made no contribution (no butyric acid present) and proteolytic clostridia a relatively small contribution, if any (modest proportion of N present as  $\text{NH}_3\text{-N}$ ).
- Although incremental doses of  $\text{KNO}_3$  were calculated to increase the nitrate concentration of grass at ensiling by 0, 520, 1030 and 1550 mg/l, and this was paralleled by incrementally increasing concentrations of K in silage DM, concentrations of  $\text{NO}_3^-$  in silage were low and not significantly related to the rates applied. In the absence of effluent production, this suggests the added  $\text{NO}_3^-$  underwent a chemical change during ensilage. This nitrate did not appear to have been substantially converted to ammonia.
- Medium and high rates of  $\text{KNO}_3$  addition reduced lactic acid concentration, but did not alter the concentration of other fermentation products (e.g. acetic acid or ethanol) or residual WSC. This suggests that the activity of homofermentative lactic acid bacteria was preferentially restricted.
- The hypothesis that high concentrations of nitrate in grass at ensiling would promote a clostridial fermentation was rejected. However, under the prevailing conditions, although high concentrations of added nitrate did not cause bad preservation ( $\text{NH}_3\text{-N} > 100 \text{ g/kg N}$ ), they did create conditions where lactic acid did not dominate the fermentation products.

**Table 12.** Silage chemical composition after over 100 days ensilage.

	Rate of nitrate addition				SEM	Significance
	None	Low	Medium	High		
Dry matter (g/kg)	205	216	218	213	2.6	*
pH	3.93	4.03	4.05	3.98	0.033	NS
Lactic acid (g/kg DM) (L)	106	98	67	68	4.0	***
Acetic acid (g/kg DM) (A)	40	42	34	43	2.7	NS
Propionic acid (g/kg DM)	0.8	0.6	2.4	2.7	0.72	NS
Butyric acid (g/kg DM)	0	0	0	0	---	---
Total VFA (g/kg DM)	42	43	37	45	3.1	NS
Ethanol (g/kg DM) (E)	43	43	30	39	4.3	NS
L/(A+E)	1.27	1.15	1.12	0.84	0.12	NS
Fermentation acids (g/kg DM) (FA)	148	141	104	113	5.6	***
L/FA	0.72	0.69	0.65	0.60	0.019	**
WSC (g/kg DM)	14	14	16	13	1.1	NS
Ammonia-N (g/kg N)	78	68	64	64	2.4	**
Crude protein (g/kg DM)	137	144	142	149	2.8	NS
Nitrate (mg/l)	0	198	310	158	98.6	NS
K (g/kg DM)	23.5	25.5	26.7	28.7	0.53	***
Buffering capacity (mEq/kg DM)	700	621	606	630	15.6	**
DM recovery (g/kg)	881	931	942	917	12.0	*

***Experiment 8: Grass nitrate concentration and its influence on silage fermentation***

**Materials and Methods.** Leafy autumn regrowth of a permanent grassland sward was precision-chop harvested and ensiled in early November either (a) with conventional good management (i.e. easy-to-preserve grass) or (b) after being treated with dirty water six days pre harvest, shaded beneath a polythene covered canopy for three days pre harvest and having the filling and sealing of the silos delayed for 24 h after nitrate treatments were imposed (i.e. difficult-to-preserve grass). For both

grass types, 6 kg grass (excluding additive) were ensiled in each of four laboratory silos for each of the following rates of nitrate addition: none, low (0.65 g KNO<sub>3</sub>/kg grass), medium (1.31gKNO<sub>3</sub>/kg) and high (1.96 gKNO<sub>3</sub>/kg). Silos were stored at approximately 15°C for 273 days, with effluent being collected, and its pH measured, on 18 occasions between days 1 and 273. Silage DM recovery and chemical composition were quantified, while aerobic stability was assessed only on the easy-to-preserve grass. Data were considered as a 2 X 4 factorial arrangement of treatments and subjected to two-way analysis of variance, except for aerobic stability where the completely randomised design was subjected to one-way analysis of variance.

**Results and Discussion.** The mean (standard deviation (s.d.)) composition of the clean grass at harvesting was DM 149 (3.4) g/kg, pH 5.8 (0.08), crude protein 139 (12.9) g/kg DM, DM digestibility (DMD) 677 (12.2) g/kg, organic matter digestibility 689 (9.8) g/kg, ash 144 (10.5) g/kgDM, water soluble carbohydrates (WSC) 17 (1.3) g/l, nitrates 175 (82.2) mg/l and buffering capacity 269 (19.1) mEq/kgDM. The corresponding values for the contaminated grass were 143 (5.0) g/kg, 6.03 (0.15), 140 (5.0) g/kg DM, 604 (11.8) g/kg, 654 (8.1) g/kg, 213 (7.5) g/kg DM, 12 (1.1) g/l, 50 (0) mg/l and 283 (11.6) mEq/kg DM. The initial rate of forage pH decline (obtained using daily effluent samples) was faster ( $P < 0.001$ ) for clean than contaminated forage, and when additional nitrate was not applied compared to when it was applied. Total effluent production was not altered ( $P > 0.05$ ) by nitrate addition. Table 13 indicates that clean grass underwent ( $P < 0.001$ ) a more lactic acid dominant fermentation than contaminated grass. Elevated rates of nitrate addition to clean grass reduced ( $P < 0.05$ ) silage pH and increased ( $P < 0.05$ ) lactic acid and nitrate concentrations, and buffering capacity. In contrast, elevated rates of nitrate addition to contaminated grass resulted in elevated ( $P < 0.05$ ) silage pH and buffering capacity, and a reduced ( $P < 0.0$ ) concentration of lactic acid. Nitrate addition reduced aerobic deterioration of silage made from easy-to-preserve grass.

**Conclusions.** Increasing the nitrate concentration of grass at ensiling had differential effects on fermentation depending on grass ensilability. With an easy-to-ensile grass it encouraged a more extensive lactic acid fermentation whereas with difficult-to-ensile grass it resulted in a reduced content of lactic acid and evidence of increased clostridial activity.



**Table 13.** Influence of grass type and rate of nitrate addition on silage composition.

Grass type	Nitrate rate	pH	NH <sub>3</sub> -N (g/kgN)	Lactic acid (g/kg DM)	Acetic acid (g/kg DM)	Propionic acid (g/kg DM)	Butyric acid (g/kg DM)	B.capacity (mEq/kg DM)	Nitrate (mg/l)
Clean	None	3.95	87	86	55	5.6	0.9	653	<1
	Low	4.05	90	74	59	7.1	1.3	643	8
	Medium	3.83	85	125	37	3.9	1.9	757	473
Contaminated	High	3.65	77	129	37	3.7	<0.1	798	876
	None	4.20	111	59	51	7.8	6.5	626	<1
	Low	4.70	210	16	53	8.0	17.1	690	<1
	Medium	4.65	147	15	71	9.5	6.2	720	<1
	High	4.73	158	12	63	8.5	6.3	741	<1
Significance	Grass	***	***	***	***	***	***	NS	***
	(G)								
	Nitrate	*	NS	*	NS	NS	NS	***	***
	(N)								
	G x N	***	NS	***	***	*	NS	NS	***
<sup>1</sup> s.e.		0.077	19.3	10.2	4.7	0.80	2.39	31.4	22.3

<sup>1</sup>G x N interaction; error df = 24

## (b) Manipulating fermentation by wilting and use of contrasting additives

### *Experiment 9: Conservation of unwilted and wilted grass treated with different additives and ensiled in laboratory silos*

Farmers should invest in silage additives if doing so improves their profits. Thus, the additives must result in adequate quantitative or qualitative improvements in conservation efficiency, animal productivity or both. The opportunities for different types of additives to improve conservation efficiency may vary with ensiling conditions. Wet crops may be difficult to preserve properly, may ferment extensively and may undergo sizeable losses via effluent (O'Kiely and Muck, 1998). Wilting restricts the extent of fermentation, but may also restrict the opportunities for some additives to markedly improve conservation characteristics.

The objective was to quantify the relative effects of specific additives on the conservation characteristics of unwilted and wilted grass ensiled in laboratory silos.

**Materials and methods.** The primary growth of a *Lolium perenne* dominant sward was precision-chop harvested unwilted or after 24 hours wilting. Representative samples of unwilted (6 kg) and wilted (5 kg) grass were allocated among six additive treatments and ensiled in laboratory silos (four per treatment). Additive treatments were (1) no additive, (2) formic acid (850 g kg<sup>-1</sup>; 3 ml kg<sup>-1</sup> grass), (3) ammonium tetraformate (Add SafeR, Trouw Nutrition Ltd.; 4 ml kg<sup>-1</sup> grass), (4) ammonium tetraformate (GrasAAT; Norsk Hydro ASA; 4 ml kg<sup>-1</sup> grass), (5) a mixture of formate, sulphite and benzoate (Norsk Hydro ASA; 3 ml kg<sup>-1</sup> grass) and (6) *Lactobacillus plantarum* (Ecosyl, Zeneca Bioproducts and Fine Chemicals Ltd; 3 ml kg<sup>-1</sup> grass). The laboratory silos were stored for over 100 days at about 15°C.

**Results and discussion.** *Grass composition.* The mean (s.d.) composition of unwilted grass was DM 163 (14.0) g kg<sup>-1</sup>, ash 98 (4.7) g kg<sup>-1</sup> DM, *in vitro* DM digestibility (IVDMD) 788 (4.0) g kg<sup>-1</sup>, water soluble carbohydrates (WSC) 14 (1.4) g l<sup>-1</sup>, buffering capacity 577 (23.0) mEq. kg<sup>-1</sup> DM and nitrates 310 (39.5) g l<sup>-1</sup>. The corresponding values for wilted grass were 259 (10.9) g kg<sup>-1</sup>, 95 (2.7) g kg<sup>-1</sup> DM, 807 (21.3) g kg<sup>-1</sup>, 34 (2.9) g l<sup>-1</sup>, 533 (9.6) mEq. kg<sup>-1</sup> DM and 340 (56.2) g l<sup>-1</sup>. The low concentration of WSC and high buffering capacity of unwilted grass indicate that it was difficult to preserve whereas the higher DM and WSC concentrations in the wilted grass suggest an easier crop to preserve.

*Silage composition.* Unwilted silage made without an additive preserved badly, having high concentrations of acetic acid and ammonia-N and a low concentration of lactic acid (Table 14). All the additives improved these fermentation characteristics, but the effects were most marked with the four additives containing formic acid/formate. Wilting resulted in a lactic acid dominant fermentation. The effects of additives were less marked for wilted compared to unwilted silage. All additives applied to wilted grass reduced the concentrations of both acetic and propionic acids and increased the concentration of water soluble carbohydrates in silage.

*Conservation losses.* Only unwilted grass produced effluent, but the quantities involved were not effected by additive treatment. However, formic acid and the mixture of formate, sulphite and benzoate increased effluent dry matter concentration. Dry matter recovery rates from the silo were improved by wilting. The positive effects of additives were greater with unwilted silages, where the four products containing formate supported the largest increases. Silage was generally stable under aerobic conditions, with most additives having relatively minor effects. However, the mixture of formate, sulphite and benzoate applied to unwilted forage, and in particular the inoculant of lactic acid bacteria applied to wilted forage, were associated with greater aerobic instability.

**Conclusions.** The four additives containing formic acid/formate were more effective at improving the preservation and in-silo DM recovery of unwilted grass than the *L. plantarum* additive. The effects of additives on conservation characteristics were generally smaller with wilted than unwilted forages. *L. plantarum* improved the fermentation of wilted grass more than other additives, but produced an aerobically less stable silage.

**Table 14.** Silage composition and conservation characteristics - individual treatment effects.

	Unwilted					Wilted					SEM	Sig.		
	NA	FA	AS	GA	FSB	LP	NA	FA	AS	GA			FSB	LP
<b>Silage composition</b>														
Dry matter (g/kg)	138	157	157	153	157	146	222	228	233	231	230	232	1.7	***
Crude protein (g/kg DM)	174	172	179	180	166	176	164	159	161	165	155	154	1.7	**
<i>In vitro</i> DMD (g/kg)	689	753	757	735	759	717	748	773	777	772	782	772	8.5	NS
pH	4.60	3.78	3.83	3.83	3.93	4.25	3.88	3.85	3.88	3.83	3.90	3.90	0.025	***
Lactic acid (g/kg DM)	19	129	123	136	140	80	142	126	125	133	139	137	4.5	***
Acetic acid (g/kg DM)	111	32	33	33	25	73	55	28	27	28	24	18	2.8	***
Propionic acid (g/kg DM)	7.6	3.0	2.9	3.3	4.0	7.7	5.2	1.0	0.9	0.9	0.9	0.3	0.70	**
Butyric acid (g/kg DM)	0.29	0.05	0.04	0.05	0.39	0.06	0.07	0.08	0.08	0.05	0.18	0.07	0.053	NS
Total VFA (g/kg DM)	119	35	36	36	29	80	61	30	29	28	25	18	3.4	***
WSC (g/kg DM)	17	19	17	16	17	13	19	25	28	25	28	34	1.5	***
Lactic/fermentation acids	0.14	0.79	0.77	0.79	0.83	0.50	0.70	0.81	0.82	0.82	0.85	0.88	0.021	***
Ammonia-N (g/kg N)	146	70	112	108	76	77	69	60	96	95	70	55	2.1	***
<b>Effluent</b>														
- production (g/kg grass ensiled)	185	172	171	174	178	170	---	---	---	---	---	---	8.5	NS
- DM concentration (g/kg)	40	48	45	43	48	38	---	---	---	---	---	---	2.0	*
- pH	5.10	4.00	4.35	4.33	4.35	5.23	---	---	---	---	---	---	0.104	***
<b>Recovery</b>														
g silage DM/kg grass DM ensiled	674	786	788	764	772	729	842	871	889	882	880	886	11.1	**
<b>Aerobic stability</b>														
Days to pH rise	8.0	4.5	6.5	6.0	5.0	5.0	9.5	7.5	8.0	8.0	9.0	3.0	1.44	NS
Days to pH max.	8.0	8.0	8.0	8.0	8.0	8.0	10.5	10.0	11.0	11.0	10.5	9.5	0.48	NS
Max. pH rise	1.0	1.0	2.3	3.7	4.8	4.6	0.7	3.1	5.5	1.5	2.3	5.8	1.11	NS
Days to temperature rise	3.0	4.0	3.0	3.5	3.5	3.0	4.0	4.0	4.5	4.5	3.5	2.0	0.66	NS
Days to temperature max.	8.5	7.0	7.0	8.0	7.5	7.0	10.5	8.5	10.0	10.0	11.0	7.5	0.66	NS
Max. temp. rise (°C)	21.5	17.0	20.0	22.5	28.0	24.5	25.5	20.0	40.0	24.0	23.5	25.0	3.18	*
Accumulated temp. rise to Day 5 (°C)	13	11	12	17	34	14	10	12	10	10	11	64	1.7	***

NA = no additive, FA = formic acid, AS = Add SafeR (ammonium tetraformate based), GA = GrasAAT (ammonium tetraformate based), FSB = formate + sulphite + benzoate and LP = *Lactobacillus plantarum*. Note that ammonia added in the AS and GA additives will have contributed to g NH<sub>3</sub>-N/kg N

### (c) Strains and species of lactic acid bacteria

This research was conducted in collaboration with Dr. Michael O'Connell and co-workers from Dublin City University

#### ***Experiment 10: Conservation characteristics of grass ensiled in laboratory silos following treatment with different strains and species of lactic acid bacteria***

The direction of silage preservation reflects the microbiological, chemical and physical characteristics of the crop ensiled, ambient environmental conditions and the silage-making and storing practices imposed. Considerable interest exists in the use of inoculants of homofermentative lactic acid bacteria to improve this process. Most silage inoculants marketed in Ireland contain *Lactobacillus plantarum*, and possibly other bacteria, enzymes, substrates and additional ingredients. Fitzsimons *et al.* (1992) and Duffner (1993) examined the potential of strains of *lactobacilli* and *pediococci* selected from Irish silages or obtained from bacterial collections from different laboratories to utilise a range of substrates and to decrease pH during ensilage. They identified one strain of *Pediococcus* spp. (G 24) and a strain of *Lactobacillus plantarum* (DCU 101) for further investigation. When each of these bacteria were separately inoculated onto grass of adequate fermentable carbohydrate content, they improved the rate of pH decline in the early stages of ensilage. The objective of the present experiment was to determine if such effects occurred when grass of inadequate fermentable carbohydrate content was inoculated at ensiling with these bacteria, separately or in combination.

**Materials and Methods.** A regrowth of grass harvested from a *Lolium perenne* dominant sward in mid July was precision-chopped immediately after mowing, and ensiled in laboratory silos (12 per treatment) with (A) no additive, (B) *Lactobacillus plantarum* (Ecosyl at 3 ml/kg grass; Zeneca BioProducts and Fine Chemicals Ltd.; control), (C) *Lactobacillus plantarum* (DCU 101; 3 ml/kg grass), (D) *Pediococcus* spp. (G 24; 3 ml/kg grass) and (E) *L. plantarum* (DCU 101) plus *Pediococcus* spp. (G 24) at 3 ml/kg grass. The target application rate for the bacteria added in treatments (B), (C) and (D) was  $10^6$  colony forming units (CFU)/g grass, while the target for each bacteria in treatment (E) was  $5 \times 10^5$  CFU/g grass. Silos were opened after 28, 100 and 400 days ensilage, and subjected to physical and chemical analyses. Silage conservation data were analysed as a 5 (additive type) x 3 (silo opening time) factorial completely randomised design.

**Results.** Mean (s.d.) composition of the grass at ensiling was dry matter (DM) 114 (10.0) g/kg, crude protein 198 (13.6) g/kg DM, *in vitro* DM digestibility 725 (13.3) g/kg, ash 111 (19.3) g/kg DM, water soluble carbohydrates (WSC) 8 (4.3) g/l aqueous phase and buffering capacity 426 (22.3) mEq/kg DM. There was no significant effect of additive treatment on any measured aspect of silage conservation, either as a main effect or at any of the days of opening (Table 15). In contrast, day of opening affected ( $P < 0.001$ ) silage pH, the concentrations of lactic acid, propionic acid, butyric acid, ethanol and ammonia-N, and the recovery of ensiled DM. Day of opening also affected ( $P < 0.05$ ) the concentrations of DM, acetic acid and WSC. On average, as ensilage proceeded from day 28 to 100 to 400, there was a progressive increase ( $P < 0.05$ ) in pH and in the concentrations of acetic acid, propionic acid and ammonia-N, and a corresponding decrease ( $P < 0.05$ ) in the concentration of lactic acid and in the recovery of ensiled DM. The concentration of acetic acid was lowest ( $P < 0.05$ ) on day 28 and highest ( $P < 0.05$ ) on day 100. The concentration of WSC was highest ( $P < 0.05$ ) on day 28 while the concentration of butyric acid was highest ( $P < 0.05$ ) on day 400.

**Table 15.** Effects of silage additive treatment and day of opening on the composition of herbage ensiled in laboratory silos (g/kg DM unless otherwise stated).

Days of opening	28					100					400					sem	SxC
	A	B	C	D	E	A	B	C	D	E	A	B	C	D	E		
Dry matter (g/kg)	141	137	140	140	143	134	131	138	138	136	139	135	130	130	132	4.4	NS
pH	3.88	3.97	3.83	4.20	3.87	4.40	4.53	4.33	4.27	4.43	4.60	4.70	4.60	4.63	4.67	0.100	NS
Lactic acid108		101	108	88	108	51	28	50	50	36	1	1	1	1	3	8.2	NS
Ethanol	7	7	7	8	8	16	31	23	25	32	15	17	10	9	12	3.7	NS
Acetic acid	14	19	16	23	17	55	63	50	51	59	91	85	115	100	88	6.4	NS
Propionic acid	0	1	0	1	1	4	5	3	3	3	17	14	23	22	16	2.1	NS
Butyric acid	0	0	0	0	0	0	0	0	0	0	5	6	1	2	6	1.0	NS
WSC(g/l)	1	1	1	1	2	1	1	1	1	0	1	0	1	1	1	0.3	NS
WSC	6	8	8	6	10	6	1	4	8	2	4	2	6	7	4	1.9	NS
NH <sub>3</sub> -N	1.7	1.8	1.6	2.3	1.6	3.6	2.8	2.6	2.5	2.8	4.4	5.3	5.8	4.2	4.6	0.40	NS
DM recovery (g/kg)	908	895	931	890	916	825	815	844	851	844	769	779	736	759	742	25.6	NS

A - No additive ; B- *L. plantarum* (Control); C - *L. plantarum* (DCU101) ; D - *Ped. spp* (G24); E-G24+DCU101.

**Conclusion.** Although a lactic acid dominant primary fermentation occurred in the earlier stages of ensilage, this was progressively replaced by a secondary fermentation which resulted in a decrease in lactic acid concentration and a simultaneous increase in pH and the concentrations of acetic, propionic and butyric acids and ammonia-N. Under these conditions of inadequate fermentable carbohydrate content, none of the bacterial inoculant treatments improved silage fermentation or DM recovery rate.

#### (d) Inhibition of *E.coli* in grass silage

This research was conducted in collaboration with Dr. Cairiona Byrne, Dr Declan Byrne and co-workers from the Teagasc, National Food Centre.

#### **Experiment 11: Effects of *Escherichia coli* 0157:H7 added to grass at ensiling on the early stages of silage fermentation**

Enterobacteria are frequently present on grass at ensiling where contamination with animal manure or soil has occurred. These gram negative, non-sporing, rod-shaped and facultatively anaerobic bacteria ferment carbohydrates to short-chain organic acids (especially acetic acid) or butanediol. They have weak proteolytic activity but can deaminate and decarboxylate some amino acids. Most species can reduce nitrate via nitrite to ammonia, and some can also produce nitrous oxide. Growth limiting factors for enterobacteria include pH <4.5 to 5.0,  $a_w$  <0.95 and temperature <8°C. From among the enterobacteria, *E. coli* is particularly important because of the virulence of some strains towards livestock and humans. The objectives of the present experiment were to trace the development of a specific non-infectious strain of *E. coli* 0157:H7 in the early stages of silage fermentation, to determine its influence on the fermentation profile and to determine the effects on *E. coli* 0157:H7 numbers of altering the ensilage conditions.

**Materials and Methods.** A leafy regrowth of unwilted permanent grass was harvested in mid September and precision-chopped. Sixty samples of 6 kg grass were randomly assigned to the following additive treatments: (a) no additive, (b) *E. coli* 0157:H7, (c) formic acid (850 g/kg; 3 ml/kg grass), and (d) *E. coli* 0157:H7 plus formic acid (i.e. (b) + (c)). A non-infectious strain of *E. coli* 0157:H7 was used, and was applied at  $\log_{10}$  4.5 colony forming units/g forage. Additives were applied manually before ensiling in laboratory silos. Three silos per treatment were opened after 0, 1, 2, 3 and 10 days ensilage, and subjected to chemical and microbiological analyses.

**Results.** Forage microbiological and chemical analysis data are summarised in Table 16. Mean (s.d.) forage dry matter digestibility at ensiling was 722 (14.1) g/kg. Silage made without additive underwent a rapid, lactic acid dominant fermentation during the 10 days of ensilage. Formic acid restricted fermentation, reducing ( $P<0.001$ ) the concentration of lactic acid, acetic acid and ammonia-N, as well as pH and buffering capacity, and increasing ( $P<0.001$ ) the concentration of water soluble carbohydrates and nitrate. Inoculation of forage did not alter ( $P>0.05$ ) silage fermentation. In the absence of formic acid, counts of Enterobacteria (which were high on day 0) and *E. coli* 0157:H7 decreased rapidly, and were absent after 10 days ensilage. Formic acid increased ( $P<0.001$ ) the initial rate of decrease in Enterobacteria counts, but resulted in higher ( $P<0.001$ ) counts on day 10. However, these latter values were relatively low and appeared to be decreasing. Similarly, formic acid increased the initial rate of decline in counts of *E. coli* 0157:H7, but by day 10 was associated with a higher count than when formic acid was not applied.

**Conclusions.** Grass ensiled with or without formic acid provided contrasting ensiling conditions. Addition of *E. coli* 0157:H7 to grass at ensiling did not influence the fermentation profile up to day 10, either in the presence or absence of formic acid. Altering the ensilage conditions by using formic acid changed the survival pattern of *E. coli* 0157:H7 and of Enterobacteria. Both Enterobacteria and the inoculated *E. coli* 0157:H7 rapidly decreased to low levels or disappeared within the time-frame studied.

**Table 16.** Forage chemical and microbiological composition during the early stages of fermentation.

	Dry matter (g/kg)	Crude protein (g/kg DM)	PH (g/kg DM)	Ammonia-N (g/kg N)	Lactic acid (g/kg DM)	Acetic acid (g/kg DM)	Prop. (g/kg DM)	Butyric acid (g/kg DM)	Total VFA (g/kg DM)	WSC (g/kg DM)	B.cap. (mEq/kg DM)	Nitrate (mg/l)	EB	E.c.
Day 0														
No additive	185	150	6.27	29	5	5	0.08	0	5	62	430	54	8.12	0
<i>E. coli</i> (E.c.)	181	154	6.37	31	4	5	0	0	5	59	434	92	7.77	4.57
Formic acid (FA)	185	149	4.63	16	3	2	0.03	0	2	71	445	143	7.62	0
E.c. + F.A.	190	146	4.57	13	3	1	0	0.35	1	66	419	167	7.26	4.50
Day 1														
No additive	179	166	5.13	28	17	16	0	0.1	17	43	468	122	8.15	0
<i>E. coli</i>	170	158	5.07	32	17	19	0.17	0	19	41	461	132	7.67	4.89
Formic acid	188	142	4.47	15	5	2	0	0	2	69	419	128	6.18	0
E.c. + F.A.	186	143	4.53	15	4	2	0	0	2	78	433	123	6.11	3.64
Day 2														
No additive	181	146	4.90	38	36	12	0	0	12	39	490	13	7.21	0
<i>E. coli</i>	178	147	4.90	42	40	12	0	0	12	40	494	13	6.37	3.64
Formic acid	182	148	4.47	21	11	3	0	0	3	72	460	39	5.95	0
E.c. + F.A.	184	144	4.40	22	9	3	0	0	3	75	436	30	6.02	3.05
Day 3														
No additive	188	144	4.70	42	38	11	0	0	11	34	556	0	4.16	0
<i>E. coli</i>	190	147	4.70	42	40	13	0	0	13	36	549	3	4.91	2.79
Formic acid	192	143	4.37	23	10	3	0	0	3	72	476	69	4.12	0
E.c. + F.A.	192	137	4.40	22	11	3	0	0	3	70	469	37	4.61	2.59
Day 10														
No additive	179	143	4.30	54	69	16	0	0	16	29	622	3	0	0
<i>E. coli</i>	180	148	4.23	56	73	16	0	0	16	30	636	1	0	0
Formic acid	195	138	4.23	32	22	5	0	0	5	68	476	37	1.36	0
E.c. + F.A.	188	147	4.33	30	19	4	0	0	4	72	486	62	1.39	0.54
SEM (3-way interaction)	4.0	3.9	0.061	1.8	2.1	0.6	0.04	0.05	0.6	3.1	12.7	16.3	0.520	0.192
Significance	**	**	***	***	***	***	NS	**	***	***	***	***	***	***
Day (D)	***	***	***	***	***	***	NS	*	***	***	***	***	***	***
Formic (F)	***	***	***	***	***	***	NS	*	***	***	***	***	NS	***
<i>E. coli</i> (E)	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	*
D x F	NS	*	***	**	***	***	NS	**	***	***	***	*	NS	***
D x E	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	**	*
F x E	NS	NS	NS	NS	NS	*	NS	NS	*	NS	NS	NS	NS	*
D x F x E	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	*

EB = Enterobacteria ( $\log_{10}$  colony forming units (CFU)/g) recovered on VRBGA; E.c. = *E. coli* 0157:H7 ( $\log_{10}$  CFU/g) recovered on TSA/SMAC

### **Experiment 12: Survival of *Escherichia coli* 0157:H7 added to grass at ensiling and its influence on silage**

Enterobacteria can negatively influence silage fermentation, particularly where the initial rate of pH decline is slow. These gram negative, non-sporing, rod shaped and facultatively anaerobic bacteria ferment monosaccharides to short-chain organic acids (especially acetic acid) or butanediol. They have weak proteolytic activity but can deaminate and decarboxylate some amino acids. Most species can reduce nitrate via nitrite to ammonia, and some can also produce nitrous oxide. Growth limiting factors for enterobacteria include pH < 4.5 to 5.0,  $a_w$  < 0.95 and temperature < 8°C (O’Kiely and Muck, 1998). Enterobacterial activity is more likely where forage of low water soluble carbohydrate (WSC) concentration or high buffering capacity occur together with contamination of grass with sources of inoculum such as animal manure or soil. From among the Enterobacteria, *Escherichia coli* is particularly important because of the virulence of some strains towards livestock and humans. The objectives of the present experiment were to trace the development of a specific, non-infectious strain of *E. coli* 0157:H7 throughout silage fermentation, to determine its influence on the fermentation profile and to determine the effects of altered ensilage conditions on *E. coli* 0157:H7 numbers.

**Materials and Methods.** Unwilted, clean, precision-chop grass was ensiled (6 kg grass per silo) in laboratory silos (O’Kiely and Wilson, 1991) in mid June under the following treatments: (a) no additive (control), (b) *E. coli* 0157:H7, (c) formic acid (850 g kg<sup>-1</sup>; 3 ml kg<sup>-1</sup> grass), and (d) *E. coli* 0157:H7 plus formic acid (i.e. (b) + (c)). A non-infectious strain of *E. coli* 0157:H7 was used, and was applied at log<sub>10</sub> 4.5 colony forming units/g forage. Additives were applied manually immediately before ensiling and silos were stored at 15°C. There were 18 silos per treatment, three of which were opened 0, 2, 5, 9, 19 and 180 days post ensiling, and subjected to physical, chemical and microbiological analyses. Enterobacteria were recovered in VRBGA (Violet Red Bile Glucose Agar) while *E. coli* 0157:H7 were recovered on TSA (Tryptone Soya Agar – to recover injured cells) at 37°C for 2 hours and then overlaid with SMAC (Sorbitol MacConkey agar) to select for 0157. Forage dry matter (DM) concentration was determined after drying of samples at 40°C for 48 h in an oven with forced air circulation. Dried samples were milled through a sieve with 1 mm holes, with buffering capacity being determined according to Playne and McDonald (1966) and crude protein (N x 6.25) according to AOAC (1990) using a nitrogen analyser (LECO FP 428). Juice extracted from silage samples was assayed for lactic acid (Ciba-Corning Diagnostics 550 Express clinical chemistry analyser using the method of Boehringer Mannheim (Catalogue number 139004)), and for volatile fatty acids, WSC, ammonia-N and pH as described by O’Kiely and Wilson (1991). Data were statistically analysed by 3-way analysis of variance for a 2 (*E. coli*) x 2 (formic acid) x 6 (days of opening) factorial design.

**Results and Discussion.** Forage microbiological and chemical analysis data are summarised in Table 17. The low buffering capacity of the grass used in this experiment probably reflects its low crude protein concentration (Muck *et al.*, 1991), and compensated for the correspondingly low concentration of WSC to indicate a forage that was not difficult to preserve by ensilage. The low buffering capacity also facilitated a rapid rate of pH decrease in response to lactic acid production. Silage made without additive had a relatively rapid rate of pH decline and underwent a lactic acid dominant fermentation. The mean concentration of crude protein was 100 g kg<sup>-1</sup> DM and the concentrations of propionic and butyric acids and nitrates were each below 1 g kg<sup>-1</sup> DM throughout. Numbers of indigenous Enterobacteria were high initially but declined progressively until colonies were no longer recoverable by day 19 of ensilage. This was reflected in the relatively low concentrations of acetic acid and ammonia-N found in the control silage during the early stages of ensilage. No indigenous *E. coli* 0157:H7 appeared to be present at any stage of ensilage. Grass ensiled with or without formic acid provided contrasting ensiling conditions. Formic acid restricted fermentation, reducing (P<0.001) buffering capacity and the concentration of lactic acid and increasing (P<0.001) the concentration of WSC. Formic acid addition increased (P<0.05) the initial rate of decline in enterobacterial numbers. This probably reflected both its immediate effect of reducing forage pH together with the specific antibacterial effect of the undissociated acid (McDonald *et al.*, 1991). Inoculation of forage with *E. coli* 0157:H7 elevated (P<0.001) the numbers of *E. coli* 0157:H7 present on the forage. The latter numbers declined rapidly and appeared to have disappeared by day 5 of ensilage. That *E. coli* 0157:H7 cfu dropped to zero sooner than for Enterobacteria may have been largely due to the substantially higher counts of the indigenous Enterobacteria compared to the



inoculated *E. coli* bacteria at the start of ensilage, as well as to possible differences in their relative tolerance of the prevailing ensilage conditions. The addition of formic acid accelerated ( $P < 0.001$ ) this rate of decline after day 0 of ensilage. Inoculation of forage with *E. coli* 0157:H7 did not alter ( $P > 0.05$ ) silage fermentation or the rate of decline in the number of colonies of Enterobacteria detected. This is not surprising given the speed with which conditions within the silo became inhibitory for the inoculated *E. coli*.

The above results agree with a previous study (O'Kiely, Byrne and Bolton, 1999) in which the effects of inoculating grass with *E. coli* 0157:H7 at ensiling were quantified during the first ten days of ensilage. Due to the rapid rate of pH decline in both studies, there remains the need to determine the survival of *E. coli* 0157:H7 during ensilage and its influence on silage fermentation when it has been inoculated onto grass of high buffering capacity and relatively low WSC concentration, and possibly at higher numbers than those used in this experiment.

In conclusion, the number of colonies of both inoculated *E. coli* 0157:H7 and indigenous Enterobacteria decreased rapidly to undetectable levels where unwilted precision-chopped grass was ensiled and underwent a rapid lactic acid dominant fermentation. Altering the ensiling conditions by adding formic acid increased the rate of decline of both *E. coli* 0157:H7 and Enterobacteria. Under these conditions, inoculation of grass at ensiling with *E. coli* 0157:H7 did not influence the fermentation during 180 days of ensiling.

**Table 17.** Forage chemical and microbiological composition throughout ensilage.

	Dry Matter (DM) (g kg)	PH	NH <sub>3</sub> -N (g/kg N)	Lactic acid (g kg <sup>-1</sup> DM)	Acetic Acid (g kg DM)	WSC <sup>1</sup> (g kg DM)	Buffering capacity (mEq. kg DM)	E. coli log <sub>10</sub> CFU <sup>2</sup> g)	Enterobacteria (log <sub>10</sub> CFU <sup>2</sup> g)
Day 0									
No additive	197	4.40	32	14	10	89	331	0	8.12
<i>E. coli</i> (Ec.)	205	4.40	33	12	9	98	330	3.60	7.80
Formic acid (F.A.)	207	4.23	15	1	4	111	289	0	6.32
E.c. + F.A.	205	4.20	14	1	2	115	293	3.53	6.48
Day 2									
No additive	194	4.00	28	54	10	57	432	0	5.13
<i>E. coli</i>	197	4.00	28	56	13	52	394	3.49	5.16
Formic acid	201	4.03	14	11	1	94	357	0	4.67
E.c. + F.A.	202	4.07	22	14	3	84	316	2.55	4.85
Day 5									
No additive	197	3.83	41	79	11	45	467	0	3.34
<i>E. coli</i>	197	3.80	39	77	11	38	465	0	4.37
Formic acid	195	4.00	24	22	4	71	338	0	3.96
E.c. + F.A.	193	4.00	25	24	3	68	335	0	3.12
Day 9									
No additive	191	3.70	44	105	16	28	596	0	1.71
<i>E. coli</i>	192	3.70	42	103	15	25	596	0	0.38
Formic acid	187	3.83	31	45	8	36	446	0	1.61
E.c. + F.A.	179	3.80	34	52	8	31	447	0	0.56
Day 19									
No additive	194	3.60	47	111	17	21	653	0	0
<i>E. coli</i>	197	3.60	47	110	19	23	627	0	0
Formic acid	187	3.73	35	56	42	29	463	0	0
E.c. + F.A.	184	3.73	32	62	46	28	459	0	0
Day 180									
No additive	172	3.70	50	129	21	16	668	0	0
<i>E. coli</i>	173	3.70	51	132	20	16	721	0	0
Formic acid	175	3.73	45	88	14	24	638	0	0
E.c. + F.A.	172	3.67	47	92	16	20	666	0	0
s.e.m. (E.c.xFAxday)	7.4	0.033	1.7	3.1	1.7	3.3	15.3	0.054	0.428
Significance									
E.c.	NS	NS	NS	NS	NS	NS	NS	***	NS
F.A.	NS	***	***	***	*	***	***	***	*
E.C. x F.A.	*	NS	NS	NS	NS	NS	NS	***	NS
Day	***	***	***	***	***	***	***	***	***
E.c. x day	NS	NS	NS	NS	NS	*	NS	***	NS
F.A. x day	***	***	***	***	***	***	***	***	NS
E.c. x F.A. x day	NA	NS	NS	NS	NS	NS	NS	***	NS

<sup>1</sup>WSC = water soluble carbohydrates<sup>2</sup>CFU = colony forming units**(e) Exposure of calves to nitrogen dioxide in silage gas**

This research was conducted in collaboration with Mr. Tomas Turley, Teagasc Advisory Service, Kilkenny and Mr. Phil Rogers, Grange Research Centre.

Large quantities of gas are produced in the first days of silage fermentation, especially when wet grass ferments extensively. The gas consists mainly of carbon dioxide, as well as a mixture of oxides of nitrogen such as nitrous oxide (N<sub>2</sub>O), nitric oxide (NO), nitrogen dioxide (NO<sub>2</sub>), nitrogen trioxide (N<sub>2</sub>O<sub>3</sub>) and dinitrogen tetroxide (N<sub>2</sub>O<sub>4</sub>) (Grayson 1956, Peterson *et al*, 1958; Giddens *et al*, 1970, McDonald *et al*, 1991).

In the first hours of ensiling, reduction of forage nitrate via nitrite to ammonia, or NO or N<sub>2</sub>O, can commence (McDonald *et al*, 1991). It is mediated mainly by indigenous bacteria and usually results in a rapid decrease in nitrate concentration. NO is a colourless gas and is the main oxide of nitrogen produced in the process. It spontaneously oxidises to NO<sub>2</sub> on exposure to air. NO<sub>2</sub> is a reddish-brown

heavy gas with an irritating odour (The Merck Index, 1976), and is the main cause of silo gas toxicity (McLoughlin *et al.*, 1985). NO and NO<sub>2</sub> react readily with water, to form nitrous acid (HNO<sub>2</sub>) and nitric acid (HNO<sub>3</sub>), respectively (Whitehead, 1995). Thus, by dissolving in the aqueous film lining the respiratory tract, NO<sub>2</sub> can cause severe respiratory irritation. The lesions can include pulmonary oedema, haemorrhage and emphysema, and metabolic imbalances (McLoughlin *et al.*, 1985).

A permanent grassland sward received 34000 l cattle slurry/ha in February 1998, followed by 140 kg inorganic fertiliser N/ha spread over two applications in mid-February and mid-March. Approximately 36 ha mown on 22 and 23 May was precision-chop harvested on 24 May and ensiled without additive in two adjoining, unroofed, walled silos. The concrete-floored silos were each 19.5 m long and 9.2 m wide, with mass concrete walls 2.4 m high. Before filling, a sheet of plastic had been placed on the inner surface of each silo wall. It was folded onto the top of the ensiled grass when the silo had been filled. The forage was then immediately covered by one layer of black 0.125 mm polythene sheeting, which was weighted by a full layer of tyres on 25 May. During silo filling and sealing, the prevailing weather conditions were dry, warm and calm, and relatively little effluent was produced subsequently.

The concrete silo wall common to the silo and the calf-shed had a single small vertical crack, as well as a series of plastic tubes (0.6 cm high x 1.3 cm wide) through it. The tubes related to the construction of the wall, and were located 0.7 m apart, in rows 0.3, 0.6 and 0.9 m above the calf-shed floor. The tubes were less frequent above 0.9 m, and were irregularly located in the wall. Many of these appeared to be blocked by debris.

A lean-to cattle-shed adjoined the outer wall of each silo. Both cattle-sheds were 19.5 m long and 6.1 m wide, with the roof sloping from 3.66 m high over the silo wall to 3.05 m high at the far side. There were four openings, each 0.61 m high x 2.44 m long, between the top of each silo wall and the roof of each cattle-shed. The grass in each silo was stored to a height that exceeded that of the roofs of the cattle sheds. Each shed had a large steel door at both ends.

Ten calves, 5 to 9 weeks old, were accommodated in one lean-to shed. On May 25, no problems were evident when cows were brought to the calves for suckling between 0700 to 0830h. A yellow haze was noticed around the silos during the morning, together with occasional coughing by the calves around 1300h. On entering the building at 1430h in response to an abnormal level of coughing by the calves, the farmer observed them coughing, choking, standing with tongues fully extended and frothing from the mouth. Calves furthest from the entrance exhibited the most severe symptoms. The farmer detected a strong bleach-like smell and noticed a yellow-brown haze. He found it quite difficult to breathe and experienced a choking sensation for some days afterwards. The farmer released all calves immediately into an open yard. Six calves recovered quickly, but four required veterinary treatment later that afternoon and for a further number of days. The veterinarian diagnosed inhalation pneumonitis and prescribed an antibacterial agent (Baytril : Bayer) and a nonsteroidal anti-inflammatory drug (Flunixin : Interchem). Initially, the condition of the calves was adjudged to be life-threatening, but they had fully recovered by May 28. The distinctive yellow-brown colour and smell of the gas were observed in the (empty) building on the night of May 25 and around the silos on May 26. On May 26, two adult wood-pigeons were found dead beneath their nest, which was located on the wall-plate between the lean-to building and the silo.

On May 25, some intensely yellow forage was noted under the polythene sheet at the front of the silo. It contrasted with the greener colour of the rest of the fermenting forage. On May 28, after heavy rain, two samples of forage were taken for chemical analysis. These samples were of: (a) the yellow-coloured forage that protruded under the polythene sheet and (b) forage of more normal colour from immediately beneath the polythene but 0.5 m further into the silo. The normal coloured forage was at an intermediate stage of an extensive fermentation (Table 18). It had a high crude protein concentration, reflecting the high rate of nitrogen applied to the crop. The intensely yellow coloured forage had a higher water content, and was taken from a position where much of the gaseous output of the silo would pass. The extremely low pH, allied to the virtual absence of fermentation, suggested a high presence of strong mineral acid. The high concentration of crude protein, reflecting elevated N concentrations, implied that nitric acid derived from nitrogen dioxide dissolving in water was probably the mineral acid. The low DM digestibility may reflect *in situ* digestion of forage organic matter by the mineral acid. The low

buffering capacity may reflect the low pH and possible acid digestion of organic and amino acids, the primary sources of buffering in the crop.

**Table 18.** Chemical composition of intensely yellow and normal coloured forage taken from the front of the silo 4 days after ensiling.

	Normal colour	Intensely yellow
Dry matter (DM) (g/kg)	214	194
PH	4.3	1.1
Crude protein (g/kg DM)	193	294
in vitro DM digestibility (g/kg)	700	573
Ammonia-N (g/kg N)	94	31
Nitrate (mg/l)	400	501
Lactic acid (g/kg DM)	66	9
Acetic acid (g/kgDM)	21	8
Propionic acid (g/kgDM)	3	3
Butyric acid (g/kgDM)	0.2	0.3
Ethanol (g/kgDM)	6	18
Buffering capacity (mEq/kg DM)	686	97

In conclusion, formation of toxic gaseous oxides of nitrogen can occur during the early stage of fermentation of unwilted grass silage in horizontal silos. Under certain unusual conditions, these gases can pose a threat to humans and livestock in the immediate vicinity of the silo. In the above case, the quick action of the farmer (who had never encountered such a case before) prevented fatalities, but the incident underlines the need for vigilance and common sense near silos. Ideally, livestock should not be accommodated in buildings adjoining silos during silo filling or during the following week. If animals must be housed near such silos, they should be inspected very often in the first few days after ensiling.

## 5. AEROBIC STABILITY OF GRASS SILAGE MIXED WITH CONCENTRATES AT FEEDOUT

Three experiments were conducted to investigate aspects of the aerobic stability of grass silage mixed with concentrates at feedout. They were conducted in collaboration with Dr. Evelyn Doyle and co-workers from the Department of Industrial Microbiology at University College Dublin.

### *Experiment 13: Aerobic stability of grass silage with different rates and forms of wheat grain added at feedout*

The conservation characteristics of silage generally stabilise at some stage of ensilage provided anaerobic conditions are maintained. However, respiration recommences and silage becomes inherently unstable during the aerobic conditions that prevail during feedout. This results in quantitative and qualitative losses both at the open face of the silos and in the feed manger. Aerobic deterioration of silage is usually initiated by yeast, with moulds and possibly bacteria succeeding them. Yeast readily respire silage sugars or fermentation acids, but can also respire some of the constituents in energy or protein-rich feedstuffs. It can be hypothesised that the rate and extent of aerobic deterioration of silage in the feed manger will increase where the silage is mixed with supplementary concentrates (as sources of respirable substrate and/or of inoculum). Such mixing is typical of total mixed rations (TMR's) produced using feeder wagons. Two experiments were conducted to quantify the effects of the rate of addition and form of processing of wheat grain on the aerobic deterioration of two contrasting silages with which they were mixed.

**Materials and Methods.** In each of two experiments (Experiments 13 and 14), unwilted, well preserved, precision-chop grass silage was removed from the well-managed face of a horizontal bunker silo using a shear-grab. Each block of silage was thoroughly mixed, and 6 kg weighed into each of 48 polythene-lined polystyrene boxes with loosely fitted lids. From 100 kg dry wheat grain in each experiment, representative samples were (a) left unprocessed, (b) passed through a roller mill with x mm spacing, (c) passed through a hammer mill with a 2 mm screen, (d) treated with sodium hydroxide, or (e) treated with urea. For both the sodium hydroxide and urea treatments, water was added to the wheat grain at a rate calculated from the scale: +100, 140 or 180 ml water per kg grain with a moisture content of 200, 160 or 120 g/kg, respectively. Sodium hydroxide (30 g/kg grain) or urea (30 g/kg grain) were dissolved in the water, thoroughly mixed with whole grain and stored for at least 4 days in an insulated container (stirred once daily). Triplicate polystyrene containers of silage per treatment were stored for 6 (Experiment 13) or 14 (Experiment 14) days in a chamber maintained at 20°C. The 16 treatments were silage alone or with each of the five processed forms of wheat integrally mixed with the silage at 400, 800 and 1200 g grain/6 kg silage. Physical, chemical and microbiological measurements were made.

**Results.** The silage in Experiment 13 had a mean (s.d.) dry matter (DM; 40°C for 48 h) concentration of 190 (18.4) g/kg, pH 4.35 (0.071), yeast count of  $3 \times 10^3$  colony forming units (cfu)/g and no detectable mould. The corresponding values in Experiment 14 were 244 (16.3) g/kg, 4.0 (-),  $4 \times 10^2$  cfu/g and  $3.9 \times 10^2$  cfu/g. The wheat had a DM (40°C, 48 h concentration) of 890 (2.1) and 929 (0.7) g/kg in Experiments 13 and 14, respectively. The pH of the whole, rolled, ground, NaOH and urea wheat grain were 6.84 (0.028), 6.33 (0.014), 6.43 (0.028), 10.60 (0.071) and 8.68 (0), respectively. The silage in Experiment 13 was aerobically much less stable than that in Experiment 14. Tables 19 and 20 summarise the other results. Whole wheat did not alter aerobic stability, heat production or aerobic losses. Rolling or grinding wheat increased total heat production, but did not necessarily cause deterioration to occur earlier. Sodium hydroxide increased aerobic losses in Experiment 14. Urea delayed aerobic deterioration in Experiment 1 but accelerated the rate of deterioration in Experiment 14.

**Conclusions.** Silage aerobic stability was not compromised by the addition of whole wheat grain at feedout. Rolling or grinding wheat increased total heat production, but did not necessarily make the silage aerobically less stable. There was not a clear dose response to the rate of wheat used. The added wheat was not an influential source of microbial inoculum. The micro-organisms in the silage that initiate aerobic deterioration initially had adequate substrate in the silage. Physically or chemically disrupting the seed coat of the wheat grain made aerobically metabolisable substrate available, and thus

allowed increased total heat production rather than earlier heat production. Raising pH by alkali addition encouraged instability. Urea, as a source of ammonia, could improve (with unstable silage) or disimprove (with stable silage) aerobic stability.

**Table 19.** Aerobic deterioration of silage with different rates of addition and processed forms of wheat grain added (Experiment 13).

Treatment	Days to °C rise	Days to max. °C	Days from °C rise to max.	Max. °C rise	Acc. °C rise to day 5 <sup>1</sup>	Rate of °C rise (°C/day) <sup>2</sup>
Silage alone	1	2.7	1.7	20.4	64	8.7
Silage+whole wheat : low	1	2.7	1.7	23.6	75	10.2
: medium	1	2.7	1.7	23.4	73	10.4
: high	1	2.0	1.0	21.6	71	10.8
Silage+rolled wheat : low	1	2.0	1.0	25.5	81	12.7
: medium	1	2.0	1.0	25.0	82	12.5
: high	1	2.7	1.7	24.3	79	10.5
Silage+ground wheat : low	1	2.0	1.0	23.5	82	11.7
: medium	1	2.7	1.7	24.4	84	10.7
: high	1	2.0	1.0	23.7	81	11.9
Silage+NaOH wheat : low	1	3.3	2.3	25.2	87	10.6
: medium	1	2.7	1.7	28.1	91	12.4
: high	1	2.0	1.0	27.0	93	13.5
Silage+urea wheat : low	1	3.3	2.3	22.1	72	8.7
: medium	1	5.3	4.3	25.7	57	4.8
: high	1	4.7	3.7	24.8	64	5.4
SEM	-	0.58	0.58	3.04	5.8	1.96
Significance	-	**	**	NS	**	NS

<sup>1</sup>Accumulated temperature rise to day 5 (°C); <sup>2</sup>Rate of rise in temperature from start to maximum temperature

**Table 20.** Aerobic deterioration of silage with different rates of addition and processed forms of wheat grain added (Experiment 14).

Treatment	Days to °C rise	Days to max °C	Days from °C rise to max.	Max °C rise	Acc. °C rise to day <sup>1</sup>			Rate of DM <sup>2</sup>		Rate of °C rise <sup>3</sup>
					5	7	12	Loss	Recovery	
Silage alone	4.3	13.0	8.7	21.7	5.2	11.7	66.1	166	834	1.7
Silage+whole Wheat : low	4.7	12.0	7.3	22.7	4.7	12.3	77.0	174	826	1.9
: medium	4.0	11.7	7.7	22.1	4.6	11.9	80.3	179	821	1.9
: high	4.7	11.7	7.0	22.7	4.6	13.3	88.8	176	824	1.9
Silage+rolled Wheat : low	4.7	11.7	7.0	21.4	5.7	15.3	88.9	108	892	1.9
: medium	3.7	12.0	8.3	26.3	4.7	14.6	89.6	183	817	2.2
: high	4.0	12.0	8.0	29.4	6.2	19.3	114.4	181	819	2.4
Silage+ground Wheat : low	4.0	12.0	8.0	26.5	4.2	13.7	89.1	202	798	2.2
: medium	3.7	11.7	8.0	24.7	5.0	23.8	97.5	203	797	2.1
: high	3.3	11.3	8.0	25.1	5.4	23.9	111.2	204	796	2.2
Silage+NaOH wheat : low	3.7	9.0	5.3	22.3	7.8	30.3	124.7	306	694	2.4
: medium	3.7	8.0	4.3	20.5	10.4	40.7	127.1	301	699	2.6
: high	3.3	8.3	5.0	27.3	14.5	53.9	180.2	198	802	3.3
Silage+urea wheat : low	4.7	11.7	7.0	33.1	2.8	13.8	113.6	270	730	2.8
: medium	4.0	11.3	7.3	25.2	4.1	16.2	109.6	249	751	2.3
: high	4.0	10.3	6.3	20.7	4.0	18.5	104.9	404	596	2.0
SEM	0.55	0.68	0.72	3.27	0.81	2.97	10.18	46.5	46.5	0.23
Significance	NS	***	**	NS	***	***	***	*	*	**

<sup>1</sup>As in Table 1; <sup>2</sup>g/kg; <sup>3</sup>as <sup>2</sup>in Table 1

**Experiment 15: Aerobic stability of grass silage mixed with a range of concentrate feedstuffs at feed-out**

The conservation characteristics of silage generally stabilise at some stage of ensilage provided anaerobic conditions are maintained. However, respiration by indigenous micro-organisms commences and silage becomes inherently unstable during the aerobic conditions that prevail during feedout. This results in quantitative and qualitative losses both at the open face of bunker silage and in the feed manger. Furthermore, fungal growth in silage poses health hazards in terms of the challenge from airborne spores to the eyes and respiratory tract, and metabolic disorders associated with the ingestion of mycotoxins (Wilkinson, 1999). Aerobic deterioration of silage is usually initiated by yeast, with moulds and possibly bacteria succeeding them (Driehuis et al., 1999). Yeast readily respire silage sugars or fermentation acids, but can also respire some of the constituents in energy or crude protein-rich concentrate feedstuffs (McDonald et al., 1991). It can be hypothesised that the rate and extent of aerobic deterioration of silage in the feed manger will increase and its aerobic stability will decrease where the silage is mixed with supplementary concentrates (as sources of respirable substrate and/or of inoculum). Such mixing is typical of total mixed rations (TMR's) produced using feeder wagons. The aim of the present experiment was to quantify the effects of concentrate feedstuffs differing in their contents of starch, oil, sugar and crude protein on aerobic stability and deterioration when mixed with silage at feedout.

**Materials and Methods.** Unwilted, well preserved, precision-chop grass silage was removed from a horizontal bunker silo using a shear grab. The 700 kg sample was thoroughly mixed and subsamples (each 6 kg) were placed in 52 polythene-lined polystyrene (2.5 cm thick) boxes (59 x 39 x 22 cm) with a polystyrene lid loosely fitted. Four containers of silage were allocated to each of the following 13 treatment, with 400 g of each ingredient being manually mixed with the appropriate sub sample of silage (i.e. 400 g ingredient to 6 kg silage): no added ingredient, wheat grain, barley gain, maize grain, molassed beet pulp, citrus pulp, molasses, soyabean meal, maize gluten, sunflower meal, rapeseed meal, dry distillers grains and sunflower soil. The 10 solid ingredients were each ground through a 2 mm sieve in a hammer mill before being mixed with silage. The containers of mixed feedstuff were stored at 20°C for 6 days and mean temperatures were recorded daily. Temperature results were expressed as (1) interval in days until the temperature rose more than 2°C above the reference temperature, (2) interval in days until the maximum temperature was reached, (3) interval in days between commencement of temperature rise and reaching maximum temperature, (4) maximum temperature rise (°C), (5) accumulated temperature rise in the first 5 days of aerobiosis (°C) and (6) rate of temperature rise during (3) above (°C day<sup>-1</sup>). Yeast and mould counts were made on malt extract agar (pH 3.5) using the double-layered pour-plate technique. Data were statistically analysed by one way analysis of variance.

**Results and Discussion.** The mean chemical composition of the silage when removed from the silo was dry matter (DM) 216 g kg<sup>-1</sup>, crude protein 162 g kg<sup>-1</sup>DM, in vitro DM digestibility 697 g kg<sup>-1</sup>, buffering capacity 1132 mequiv kg<sup>-1</sup> DM, pH 4.0, lactic acid 89 g kg<sup>-1</sup> DM and ammonia-N 64 g kg<sup>-1</sup> total N. Correspondingly the counts of yeast and mould were 9.8 x 10<sup>7</sup> and 0 colony forming units (cfu) g<sup>-1</sup>, respectively. The 12 concentrate feedstuffs were all of high DM concentration (Table 21) and, with the exception of distillers grains (pH 4.4), all had pH values between 5.2 and 6.5. The feedstuffs represented a wide range in concentrations of crude protein (< 2 to 485 g kg<sup>-1</sup>), ash (< 1 to 139 g kg<sup>-1</sup>), oil A (<1 to 990 g kg<sup>-1</sup>), starch (<1 to 592 g kg<sup>-1</sup>) and sugar (<1 to 477 g kg<sup>-1</sup>). Similarly, there was a wide range in the numbers of yeast (<10 to 8.7 x 10<sup>7</sup> cfu g<sup>-1</sup>) and mould (<10 to 5.5 x 10<sup>6</sup> cfu g<sup>-1</sup>). The aerobic stability of the silage was normal for a well preserved, unwilted, precision-chop silage, with an accumulated temperature rise to day 5 of 57°C (Table 22) and a corresponding mean loss of DM during aerobiosis (6 days) of 152 g kg<sup>-1</sup>. Under the circumstances prevailing in this experiment, none of the added concentrate feedstuffs altered any of the indices of aerobic stability, rate of deterioration or extent of deterioration (P>0.05).

Yeast are the most frequent initiators of the aerobic deterioration of silage (Driehuis et al., 1999). Yeast numbers on silage were higher than on any of the concentrate feedstuffs. As none of the added feedstuffs initiated an earlier commencement of aerobic deterioration, this suggests that none of the feedstuffs supplied a quantity or type of yeast capable of altering the effects of the indigenous yeast population on the silage. In the present experiment there were sufficient yeast present in the silage at feedout capable of initiating aerobic deterioration within a relatively short time frame. It is unclear what the outcome would have been in this regard if the indigenous population of yeast on the silage at feedout

were much lower. Although the added concentrate feedstuffs introduced moulds to the silage, in some cases in quite high amounts, these clearly did not lead to an earlier initiation of aerobic deterioration or an alteration in the extent of heat production. The concentrate feedstuffs were either liquids or finely milled solids, and were intimately mixed with the silage at the commencement of aerobic conditions. They supplied different forms and quantities of energy and crude protein without significantly altering silage aerobic stability or deterioration. The absence of an effect of their addition on aerobic stability or deterioration suggests that a supply of readily available, respirable substrate did not limit the activity of yeast throughout the duration of exposure of silage to air. Clearly, adequate silage fermentation products such as lactic acid, as well as residual WSC, were available to yeast for respiration under the aerobic conditions and duration of this study.

It is concluded that in the present experiment, mixing concentrate feedstuffs with grass silage did not alter silage aerobic stability or the rate or extent of aerobic deterioration. This suggests that adequate respirable substrate and yeast numbers were present in the silage to allow rapid and extensive respiration to occur, resulting in a relatively short duration of aerobic stability and rapid and extensive aerobic deterioration. This experiment should be repeated using grass silage containing substantially lower yeast counts at the commencement of feedout.

**Table 21.** Microbiological and chemical composition of concentrate feedstuffs.

Feedstuff	Yeast (cfu g <sup>-1</sup> )	Mould (cfu g <sup>-1</sup> )	Dry matter (g kg <sup>-1</sup> )	Crude protein (g kg <sup>-1</sup> )	Ash (g kg <sup>-1</sup> )	Oil A (g kg <sup>-1</sup> )	Starch (g kg <sup>-1</sup> )	Sugar (g kg <sup>-1</sup> )	pH
Wheat	6.1 x 10 <sup>7</sup>	1.1 x 10 <sup>6</sup>	863	91	16	14	592	35	6.0
Barley	8.7 x 10 <sup>7</sup>	2.0 x 10 <sup>3</sup>	850	94	18	16	489	22	5.7
Maize	2.7 x 10 <sup>6</sup>	2.4 x 10 <sup>6</sup>	862	80	12	32	588	16	5.9
Beet pulp	4.5 x 10 <sup>5</sup>	3.5 x 10 <sup>2</sup>	902	102	61	4	18	211	5.3
Citrus pulp	6.0 x 10 <sup>3</sup>	6.5 x 10 <sup>3</sup>	904	67	56	19	<1	193	5.2
Molasses	8.6 x 10 <sup>3</sup>	<10	732	41	139	<1	<1	477	5.2
Soyabean meal	2.9 x 10 <sup>5</sup>	1.0 x 10 <sup>3</sup>	880	485	63	20	42	89	6.5
Maize gluten	4.5 x 10 <sup>4</sup>	<10	890	204	65	32	191	30	6.0
Sunflower meal	3.6 x 10 <sup>7</sup>	5.5 x 10 <sup>6</sup>	878	288	66	16	13	55	5.8
Rapeseed meal	5.0 x 10 <sup>5</sup>	<10	882	339	68	15	44	85	5.6
Distillers grains	4.0 x 10 <sup>5</sup>	3.0 x 10 <sup>2</sup>	898	259	62	81	19	29	4.4
Sunflower oil	<10	<10	993	<2	<1	990	<1	<1	-



**Table 22.** Indices of aerobic deterioration of silage with added concentrate feedstuffs.

Concentrate feedstuff	Interval (days)			Max. temp. to rise (°C)	Accum. temp. rise to day (°C)	Rate of temp. rise (°C day <sup>-1</sup> )
	To temp. rise max	To temp. max.	From temp. rise			
None	2.0	3.8	1.8	18.4	57	11.3
Wheat	2.0	3.5	1.5	20.0	58	14.8
Barley	2.0	4.5	2.5	21.3	57	8.7
Maize	2.3	3.5	1.5	18.2	54	13.9
Beet pulp	2.0	4.0	2.0	18.2	58	9.1
Citrus pulp	2.0	3.3	1.3	18.4	59	16.1
Molasses	2.3	3.5	1.3	19.1	50	16.9
Soyabean meal	2.0	3.8	1.8	19.1	57	15.1
Maize gluten	2.3	4.3	2.3	18.5	53	10.9
Sunflower meal	2.0	4.0	2.0	20.8	61	13.5
Rapeseed meal	2.0	3.8	1.8	18.4	52	13.0
Distillers grains	2.0	3.8	1.8	19.1	51	12.0
Sunflower oil	2.0	3.5	1.5	19.2	58	14.2
s.e.m.	0.11	0.44	0.43	1.30	3.05	2.68
Significance	NS	NS	NS	NS	NS	NS

NS = no significant difference

**Experiment 16: Dose response of unwilted silage pH to sodium bicarbonate, and effects of three sodium sources on silage aerobic stability**

In circumstances where unwilted grass silages are considered to present an excessive acid load to ruminants, supplementation with sodium bicarbonate (NaHCO<sub>3</sub>) is sometimes suggested as a therapy. However, published scientific literature reports a wide range of changes in silage intake in response to such supplementation (Shaver et al., 1984, 1985). An aim of the present experiment was to quantify the effects of incremental rates of addition of NaHCO<sub>3</sub> and NaCl on silage pH, and to determine the effects of the time interval between mixing NaHCO<sub>3</sub> with silage and recording silage pH. Because different sodium sources added to silage can differentially affect pH and osmotic pressure, a further aim was to quantify the effects of three sodium compounds on silage aerobic stability.

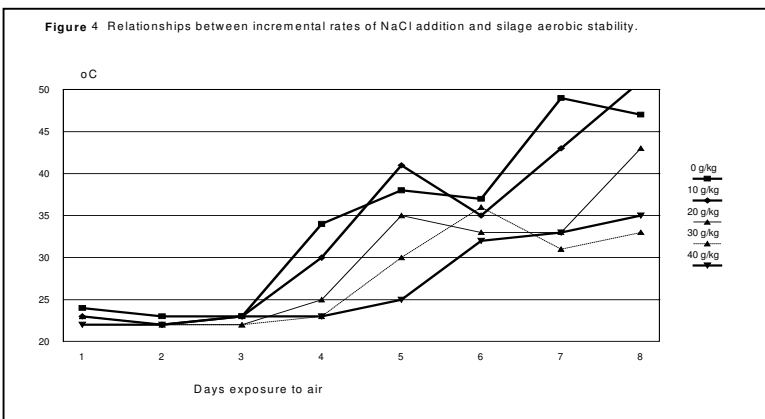
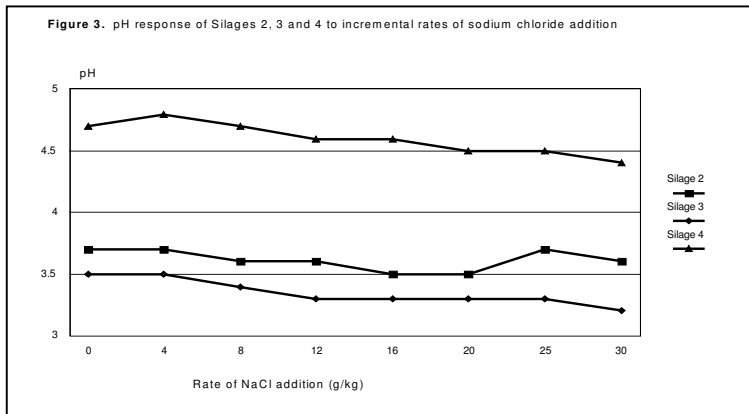
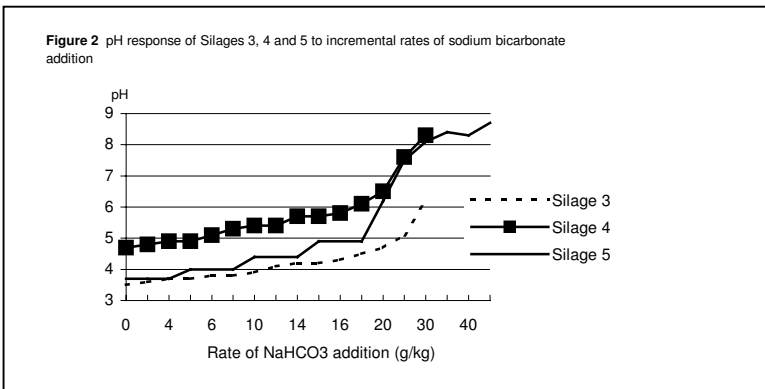
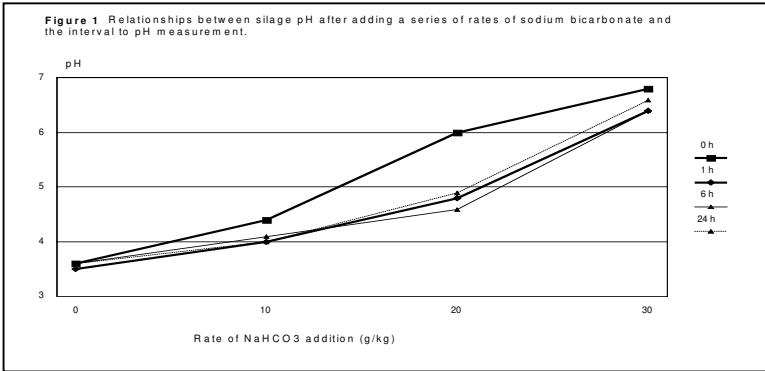
**Materials and Methods.** Triplicate sub-samples of Silage 1 were treated with NaHCO<sub>3</sub> added at 0, 10, 20 or 30 g/kg, and had their pH determined after 0, 1, 6 or 24 hours (treated silages stored at 4°C and then equilibrated to room temperature before pH determination). Triplicate sub-samples of Silages 2, 3 and 4 had NaCl applied at 0, 4, 8, 12, 16, 20, 25 and 30 g/kg, while triplicate sub-samples of Silages 3, 4 and 5 had NaHCO<sub>3</sub> mixed at 0, 2, 4, 6, 8, 10, 12, 14, 16, 18, 20, 25 and 30 (35, 40 and 45 for Silage 5) g/kg. Silage pH values were determined after overnight storage at 4°C and equilibration to room temperature. The aerobic stability of each of triplicate sub-samples of Silage 6 was assessed during 8 days storage at 21°C following the addition of NaHCO<sub>3</sub>, NaCl or NaOH at 0, 10, 20, 30 or 40 g/kg silage.

**Results.** Without NaHCO<sub>3</sub>, silage pH did not differ due to time of pH recording, whereas when applied at 10, 20 or 30 g/kg silage, the pH values were similarly lower after 1, 6 or 24 hours compared to at 0 hours (Figure 1). Silages 1 to 5 had initial pH values of 3.5, 3.7, 3.6, 4.7 and 3.7, respectively, and for each silage the precise pH response to added NaHCO<sub>3</sub> was unique. However, the general patterns were of buffer curves, with added NaHCO<sub>3</sub> increasing (P<0.001) pH for each silage (Figures 1 and 2). Added NaCl caused a small but significant (P<0.001) decrease in silage pH (Figure 3).

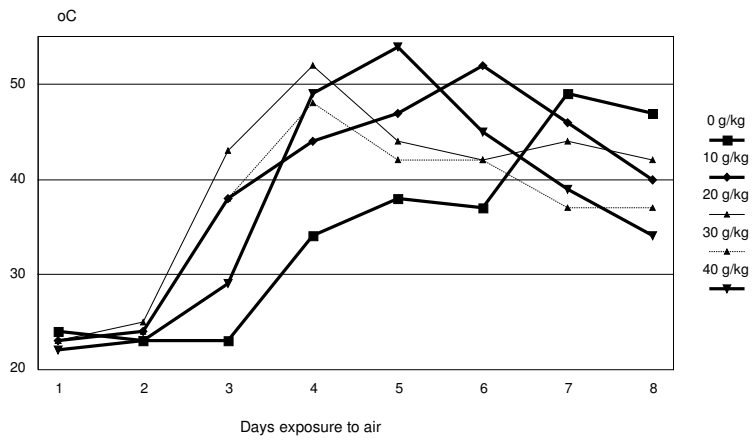
Silage 6 had dry matter (DM) 191 g/kg, pH 3.97, crude protein 166 g/kg DM and in vitro DM digestibility 742 g/kg. Addition of NaCl at 10 g/kg silage did not alter aerobic stability, whereas the 20,

30 and 40 g/kg rates progressively improved aerobic stability, as evidenced by the delay until temperature rise was detected (Figure 4). In contrast, NaHCO<sub>3</sub> disimproved silage aerobic stability, causing a quicker temperature rise than for untreated silage (Figure 5). The initial rate of aerobic deterioration was progressively increased by the 10 and 20 g/kg rates of NaHCO<sub>3</sub> addition, before being then progressively decreased by the 30 and 40 g/kg rates. Addition of NaOH to silage caused an immediate rise in temperature (Figure 6) reflecting the exothermic reaction taking place. Increasing rates of addition lead to progressively larger immediate temperature rises, but the silage temperatures then returned to the values of an untreated silage in the following 1 or 2 days. The addition of 10 g NaOH/kg silage initiated earlier and more rapid aerobic deterioration, whereas the highest rate of addition (40 g/kg) conferred extreme stability. The intermediate rates (20-30 g/kg) caused effects between the extremes described for the 10 and 40 g/kg rates.

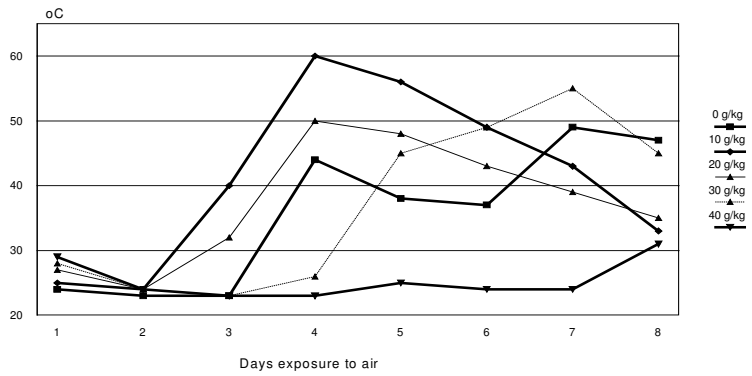
**Conclusions.** Silage pH can be adjusted upwards by adding NaHCO<sub>3</sub>, the rate of addition to achieve a particular unit change being specific to each silage (probably strongly related to the buffering constituents of the silage). Silage pH values after adding NaHCO<sub>3</sub> are better measured one hour or more after mixing rather than immediately, to avoid over-estimating the pH elevating effects of NaHCO<sub>3</sub>. Addition of NaCl did not increase silage pH, but would have increased osmotic pressure - incremental rates of addition of NaHCO<sub>3</sub> and NaOH would also have the latter effect. Added NaCl improved aerobic stability by increasing silage osmotic pressure (reducing water activity ( $a_w$ )) and rather than by altering pH. Sodium bicarbonate, by increasing silage pH, made it aerobically more unstable, although the effect was progressively less amplified for the two higher rates of addition possibly due to increasing osmotic pressure effects. In contrast, the low rate of NaOH increased aerobic instability, with increasing rates of addition progressively increasing stability through to the 40 g/kg rate which conferred apparently complete stability through to day 7.



**Figure 5** Relationships between incremental rates of NaHCO<sub>3</sub> addition and silage aerobic stability.



**Figure 6** Relationships between incremental rates of NaOH addition and silage aerobic stability.



## 6. NUTRITIVE VALUE OF GRASS SILAGES

Three experiments were conducted to study the nutritive value of unwilted grass silages offered to beef cattle. In the first two experiments, the effects of using contrasting species and strains of lactic acid bacteria were investigated. In the third experiment, the interaction of the effects of silage additives used to restrict or modify fermentation and the effects of offering supplementary concentrates to cattle were quantified. These experiments were conducted in collaboration with Dr. Patrick Shiels; Dr. Michael O'Connell and co-workers from Dublin City University ; Prof. Patrick Caffrey from the Faculty of Agriculture at University College Dublin.

### *Experiment 17: Digestibility and nitrogen retention in cattle offered silages made with different species and strains of lactic acid bacteria*

Although there are many published examples of improvements in silage nutritive value following inoculation with homofermentative lactic acid bacteria at ensiling, few of these experiments were conducted under the conditions of inadequate to marginal fermentable carbohydrate supply that frequently prevail in Ireland. Fitzsimons *et al.* (1992) examined the potential of strains of *Pediococcus acidilactici* isolated from Irish silages or obtained from bacterial collections from different laboratories to utilise a range of substrates and to decrease pH during ensilage. They identified *Pediococcus* spp. (G 24) as capable of causing a more rapid drop in pH at the commencement of silage fermentation, when applied to grass of adequate fermentable carbohydrate content and ensiled in laboratory silos. Duffner (1993) and Duffner *et al.* (1994) demonstrated the ability of *Lactobacillus plantarum* (DCU 101), isolated from an Irish silage and added at ensiling to grass of adequate fermentable carbohydrate content, to increase the rate or extent of pH decline. The objectives of the present experiment were to determine if the effects of *Pediococcus* spp. (G 24) and *L. plantarum* (DCU 101) when used separately or in combination were manifest on a farm-scale with grass of low fermentable carbohydrate content, and if digestibility and nitrogen retention in cattle subsequently fed the silage were improved.

**Materials and Methods :** Grass was ensiled from the first regrowth of a *Lolium perenne* sward in mid-July. Alternate pairs of loads of grass were ensiled following treatment with: (A) no additive, (B) *L. plantarum* (Ecosyl; Zeneca BioProducts and Fine Chemicals Ltd.; Control) at 3.2 l/t, (C) *L. plantarum* (DCU 101) @ 3.1 l/t, (D) *Pediococcus* spp. (G 24) @ 3.3 l/t or (E) *L. plantarum* + *Pediococcus* spp. (DCU 101 + G 24) @ 3.3 l/t. The target application rate for the bacteria added in treatments (B), (C) and (D) was  $10^6$  colony forming units (CFU)/g grass, while the target for each bacteria in treatment (E) was  $5 \times 10^5$  CFU/g. Following 47 days storage, silages were offered *ad libitum* to crossbred continental heifers (417 (s.d. 23.4) kg mean initial liveweight; 14/treatment) and supplemented with 2 kg concentrates per head daily for 112 days. Simultaneously, *in vivo* coefficients for the digestibility of the various dietary fractions, together with nitrogen retention and the concentrations of blood metabolites, were determined using Friesian steers (425 (s.d. 32.3) kg mean initial liveweight) in a 5 (additive treatment) x 5 (period) Latin Square design experiment. Each period was of 28 days duration. For the final 10 days of each period, each steer was offered the appropriate silage at 0.9 of *ad libitum* intake and the quantity of concentrates offered was adjusted to a similar forage:concentrate ratio to the heifers. On day 18 of each period, blood samples were collected via jugular catheter 0.5 hours prior to feeding and 0.5, 1.0, 1.5, 2.0, 2.5, 3.0, 3.5, 5.5, 7.5, 11.5, 15.5 and 23.5 hours post-feeding. Faeces and urine collection took place between days 21 to 28.

**Results.** Mean (s.d.) composition of the grass at ensiling was dry matter (DM) 138 (18.2) g/kg, *in vitro* DM digestibility 746 (20.7) g/kg, water soluble carbohydrates (WSC) 9 (3.7) g/l aqueous phase and buffering capacity 522 (38.1) mEq/kg DM. Total lactic acid bacteria on untreated grass were  $1.1 \times 10^6$  CFU/g. The recovery rates of edible silage DM were 650, 700, 710, 720 and 760 kg/t DM ensiled for treatments (A) to (E), respectively. Mean silage DM intake by the heifers was 5.97, 5.91, 5.60, 5.85 and 5.87 (s.e.m. 0.135;  $P > 0.05$ ) kg/day for treatments (A) to (E), respectively, with corresponding carcass gains of 478, 473, 485, 483 and 506 (s.e.m. 18.6;  $P > 0.05$ ) g/day. The mean (s.d.) composition of the concentrates offered to the steers was DM 849 (4.2) g/kg, crude protein 146 (12.1) g/kg DM, ash 32 (1.6) g/kg DM, crude oil 27 (0.5) g/kg DM, crude fibre 58 (17.1) g/kg DM, neutral detergent fibre 263 (24.3) g/kg DM and acid detergent fibre 48 (7.7) g/kg DM. The mean composition of the silages offered to the steers is summarised in Table 23, and feed intake, dietary *in vivo* digestibility, nitrogen retention and blood composition are summarised in Table 24. Although all silages underwent a lactic acid dominant

primary fermentation, a secondary fermentation proceeded throughout feedout, with pH values and concentrations of acetic acid and ammonia-N progressively increasing (data not shown).

**Table 23.** Mean (s.d.) composition of silages offered to the steers in the *in vivo* digestibility and nitrogen retention experiment.

	No additive	<i>L. plantarum</i> (Control)	<i>L. plantarum</i> (DCU 101)	<i>Pediococcus</i> spp. (G 24)	<i>L.p. + P. spp.</i> (DCU 101 + G 24)
Dry matter <sup>1</sup>	170 (7.0)	163 (4.6)	168 (19.0)	165 (9.1)	168 (4.2)
Crude protein <sup>2</sup>	151 (2.5)	161 (12.5)	157 (8.4)	155 (8.7)	157 (8.7)
<i>in vitro</i> DMD <sup>1</sup>	699 (24.0)	666 (8.2)	668 (19.9)	646 (43.9)	668 (23.6)
NDF <sup>2</sup>	650 (34.0)	619 (52.6)	617 (71.9)	565 (44.2)	587 (21.5)
ADF <sup>2</sup>	366 (18.9)	354 (7.8)	358 (12.1)	374 (7.5)	368 (11.1)
Ash <sup>2</sup>	93 (4.0)	90 (2.1)	92 (3.6)	98 (3.8)	99 (12.2)
PH	4.30 (0.638)	4.22 (0.084)	4.22 (0.311)	4.52 (0.217)	4.26 (0.378)

<sup>1</sup>=g/kg; <sup>2</sup>= g/kg DM

**Table 24.** Silage additive treatment effects on *in-vivo* digestibility, nitrogen balance and blood composition.

	No additive	<i>L. plantarum</i> (Control)	<i>L. plantarum</i> (DCU101)	<i>Ped. spp.</i> (G24)	<i>L. p. + P. spp.</i> (DCU101+G24)	s.e.m.	Sig
Silage DMI (kg/day)	4.88	5.03	5.01	4.98	5.02	0.173	NS
Concentrate DMI (kg/day)	1.56	1.61	1.61	1.54	1.57	0.054	NS
Total DMI (kg/day)	6.44	6.64	6.61	6.51	6.59	0.205	NS
<b><u>Digestibility (g/kg)</u></b>							
Dry matter	751	763	752	740	741	11.1	NS
Organic matter	769	783	772	760	776	10.4	NS
Nitrogen	693	706	689	677	677	16.4	NS
NDF	734	734	731	733	724	14.0	NS
ADF	696	695	684	684	675	13.2	NS
DOMD	706	724	713	698	698	9.2	NS
<b><u>Nitrogen balance (g/day)</u></b>							
Total N intake	154	157	158	161	157	4.7	NS
Digestible N intake	107	112	109	109	106	5.3	NS
Urine N	59	58	65	47	73	8.7	NS
N retention	48	53	44	62	33	11.4	NS
<b><u>Blood composition (mmol/litre)</u></b>							
β-hydroxybutyrate	0.39	0.49	0.46	0.47	0.44	0.072	NS
Glucose	4.32	4.27	4.25	4.29	4.30	0.066	NS
Non-esterified fatty acids	0.10	0.08	0.08	0.11	0.10	0.010	NS
Urea	3.80	3.84	3.38	3.82	3.75	0.234	NS

**Conclusions.** The crop used contained insufficient fermentable carbohydrate to sustain the dominance of a lactic acid primary fermentation. Under these conditions, none of the four bacterial inoculant treatments improved *in vivo* digestibility or nitrogen retention by steers, or altered the concentration of blood metabolites.

***Experiments 18 and 19: The potential of silage inoculants with different species and strains of lactic acid bacteria to influence rumen metabolism in steers***

Fitzsimons *et al.* (1992) suggested that *Pediococci* species could be responsible for much of the initial pH fall in silages, thereby facilitating the *Lactobacilli* which are active within a slightly lower pH range. Furthermore, a considerable range existed in the suitability of different lactic acid bacteria to the many conditions prevailing during ensilage. The present experiments evaluated the potential of *Pediococcus* species and *Lactobacillus plantarum* strains selected from Irish silages, and which performed well when

evaluated in laboratory silos, to alter fermentation and degradation kinetics in the rumen, and nitrogen utilisation and blood composition of steers.

**Materials and Methods.** Unwilted grass of low water soluble carbohydrate (WSC) concentration (16 g/kg aqueous phase) and high buffering capacity (527 m Eq/kg dry matter (DM)) and *in vitro* DM digestibility (756 g/kg) was ensiled following treatment with: (A) no additive (negative control), (B) *Lactobacillus plantarum* (Ecosyl, Zeneca Bioproducts Ltd.; positive control), (C) *L. plantarum* (DCU 101), (D) *Pediococcus* spp. (G24) or (E) *L. plantarum* (DCU 101) + *Pediococcus* spp. (G24). Additives were applied at approximately 3.1 l/t grass.

In Experiment 18, five Friesian steers (350 kg liveweight) were offered their respective silages in a 5 (treatments) x 5 (28 d periods) Latin square design study. Cattle received 2 kg concentrates per head daily. Silages were offered *ad libitum* for 19 d and intake was then restricted to 0.9 of *ad libitum*. The allowance of concentrates was restricted to ensure similar forage to concentrate ratios as observed in a parallel animal production study (Shiels *et al.*, 1996). Blood samples were collected at intervals over 24 h on day 18 via in-dwelling jugular catheters, while urine and faeces were collected between days 21 and 28. In a parallel study (Experiment 19), Friesian steers (643 kg liveweight) fitted with permanent rumen canulae were used in a 5 (treatments) x 5 (28 d periods) Latin square design to examine rumen digestion. Animals were offered silage *ad libitum* supplemented with 2 kg concentrates per head daily for 20 days, before total DM intake was reduced by proportionately 0.1 as described above, and forage:concentrate ratio adjusted to the values obtained by Shiels *et al.* (1996).

**Results.** For each of silages A to E, the mean (sd) respective compositions were as follows: DM 174 (6.4), 176 (5.3), 176 (6.9), 169 (7.0) and 171 (8.2) g/kg; pH 3.95 (0.186), 3.93 (0.129), 3.90 (0.161), 4.03 (0.161) and 3.99 (0.130); lactic acid 116 (28.1), 129 (22.2), 130 (21.6), 109 (17.3) and 115 (19.4) g/kg DM; acetic acid 34 (18.8), 32 (15.0), 28 (15.4), 39 (13.7) and 38 (15.3) g/kg DM; and ammonia-N 87 (7.4), 85 (7.6), 75 (8.0), 85 (9.7) and 86 (6.8) g/kg N. Silage additive treatments did not affect nitrogen metabolism or blood composition (Table 25), or the kinetics of DM digestion (Table 26). The *Pediococcus* spp. and the combined *Pediococcus* spp. + *L. plantarum* additive treatments increased ( $P < 0.05$ ) acetate to propionate ratio and tended to increase the non-glucogenic ratio in rumen fluid. Silage additive treatment did not affect rumen degradability of silage DM, neutral detergent fibre or nitrogen.

**Conclusions.** These results support the conclusions of Shiels *et al.* (1996) that under the prevailing conditions the additive treatments studied did not improve silage nutritive value. The small shift recorded in fermentation products was not sufficient to influence nutritive value significantly.

**Table 25.** Nitrogen utilisation and blood composition of cattle in Experiment 18

	No additive	<i>L.plant.</i> (control)	<i>L.plant.</i> (DCU101)	<i>Ped. spp</i> (G24)	<i>L.plant.+Ped. spp.</i> (DCU101 + G24)	sem	Sign.
Feed intake (kg/d)							
Silage DM	5.0	5.0	5.0	4.8	4.9	0.15	NS
Concentrate DM	1.6	1.6	1.6	1.5	1.5	0.03	NS
Nitrogen utilisation (g/d)							
Ingested N	151	151	151	149	148	3.2	NS
Digested N	106	108	107	107	106	3.8	NS
Urine N	51	44	53	41	40	4.7	NS
N balance	55	64	54	66	66	5.4	NS
Blood composition (m mol/l)							
β hydroxybutyrate	0.46	0.44	0.43	0.42	0.42	0.018	NS
Glucose	4.52	4.51	4.56	4.51	4.50	0.050	NS
Non-esterified fatty acids	0.09	0.09	0.09	0.09	0.09	0.007	NS
Urea	2.88	3.07	2.91	3.15	3.06	0.100	NS

**Table 26.** Rumen fermentation and kinetics of DM digestion in Experiment 19

	No additive	<i>L.plant.</i> (control)	<i>L.plant.</i> (DCU101)	<i>Ped. spp</i> (G24)	<i>L.plant.+Ped. Spp.</i> (DCU101 + G24)	sem	Sign.
Feed intake (kg/d)							
Silage DM	4.7	5.0	5.2	5.2	4.9	0.25	NS
Concentrate DM	1.6	1.7	1.6	1.7	1.6	0.08	NS
Rumen fermentation							
pH	6.53	6.53	6.58	6.58	6.61	0.036	NS
Lactic acid (mg/l)	66	106	65	128	73	32.8	NS
NH <sub>3</sub> (mg/l)	121	131	122	122	99	16.3	NS
Total VFA (mmol/l)	69	68	70	72	70	2.1	NS
Acetate/propionate	3.88 <sup>a</sup>	3.84 <sup>a</sup>	3.84 <sup>a</sup>	4.23 <sup>b</sup>	4.23 <sup>b</sup>	0.105	*
Non-glucogenic ratio	4.75	4.86	4.86	5.24	5.28	0.120	NS <sup>1</sup>
Kinetics of DM digestion							
Average pool size (kg)	6.35	6.48	6.40	6.27	6.18	0.462	NS
Ki (%/h)	4.60	4.73	5.23	5.23	4.85	0.446	NS
Kp (%/h)	1.40	1.21	1.37	1.32	1.30	0.141	NS
Kd (%/h)	3.20	3.52	3.86	3.91	3.55	0.356	NS

VFA = volatile fatty acids; non-glucogenic ratio = (acetate + (2 x butyrate))/propionate;

Ki = rate of intake, Kp = rate of passage; Kd = rate of digestion; <sup>1</sup>P=0.067

### **Experiments 20 and 21: Rumen digestion in steers offered three grass silages supplemented with two levels of concentrates**

Bacterial inoculants can increase the nutritive value of grass silage for beef cattle (O'Kiely, 1996), but the effects are not consistent (Shiels *et al.*, 1996). Shiels *et al.* (1996) concluded that where untreated silage was imperfectly preserved, bacterial inoculant treatment did not improve animal performance whereas formic acid did. However, the latter effect tended to be reduced when concentrate inputs were increased. The objectives of the present experiments were to determine the effects of silage additive and



of supplementary concentrates on fermentation, degradation kinetics in the rumen, nitrogen utilisation and blood composition.

**Materials and Methods.** Unwilted grass of low water soluble carbohydrate (WSC) concentration (19 g/kg aqueous phase) and high buffering capacity (593 mEq/kg dry matter (DM)), crude protein concentration (200 g/kg DM) and *in vitro* DM digestibility (793 g/kg) was ensiled without additive (N), with the addition of formic acid (850 g/kg; 2.7 l/t; F) or a lactic acid bacterial inoculant (*Lactobacillus plantarum*; Ecosyl @ 3.3 l/t; I). The silages were offered *ad libitum* together with 0 or 3 kg concentrates per head daily to six Friesian steers in each of two parallel 6 (treatments) x 6 (periods) Latin square experiments. For the final 10 days of each 28 day period, silage DM intake was restricted to 0.9 of *ad libitum* and the concentrate allowances restricted to ensure similar forage to concentrate ratios as observed by Shiels *et al.* (1996) in finishing heifers offered the same silages. In Experiment 20, blood samples were obtained from the cattle (457 kg liveweight) over a 24 h interval on day 18 via in-dwelling jugular catheters, while urine and faeces were collected between days 21 and 28 of each period. In Experiment 21, cattle (720 kg liveweight) with permanent rumen canula were used for determination of silage degradability by *in sacco* incubation. Rumen fermentation characteristics were measured on fluid samples obtained at 9 times on each of days 20 and 22 of each period. Liquid and solid phase outflow rates were estimated using Co-EDTA and Yb, respectively, while rumen pool size was determined immediately before feeding and 3 and 7 hours post feeding by manual evacuation on days 26, 23 and 28 of each period, respectively.

**Results.** The N, F and I silages had mean (sd) respective compositions as follows: DM 169 (4.4), 188 (9.6) and 172 (6.6) g/kg; pH 4.4 (0.10), 4.0 (0.08) and 4.2 (0.28); lactic acid 64 (14.3), 83 (11.2) and 93 (32.9) g/kg DM; acetic acid 60 (7.5), 17 (3.0) and 39 (14.8) g/kg DM; and ammonia-N 80 (13.4), 48 (16.6) and 79 (16.1) g/kg N. Nitrogen utilisation and blood composition results are summarised in Table 27, while rumen fermentation and kinetics of DM digestion are summarised in Table 28. Neither silage additive nor concentrate supplementation affected rumen degradability (i.e. soluble fraction, potentially degradable insoluble fraction or rate of degradation) of silage DM, neutral detergent fibre or nitrogen. Rumen liquid pool size and liquid passage rates were not affected by additive or concentrate supplementation treatments, whereas rumen DM pool size was increased by supplementation.

**Conclusions.** The results support the conclusions of Shiels *et al.* (1996) that, under the conditions prevailing, formic acid rather than inoculant treatment improved silage nutritive value, particularly in the absence of concentrate supplementation. The improved kinetics of rumen digestion and passage in response to formic acid would appear the most likely explanation for the increased silage intake and resultant animal performance reported by Shiels *et al.* (1996).

**Table 27.** Nitrogen utilisation and blood composition of cattle in Experiment 20.

	Silage additive (S)				Concentrate (C)			Significance		
	No additive	Formic acid	Inoculant	sem	0	+	sem	S	C	SxC
Feed intake (kg/d)										
Silage DM	5.9 <sup>ab</sup>	6.2 <sup>a</sup>	5.7 <sup>b</sup>	0.13	6.1	5.8	0.01	*	*	NS
Concentrate DM	1.1	1.1	1.1	0.03	0	2.3	0.02	NS	***	NS
Nitrogen utilisation (g/d)										
Ingested N	201 <sup>ab</sup>	210 <sup>a</sup>	195 <sup>b</sup>	3.5	185	220	2.8	*	***	NS
Digested N	151	158	146	3.4	139	165	2.8	NS <sup>1</sup>	***	NS
Urine N	56	58	60	7.0	60	56	5.7	NS	NS	NS
N balance	95	100	86	7.4	78	109	6.0	NS	**	NS
PD excretion (m mol/d)	170	176	195	14.4	177	183	11.8	NS	NS	NS
Microbial N (g/d)	79	83	94	8.9	84	87	7.3	NS	NS	NS
(g/kg DOMR)	25	23	29	3.0	29	22	2.5	NS	NS <sup>1</sup>	NS
Blood composition (m mol/l)										
β hydroxybutyrate	0.49	0.52	0.48	0.024	0.43	0.56	0.020	NS	***	NS
Glucose	4.42	4.42	4.44	0.036	4.36	4.50	0.029	NS	**	NS
Non esterified fatty acids	0.10	0.11	0.08	0.012	0.10	0.10	0.009	NS	NS	NS
Urea	4.39 <sup>a</sup>	4.59 <sup>a</sup>	3.96 <sup>b</sup>	0.120	4.16	4.48	0.098	**	*	NS

PD = purine derivative; DOMR = organic matter apparently digested in the rumen (= 0.65 intake of digested organic matter [ARC 1984]); <sup>1</sup>P=0.07

**Table 28.** Rumen fermentation and kinetics of DM digestion in cattle in Experiment 21.

	Silage additive (S)				Concentrate (C)			Significance		
	No additive	Formic acid	Inoculant	sem	0	+	sem	S	C	SxC
Feed intake (kg/d)										
Silage DM	5.5	6.1	5.8	0.20	6.0	5.6	0.17	NS <sup>1</sup>	NS <sup>2</sup>	NS
Concentrate DM	1.1	1.1	1.1	0.04	0	2.2	0.03	NS	***	NS
Rumen fermentation										
pH	6.59	6.51	6.53	0.046	6.60	6.49	0.037	NS	*	NS
Lactic acid (mg/l)	96	161	150	20.8	123	148	17.0	NS <sup>1</sup>	NS	NS
NH <sub>3</sub> (mg/l)	170	178	180	5.9	179	173	4.9	NS	NS	NS
Total VFA (mmol/l)	79	82	81	1.5	79	83	1.2	NS	*	NS
Acetate/propionate	4.07	3.63	3.77	0.125	3.78	3.86	0.102	NS <sup>2</sup>	NS	NS
Non-glucogenic ratio	5.19	4.77	4.84	0.151	4.79	5.08	0.123	NS	NS	NS
Kinetics of DM digestion										
Average pool size (kg)	6.8	6.4	6.6	0.20	6.4	6.8	0.17	NS	*	NS
Ki (%/h)	4.43	5.18	4.65	0.166	4.35	5.61	0.136	NS	NS	NS
Kp (%/h)	1.13 <sup>a</sup>	1.27 <sup>b</sup>	1.18 <sup>ab</sup>	0.053	1.15	1.23	0.043	**	***	NS
Kd (%/h)	3.30 <sup>a</sup>	3.91 <sup>b</sup>	3.47 <sup>a</sup>	0.147	3.20	3.93	0.120	*	***	NS

VFA = volatile fatty acids; Ki = rate of intake; Kp = rate of passage; Kd = rate of digestion; <sup>1</sup>P=0.09, <sup>2</sup>P=0.06

## 7. ENSILING ALTERNATIVE CROPS TO GRASS

A series of experiments were conducted to evaluate the conservation characteristics and/or nutritive value for beef cattle of silages made from crops other than grass. These included whole crop maize and wheat, as well as potato tubers.

### *Experiments 22 to 24: Nutritive value for beef cattle of maize silage made from physiologically immature crops*

Maize silage has been an integral component of many beef cattle and dairy cow feeding systems throughout the world for several decades. It is much less predominant in Ireland, where temperature is the primary environmental influence on its development, and crops range from those with extensive grain development to those where little or no physiological maturation of grain occurs. The latter are characterised by low concentrations of dry matter (DM) and starch, and extensive fermentations. Grass silages in Ireland also have lower DM concentrations and more extensive fermentations than in many mainland European countries. The experiments reported here compared the performance of beef cattle offered physiologically immature forage maize silage, unwilted grass silage or combinations of the two silages, and determined the effects of a bacterial inoculant additive on the nutritive value of such a maize silage.

**Materials and methods.** Forage maize was sown on May 23 (LG 2080), May 4 (LG 2080 and Diablo mixture) and May 7 (Appache) in successive years and correspondingly harvested on October 28, October 20 and October 11, in Experiments 22, 23 and 24, respectively. Crops were harvested about 10 days after ground frost, using a precision-chop harvester and stored under plastic in horizontal, roofed silos. In Experiment 23, alternate loads of forage maize were ensiled with no additive or following application of a bacterial inoculant (Ecocorn, Zeneca Bio Products Ltd.).

In Experiment 22, sixty Hereford x Friesian steers (481 kg mean starting liveweight) were offered one of four forage treatments: 100% grass silage (GS), 67% GS + 33% maize silage (MS), 33% GS + 67% MS and 100% MS. Experiment 24 had a similar design, and involved fifty-six Charolais x Friesian heifers (253 kg mean starting liveweight). In Experiment 23, sixty-nine weanling steers (265 kg mean starting liveweight) were offered untreated or treated maize silage or unwilted grass silage. Forages were offered *ad libitum*, supplemented with 3, 1 and 1.5 kg concentrates/head daily in Experiments 22, 23 and 24, respectively, for corresponding durations of 87, 126 and 105 days.

**Results.** In Experiments 22 and 23, respectively, crop yields, dry matter (DM) concentrations and effluent production were 56.5 and 43.1 t/ha, 200 and 172 g DM/kg and 130 and 103 l/t. Silage composition and animal productivity results are summarised in Tables 29 and 30, respectively. Maize silages were physiologically immature, having low concentrations of DM and starch (<10 g/kg DM), and having undergone extensive, lactic acid-dominant fermentations. Their *in vitro* digestibilities were much lower than pre-ensiling. Unwilted grass silages had undergone lactic acid type fermentations in Experiments 22 and 23, but there was evidence of extensive clostridial activity in Experiment 24. Grass silage digestibilities ranged from high (Experiment 22) to low (Experiments 23 and 24). Aerobic stability assessments of maize and grass silages gave mean (sd) accumulated temperature rise values to day 5 of 92 (3.3) and 20 (6.0), 65 (14.0) and 54 (17.2), and 75 (23.7) and 19 (0.2)°C for Experiments 22, 23 and 24, respectively. The relative nutritive value of maize and grass silages varied - maize silage was inferior to good quality grass silage (Experiment 22) and superior to inferior quality grass silages (Experiments 23 and 24). The improvement in animal liveweight gain in response to inoculant additive was not statistically significant.

**Conclusions.** Physiologically immature forage maize readily underwent a lactic acid-dominant fermentation. Maize silage was less stable aerobically than grass silage. The nutritive value for beef

cattle of physiologically immature maize silage was inferior to that reported in the literature for maize silage of high starch concentration, and was equivalent to average quality unwilted grass silage. The lactic acid bacteria inoculum did not significantly improve the nutritive value of maize silage.

**Table 29.** Mean chemical composition of silages in each experiment.

	Experiment 22		Experiment 23			Experiment 24	
	Maize	Grass	Maize		Grass	Maize	Grass
			Uninoculated	Inoculated			
<i>Silage composition</i>							
DM (g/kg)	205	208	212	201	182	200	177
PH	3.84	3.71	3.92	3.98	3.80	3.7	4.7
Lactic acid (g/kg DM)	89	130	88	91	125	101	27
Acetic acid (g/kg DM)	25	33	25	28	20	26	43
Propionic acid (g/kg DM)	0	2	1	1	2	0.3	10
Butyric acid (g/kg DM)	0	0	1	1	2	0.4	16
Ethanol (g/kg DM)	19	11	22	27	10	12	7
WSC (g/kg DM)	27	30	61	41	10	31	11
NH <sub>3</sub> -N (g/kg N)	77	80	60	59	73	64	237
Buf. Capacity (mEq/kg DM)	509	717	729	731	966	---	---
Crude protein (g/kg DM)	133	147	138	145	142	132	150
NDF (g/kg DM)	584	501	---	---	---	---	---
ADF (g/kg DM)	342	310	---	---	---	---	---
DMD <i>in vitro</i> (g/kg)	612	746	693	662	641	703	648
OMD <i>in vitro</i> (g/kg)	609	741	688	664	637	666	602
Ash (g/kg DM)	67	79	66	71	90	65	94

**Table 30.** Silage intake and animal performance.

	Liveweight gain (g/day)	Carcass gain (g/day)	Silage intake (kg/day)
Experiment 22			
0% MS	1385	870	6.1
33% MS	1384	829	7.2
67% MS	1371	745	7.1
100% MS	1068	633	6.1
SEM	62.7***	32.4***	0.18***
Experiment 23			
GS	491	---	4.4
Untreated MS	769	---	5.9
Treated MS	821	---	5.8
SEM	22.2***	---	0.05***
Experiment 24			
0% MS	746	---	4.1
33% MS	840	---	4.9
67% MS	844	---	5.1
100% MS	895	---	5.3
SEM	27.9**	---	0.15***

### ***Experiment 25 and 26: Comparison of laboratory methods for estimating dry matter digestibility of immature forage maize***

These experiments were conducted in collaboration with Dr. Thomas Keating, and with Dr. Jim O'Grady (IAWS).

Under suitable conditions forage maize can provide high yields of nutritious, readily ensilable feedstuff and is an attractive alternative to grass as a silage crop. The change in the digestibility of grass or physiologically mature maize during ensilage can be negligible where good silage-making practices prevail (Dulphy and Demarquilly, 1991), whereas bad ensilage techniques can result in a considerable decrease in digestibility (Flynn, 1981). When grown under sub-optimal conditions, maize may be physiologically immature at harvesting, resulting in a forage of low starch content. The yield, nutritive value and economic value of such crops tend to be disappointing. Previous results indicated that whereas physiologically immature maize readily underwent a lactic acid dominant fermentation and had a conservation efficiency comparable to good grass silage, the change in dry matter digestibility during ensilage, as estimated by the *in vitro* technique of Tilley and Terry (1963), was greater than anticipated. The present experiments sought to test if the change was an artifact of the assay, and compared the changes as measured using this assay with two other methods.

**Materials and methods.** Immature forage maize (< 50 g/kg crop dry matter (DM) present in cob) was ensiled in laboratory (Experiment 25) and farm (Experiment 26) scale silos. Samples were taken at ensiling and silo opening (over 100 days ensilage), dried at 40°C for 48 h, ground through a 1 mm screen, and assessed for DM digestibility by the following 3 methods:

- (a) incubation in rumen fluid (48 h) followed by acid pepsin (48 h) (Tilley and Terry, 1963 with the modification that the final residue was separated by filtration rather than centrifugation),
- (b) neutral cellulase gammanase digestion (MAFF, 1992), and
- (c) pepsin cellulase digestion (De Boever *et al.*, 1986).

**Results and Discussion.** The mean composition of the maize at ensiling in Experiment 25 was 216 g DM/kg, 100 g crude protein/kg DM, 285 g acid detergent fibre/kg DM, 60 g ash/kg DM, 171 g WSC/kg DM, a buffering capacity of 283 mEq/kg DM and no starch, while the corresponding values in Experiment 26 were 195 g DM/kg, 134 g/kg DM, 225 g/kg DM, 61 g/kg DM, 202 g/kg DM, 333 mEq/kg DM and no starch. Silages in both experiments were well preserved with no evidence of aerobic deterioration problems. Silage fermentation characteristics in Experiment 26 were pH 3.9, 64 g lactic acid/kg DM, 32 g acetic acid/kg DM, 91 g ethanol/kg DM and 51 g NH<sub>3</sub>-N/kg N, while the corresponding values in Experiment 2 were 3.92, 88 g/kg DM, 25 g/kg DM, 22 g/kg DM and 60 g/kg N. The comparison of laboratory methods for estimating digestibility is summarised in Table 31. The results indicate that the Tilley and Terry technique tended to give the highest values and the pepsin cellulase technique the lowest. All three techniques estimated considerably larger decreases in digestibility during the ensilage of immature forage maize than anticipated, based on prior experience with grass.

It is concluded that the large depression recorded in digestibility during ensilage was not an artifact of the Tilley and Terry (1963) assay.

**Table 31.** Comparison of laboratory methods for estimating digestibility of immature forage maize - mean (s.d.).

Stage of ensiling	Laboratory silos (Experiment 25)			Farm silos (Experiment 26)		
	Pre (n=3)	Post (n=3)	Change	Pre (n=3)	Post (n=6)	Change
Assay						
<i>In vitro</i> DMD <sup>1</sup> (g/kg)	697(5.5)	636(11.1)	-61	762(6.7)	693(10.6)	-69
NCGD <sup>2</sup> (g/kg)	643(12.5)	571(14.4)	-72	759(2.9)	714(8.3)	-45
PCD <sup>3</sup> (g/kg)	616(18.6)	569(13.9)	-47	730(8.7)	677(18.4)	-53
SEM	7.7	14.1		3.8	5.4	
Sig	***	*		**	***	

<sup>1</sup>Dry matter digestibility      <sup>2</sup>neutral cellulase gammanase digestibility      <sup>3</sup>pepsin cellulase digestibility

**Experiment 27: Nutritive value of maize and grass silage for beef cattle when offered alone or in mixtures**

Maize silage grown in Ireland can vary considerably in physiological maturity at harvesting. Crops of low starch content support poorer growth rates by beef cattle than top quality grass silage (O'Kiely and Moloney, 1995) whereas crops of high starch content support superior performance with dairy cows (Fitzgerald *et al.*, 1998). A mixed forage containing both maize and grass silage was concluded to be better for milk production by dairy cows than a forage of maize silage alone (Fitzgerald *et al.*, 1998). The experiment reported here quantified the nutritive value of each of three maize silages (produced under different management regimes) when offered to finishing beef heifers as the sole forage or in a mixture with grass silage, and contrasted it with a top quality grass silage offered as the sole forage. The experiment was not designed to provide a balanced contrast of the effects of crop management regime.

**Material and Methods.** Maize (Hudson) was sown in separate fields at Baltray, Co. Louth on 18 April (under plastic; M1), 21 April (M2) and 14 May (M3) and managed as in good commercial farm practice. The 3 crops were direct-cut (20cm stubble height) precision-chop (+ kernal cracker) harvested on 22 October. Representative maize plants were separated into their morphological components. Unwilted grass (G) was precision-chopped on 21 May. All silages were individually stored beneath 2 layers of polythene sheeting in roofed bunker silos at Grange Research Centre. The maize and grass silages were stored for 188 and 342 days, respectively, before feeding commenced. Conservation losses were estimated by both the total in/out and buried bag techniques and representative samples were simultaneously ensiled in lab silos. Aerobic stability was assessed at 20°C over 8 days. One hundred and five Charolais crossbred heifers were allocated among 7 forage treatments in a randomised complete block design. The forages offered were maize (three separate silages) or grass silage alone, or a 50:50 mixture (dry matter (DM) basis) of each maize with grass silage. Fresh silage was individually offered *ad libitum* to each heifer daily for 170 days. Each animal was individually offered 3kg concentrates (310 g citrus pulp, 460 g barley, 160 g soybean meal, 50 g molasses and 20 g min. + vit./kg) daily, in equal morning and afternoon feeds. All animals were blood sampled at 8 am and 2 pm on day 136. *In vivo* digestibility was determined with 12 Friesian steers on 4 occasions using the total faecal collection procedure.

**Results.** Mean yields of harvested DM were 4.8, 4.6 and 5.1 t/ha for M1, M2 and M3, respectively. All maize crops had good grain development and all silages were well preserved and of high nutritive value (Table 32). Heifers offered maize silages alone had higher ( $P<0.05$ ) intakes and carcass growth rates than those offered grass silage (Table 33). Animals offered the M1/G mixture had a higher intake ( $P<0.05$ ) but a similar carcass gain to the animals offered M1 alone. Heifers offered M2/G or M3/G had similar intakes but lower ( $P<0.05$ ) carcass gains than those offered M2 or M3, respectively. The carcass gains for M2/G and M3/G did not differ ( $P<0.05$ ) from G. *In vivo* dietary DM digestibilities were highest for G, and tended to be lowest for diets based on maize as the sole silage. Blood plasma urea concentrations were highest ( $P<0.05$ ) for heifers offered G, and tended to be lower for animals offered maize alone compared to M/G mixtures. Feed DM intake tended to be converted to carcass gain most efficiently for G, intermediary for maize alone and least efficiently for M/G mixtures.

**Conclusions.** All three maize silages had higher feeding values than a good quality grass silage. Significant improvements in animal productivity from mixing maize and grass silage prior to feeding did not occur.

**Table 32.** Mean (s.d.) fresh and ensiled maize and grass composition, and conservation characteristics.

	M1		M2		M3		G	
	Fresh	Silage	Fresh	Silage	Fresh	Silage	Fresh	Silage
<b>Physical composition</b>								
Crop height (above 20 cm stubble; cm)	215(9.8)	-	208(15.1)	-	231(8.1)	-	-	-
DM – grain + cob (g/kg)	506(14.0)	-	421(10.7)	-	381(17.3)	-	-	-
- remainder (g/kg)	268(2.1)	-	222(4.0)	-	218(3.5)	-	-	-
Proportions of crop DM in grain + cob	581(81.6)	-	529(71.9)	-	510(25.8)	-	-	-
<b>Chemical composition</b>								
Dry matter (g/kg)	362(7.4)	375(7.6)	287(2.0)	297(7.6)	253(4.3)	256(5.6)	152(4.6)	180(4.2)
Crude protein (g/kg DM)	91(2.1)	95(1.8)	101(2.1)	105(2.4)	112(3.3)	110(3.8)	-	166(7.1)
DMD <i>in vitro</i> (g/kg)	-	795(18.7)	-	793(11.7)	-	790(15.3)	-	744(15.2)
OMD <i>in vitro</i> (g/kg)	-	793(21.6)	-	788(14.7)	-	786(15.4)	-	737(17.1)
NDF (g/kg DM)	-	326(19.2)	-	366(23.4)	-	416(26.7)	-	508(30.0)
ADF (g/kg DM)	-	148(8.0)	-	171(10.0)	-	188(11.6)	-	317(21.3)
Ash (g/kg DM)	-	49(2.0)	-	53(1.7)	-	61(4.3)	-	90(11.3)
Starch (g/kg DM)	-	446(13.7)	-	379(23.3)	-	332(27.5)	-	-
WSC (g/kg DM)	-	19(5.1)	-	15(1.8)	-	16(2.0)	-	20(3.0)
Lactic acid (g/kg DM)	-	56(6.0)	-	71(6.4)	-	51(11.2)	-	100(13.8)
Ammonia-N (g/kg N)	-	74(10.7)	-	69(5.9)	-	77(13.4)	-	96(9.9)
Buffering capacity (mEq/kg DM)	213(2.4)	401(13.6)	240(2.8)	475(37.4)	240(22.3)	464(30.2)	-	613(46.6)
pH	-	3.9(0.10)	-	3.8(0.09)	-	4.1(0.09)	-	3.8(0.10)
<b>Aerobic stability</b>								
Days to pH rise	-	2.8(1.16)	-	2.8(0.46)	-	2.6(1.06)	-	1.5(0.53)
Days to pH max.	-	7.6(0.92)	-	6.5(1.69)	-	5.8(1.98)	-	4.6(1.06)
Days to °C rise	-	2.5(0.93)	-	2.1(0.35)	-	1.8(0.89)	-	1.3(0.46)
Days to °C max.	-	5.0(2.51)	-	4.5(0.93)	-	5.1(1.55)	-	3.9(0.64)
Acc. °C rise to day 5	-	77(46.1)	-	75(25.4)	-	69(17.4)	-	131(26.1)
<b>Recovery ex-silo (DM; g/kg)</b>								
Total (edible) out/in	-	990(875) <sup>1</sup>	-	981(891) <sup>1</sup>	-	905(837) <sup>1</sup>	-	739(694) <sup>1</sup>
Buried bags	-	1017(22.2)	-	961(15.2)	-	934(33.4)	-	-

<sup>1</sup>value in parentheses is mean rate of recovery of edible silage DM, and not s.d. M1, M2 and M3 are the 3 maize crops, and G is the grass crop.

**Table 33.** Intake, digestibility, growth, carcass and blood traits and efficiencies.

Forage	M1	M2	M3	M1/G	M2/G	M3/G	G	SEM	Signif.
<b>Intake of DM</b>									
- silage : kg/day	6.9	6.9	6.7	7.5	6.5	6.3	5.1	0.16	***
g/kg liveweight	13.3	13.0	12.6	14.1	12.5	12.1	10.0	0.27	***
- total <sup>1</sup> : kg/day	9.5	9.6	9.3	10.1	9.1	8.9	7.8	0.16	***
g/kg liveweight	18.3	18.0	17.6	19.1	17.6	17.2	15.1	0.26	***
<i>In vivo</i> dietary DMD (g/kg)	702	682	701	722	721	725	749	7.3	***
<b>Liveweight</b>									
- start (kg)	443	443	443	442	442	442	443	1.0	NS
- end (kg)	598	615	615	620	596	595	589	6.5	**
- daily gain (g)	912	1015	1011	1046	907	896	846	38.3	**
<b>Carcass weight (hot)</b>									
- end ((kg)	334	341	338	337	327	327	324	3.8	*
- daily gain (g)	716	756	740	738	678	677	653	22.4	*
Carcass gain/liveweight gain (g/kg)	790	748	733	712	764	766	780	17.2	*
Kill-out rate (g/kg)	559	554	550	544	550	551	552	3.6	NS
Kidney + channel fat weight (kg)	10.8	13.9	12.0	11.7	14.0	12.7	13.8	0.79	*
Carcass conformation <sub>2</sub> grade <sup>1</sup>	3.0	2.7	2.9	2.9	3.0	2.9	2.9	0.09	NS
Carcass fatness grade <sup>2</sup>	4.7	4.1	4.6	4.7	4.3	4.8	4.1	0.13	***
<b>Blood plasma (mmol/l)</b>									
- Glucose	4.2	4.1	4.1	4.1	4.0	4.0	4.2	0.08	NS
- Urea	2.2	1.9	2.5	2.8	2.6	2.8	3.4	0.16	***
<b>Feed efficiency (total)<sup>1</sup></b>									
- DM intake/liveweight gain	10.7	9.6	9.3	9.8	10.4	10.3	9.4	0.36	NS
- DM intake/carcass gain	13.5	12.8	12.8	13.8	13.6	13.4	12.0	0.38	*

<sup>1</sup>assuming 880 g DM/kg concentrates

**Experiment 28: Optimal growth stage and dry matter concentration for urea or propionic acid treatment of whole crop wheat at ensiling**

Whole crop wheat (WCW) can be successfully ensiled without additives after ear emergence. As growth stage progresses and crop dry matter (DM) concentration increases, the subsequent ensilage process would be expected to alter from an extensive lactic acid fermentation to a more restricted one. Whole crop wheat silages are often considered prone to aerobic deterioration, and additives based on propionic acid or urea (as an indirect source of ammonia) are among possible strategies for reducing the scale of this problem. Urea addition to physiologically immature WCW crops should accentuate the extensive fermentation and could facilitate clostridial activity, while with mature WCW it should lead to a high pH preserved forage. The ammonia derived from urea has a strong affinity for water, and when applied to wet WCW crops, urea addition may result in reduced DM intake. This experiment determined the effects of crop growth stage on silage fermentation, and quantified the interaction of urea or propionic acid based additives on both the estimated nutritive value and conservation characteristics of WCW.

**Materials and Methods.** Winter wheat (var. Brigadier) was sown in mid-October and managed as for commercial grain production, with the exception that a growth regulator was not applied. Representative samples of WCW were mown on 3, 17 and 31 July, 14 August and 3 September, and precision-chop harvested. On each harvesting date, sub-samples (each 1.5kg crop DM) were ensiled in laboratory silos (O’Kiely and Wilson, 1991) with the following additive treatments: no additive (NA), urea at 20 (UL) or 40 (UH) g/kg crop DM, or a propionic acid based additive at 2.5 (PL) or 5.0 (PH) ml/kg. The four silos per treatment were stored at 15°C. Data were analysed as a 5 (harvest dates) x 5 (additive treatments) factorial design.

**Results and Discussion.** For silages made from WCW on the five successive harvest dates, mean (s.e.) corresponding DM concentrations were 247, 326, 364, 484 and 615 (s.e. 2.8) g/kg, *in vitro* DM digestibilities were 670, 683, 637, 628 and 572 (s.e. 4.1) g/kg, acetic acid concentrations were 20, 11, 5, 4 and 2 (s.e. 0.4) g/kg DM and the accumulated temperature rise during 5 days aerobic exposure were 37, 2, 17, 18 and 6 (s.e. 1.1)°C. For the additive treatments NA, UL, UH, PL and PH, the accumulated temperature rises to day 5 were 21, 13, 14, 15 and 17 (s.e. 1.1)°C, respectively. Table 34 gives the results of other analyses. Silage made without additive at each harvest date was satisfactorily preserved. In parallel with increased DM concentration at successive harvest dates, fermentation became progressively restricted as evidenced by reduced concentrations of lactic acid, acetic acid and ammonia-N. Silage crude protein concentration also decreased with advancing maturity. Increased rates of urea addition progressively increased the concentrations of crude protein and ammonia-N, although this effect became smaller with the more advanced stages of crop maturity. On both 17 and 31 July, urea addition resulted in more extensive lactic acid fermentations. However, as growth stage advanced further, incremental rates of urea addition led to progressively higher silage pH values. The propionic acid based treatments had relatively minor effects on silage chemical composition. However, additives generally reduced the scale of aerobic deterioration.



**Table 34.** Individual treatment effects on selected silage composition variables.

Harvest date (H)	Additive (A)	pH	Lactic acid (g/kg DM)	NH <sub>3</sub> -N (g/kg DM)	Crude protein (g/kg DM)
3/7	NA	4.1	102	1.1	123
	UL	4.0	106	2.6	176
	UH	4.1	108	4.1	234
	PL	3.9	102	0.9	118
	PH	3.9	95	0.7	116
17/7	NA	3.9	78	1.2	114
	UL	4.2	81	5.6	168
	UH	4.3	102	8.7	212
	PL	3.9	59	1.3	117
	PH	3.8	58	1.4	118
31/7	NA	3.9	24	0.4	118
	UL	4.2	39	2.6	163
	UH	5.0	40	5.6	162
	PL	3.8	25	0.5	116
	PH	3.8	24	0.6	117
14/8	NA	4.2	17	0.3	106
	UL	5.0	23	2.9	135
	UH	7.8	13	3.2	154
	PL	4.2	17	0.4	107
	PH	4.2	15	0.4	109
3/9	NA	4.3	9	0.2	108
	UL	6.5	6	1.1	139
	UH	8.8	2	2.1	156
	PL	4.6	8	0.3	104
	PH	4.6	6	0.2	104
s.e.		0.07	3.8	0.29	3.3
Significance					
H		***	***	***	***
A		***	***	***	***
H x A		***	***	***	***

**Conclusions.** As the winter wheat crop matured through July and August, the resultant satisfactory silage preservation progressively changed from being due to a relatively high concentration of lactic acid to being due to a combination of reducing concentrations of lactic acid and increasing DM concentrations (and thus the lowering of water activity). The propionic acid based treatment had relatively little effect on fermentation characteristics. The effects of urea addition on fermentation were strongly related to the stage of crop maturity at harvest. Both additive types conferred some positive effect on aerobic stability, while the urea treatments increased silage crude protein concentration. This latter effect also interacted with the stage of crop maturity at harvest.

**Experiment 29: Aerobic stability of whole-crop wheat silage following addition of heterofermentative lactic acid bacteria**

Initiation of aerobic deterioration of silage at feedout is most commonly attributed to yeast, the latter being quite acid-tolerant. Bacterial inoculants applied at silage-making normally contain homofermentative lactic acid bacteria (LAB) which generally confer little benefit to subsequent silage aerobic stability. Inoculation of forage with heterofermentative LAB at ensiling, possibly using specific strains, might increase the concentration of acetic acid (and possibly other products). Acetic acid, even though a weaker acid than lactic acid (pKa 4.64 versus 3.75), is more inhibitory to yeast under anaerobic conditions at the pH values pertaining in silage. Whole-crop wheat silage can be prone to aerobic instability at feedout, with urea sometimes being applied as an indirect source of ammonia to contain aerobic deterioration. The aim of the present experiment was to determine the effects of a bacterial inoculant based on the heterofermentative *Lactobacillus buchneri* on the chemical and microbiological composition, conservation efficiency and aerobic stability of whole-crop wheat silage.

**Materials and Methods.** Winter wheat (var. Brigadier), sown at two sites 1.5 km apart, was cut to a 10 cm stubble height and precision-chopped on 8 August (2 sites) and 13 August (1 site). Each crop of whole-crop wheat (WCW) was ensiled in laboratory silos (6 kg WCW/silo; four silos per treatment) without additive or after treatment with an additive containing *Lactobacillus buchneri*, xylanase, galactomannanase,  $\beta$ -glucanase plus sugar and flow agents (ASW 57; Biotal Ltd., Wales). The additive was reconstituted in distilled water and applied at 4 ml/kg crop. Silos were stored at about 15°C for approximately 120 days. Aerobic stability was assessed during 8 days incubation at 20°C.

**Results.** These fermented WCW silages underwent lactic acid dominant fermentations in the absence of additive use (Table 35). Averaged over the three silages, additive treatment reduced the mean concentrations of lactic acid (45 to 35 g/kg DM; sem 2.7, P<0.05) and water soluble carbohydrates (24 to 15 g/kg DM; sem 1.3, P<0.001) and increased acetic acid (9 to 15 g/kg DM; sem 0.9, P<0.001) and starch (127 to 158 g/kg DM; sem 6.6, P<0.01). Yeast and mould were present in all silages (Table 36). Averaged over the three crops, additive treatment increased the total viable count (6.97 to 7.98 log<sub>10</sub> cfu/g; sem 0.197, P<0.01), decreased yeast count (4.96 to 2.70 log<sub>10</sub> cfu/g; sem 0.212, P<0.001) but did not significantly alter mould count (1.27 versus 0.50 log<sub>10</sub> cfu/g; sem 0.319) at silo opening. Recovery rates of ensiled DM (Table 37) were not on average altered by additive (992 versus 990 g/kg; sem 5.9). Silage aerobic stability (Table 38) for the 3 crops combined was improved by additive treatment, as shown by days to pH rise (3.8 to 7.7 days; sem 0.45, P<0.001) and accumulated temperature rise to day 5 (45 to 17°C; sem 2.9, P<0.001).

**Conclusions.** Additive treatment improved the aerobic stability of fermented WCW silage. This was likely due to the reduced numbers of yeast present in silage at silo opening, which in turn appears due to a less homolactic fermentation. The effects on animal performance remain to be quantified.

**Table 35.** Chemical composition of whole crop wheat silages.

Site	A			B			A		
	August 8			August 8			August 13		
Additive applied	None	Additive	SEM	None	Additive	SEM	None	Additive	SEM
Dry matter (g/kg)	349	343	4.1	363	372	4.2	425	420	3.0
Crude protein (g/kg DM)	111	118	1.8*	109	108	0.8	108	110	0.9
DMD (g/kg)	572	570	10.3	644	652	4.5	631	633	7.6
Starch (g/kg DM)	99	137	9.2*	118	159	14.2	163	178	10.5
Lactic acid (g/kg DM)	34	13	6.4	55	53	4.7	46	40	2.0
Acetic acid (g/kg DM)	8	12	2.5	11	19	0.6***	8	15	0.3***
Propionic acid (g/kg DM)	0.5	1.3	0.27	1.1	1.0	0.16	1.3	1.1	0.25
Butyric acid (g/kg DM)	0.1	0.3	0.09	0.3	0.3	0.12	0.1	0.2	0.07
Ethanol (g/kg DM)	10	10	1.6	8	6	0.3**	6	6	0.3
WSC (g/kg DM)	24	17	3.4	21	13	1.4**	27	16	1.2***
NH <sub>3</sub> -N (g/kg N)	59	63	5.5	79	96	3.2**	51	49	2.1
PH	3.33	3.53	0.243	3.80	4.03	0.018***	3.93	4.00	0.018*

**Table 36.** Microbiological analysis of silages (at silo opening) - log<sub>10</sub> colony forming

units/g silage.

Site	Harvest date	Assay	No additive	Additive	SEM
A	August 8	Total viable count	7.07	8.06	0.442
		Yeast count	5.29	4.69	0.436
		Mould count	1.26	0.50	0.534
B	August 8	Total viable count	7.20	8.47	0.199**
		Yeast count	4.42	1.71	0.315***
		Mould count	1.29	0.75	0.434
A	August 13	Total viable count	6.65	7.40	0.341
		Yeast count	5.18	1.70	0.336***
		Mould count	1.26	0.50	0.537

**Table 37.** Recovery rates (g DM/kg DM) of the silages.

Site	Harvest date	No additive	Additive	SEM
A	August 8	995	979	11.7
B	August 8	983	1006	11.4
A	August 13	998	986	7.2

**Table 38.** Aerobic stability of the silages produced.

Site	A			B			A		
	August 8			August 8			August 13		
Additive	No additive	Additive	SEM	No additive	Additive	SEM	No additive	Additive	SEM
Days to pH rise	5.3	7.8	1.25	3.5	7.8	0.40***	2.5	7.5	0.29***
Days to pH max.	8.3	8.8	0.25	7.8	8.0	0.18	6.0	8.0	---
Max. pH rise	2.1	0.8	0.74	3.6	2.0	0.25**	3.9	2.3	0.30**
Days to °C rise	2.3	2.3	0.25	2.0	4.5	1.02	2.3	1.5	0.40
Days to °C max.	3.3	3.8	0.25	6.0	8.0	---	5.0	8.0	0.29***
Max. °C rise	14.0	9.5	3.08	18.0	17.8	0.67	21.8	19.0	1.27
Accumulated °C rise to day 5	39	27	6.6	37	11	1.3***	60	15	5.3**

### **Experiment 30: Whole crop wheat silage for finishing beef heifers**

Whole crop wheat (WCW) silage is an alternative conserved forage to grass silage, particularly on land prone to mid-summer drought and where maize is not practicable. The EU area aid scheme also favours the crop in some circumstances. Aerobic deterioration at feedout can be a problem with this relatively high dry matter (DM), fibrous crop. The two main approaches to conservation are to harvest WCW at around 350 g DM/kg, and obtain a relatively extensive lactic acid fermentation, or at above 450 g DM/kg and, when coupled with urea addition, obtain a restricted fermentation. Urea can indirectly improve WCW silage aerobic stability, but can depress intake and performance if applied to WCW of less than 450 g DM/kg (O'Kiely and Moloney, 1995). In contrast, propionic acid can be used to improve aerobic stability in crops of high or low DM concentration. This experiment was designed to quantify the effects on conservation efficiency and nutritive value of (a) the growth stage of WCW at harvesting, (b) additives selected to improve WCW silage aerobic stability, (c) two main approaches to conserving WCW silage, and (d) WCW relative to grass silage.

**Materials and Methods.** Winter wheat (var. Brigadier) was sown in mid-October and managed as for commercial grain production with the exception that a growth regulator was not applied. It was mown and precision-chop harvested at two growth stages, the earlier (E; August 1 and 2; grain of soft cheddar texture) being ensiled alone (O) or with the addition of a propionic acid plus formic acid based additive (P; Top Form, BP Chemicals Ltd., 2 l/t; applied through the harvester) and the later cut (L; August 29

and 30; grain of hard cheddar texture) being ensiled alone (O) or with the addition of urea (U; 24.3 kg/t DM). Urea was dissolved in warm water (40 kg urea + 60 kg water) and applied through the forage harvester at 30.1 kg solution/tonne. Each WCW treatment was sealed beneath polythene sheeting in separate, roofed silos. Grass silage (GS) was made from a *Lolium perenne* dominant permanent grassland sward, precision chop harvested without wilting and treated with formic acid (2.2 l/t) on May 26 and 27. It was stored as for WCW silages. Groups of 15 Charolais crossbred heifers were offered the four WCW silages or the grass silage. Silages were individually offered *ad libitum* to each animal and supplemented with 3.0 kg concentrates (410 g barley, 497 g molassed beet pulp, 78 g soyabean meal and 15 g vitamin plus mineral premix per kg) daily. After 161 days, all animals were slaughtered, and carcass and rumen data collected. Simultaneously, ten Friesian steers (mean liveweight 388 (sd 27.3) kg) were offered the same diets and digestibility determined by the total faecal collection technique on three occasions during the experiment.

**Results.** The mean (s.d.) DM concentrations of the EO, EP, LO, LU and GS treatments at ensiling were 352 (19.1), 352 (11.2), 462 (51.0), 496 (34.6) and 154 (11.9) g/kg, respectively. The mean yields of forage DM harvested were 13.92, 13.02 and 7.54 t/ha for E, L and GS, respectively (annual yields for WCW, but not for GS). The mean weight of whole grain DM in faecal DM was 7.7, 8.2, 17.1, 12.4 and 2.0 g/kg for animals offered diets based on EO, EP, LO, LU and GS, respectively. The grass silage produced 368 l effluent/tonne grass ensiled. Other results are presented in Tables 39 and 40.

**Conclusions.** (a) E had a higher DM yield but a lower DM recovery rate from the silo than L. E and L had similar nutritive values; (b) A propionic acid based additive applied to E improved recovery rate of ensiled DM, without significantly altering subsequent nutritive value. Urea applied to L reduced recovery rate of ensiled DM, without significantly altering subsequent nutritive value; (c) EO had a better recovery than LU. Dietary DM was converted to carcass gain more efficiently from EO than LU, and (d) the WCW treatments had better recovery rates than GS, but supported poorer animal performance and were converted to carcass gain less efficiently.

**Table 39.** Silage chemical composition, aerobic stability and ex-silo DM recovery rate-mean (s.d.).

	Whole-crop wheat silage				Grass silage
	Early cut		Late cut		
	Alone	Propionic	Alone	Urea	
<u>Chemical composition</u>					
Dry matter (g/kg)	371 (16.3)	394 (26.9)	487 (20.3)	456 (25.9)	188 (5.3)
<i>In vitro</i> DMD (g/kg)	675 (14.5)	677 (28.9)	557 (12.9)	532 (13.5)	752 (38.8)
<i>In vitro</i> OMD (g/kg)	681 (14.8)	681 (36.8)	562 (14.8)	531 (12.8)	765 (41.5)
Ash (g/kg DM)	104 (6.0)	95 (15.5)	79 (4.4)	87 (6.0)	88 (2.6)
Crude protein (g/kg DM)	120 (2.7)	117 (4.0)	119 (7.0)	137 (5.1)	172 (6.7)
Lactic acid (g/kg DM)	60 (6.1)	59 (7.6)	13 (3.2)	8 (1.7)	105 (17.9)
Acetic acid (g/kg DM)	15 (2.7)	12 (1.3)	5 (1.4)	16 (1.8)	25 (5.8)
Propionic acid (g/kg DM)	2 (0.7)	4 (1.1)	1 (0.4)	5 (0.7)	6 (1.3)
Butyric acid (g/kg DM)	1.7 (0.40)	0.5 (0.25)	11.9 (1.56)	24.3 (6.16)	0.7 (0.84)
Ethanol (g/kg DM)	9 (2.0)	8 (3.0)	5 (0.7)	7 (1.4)	17 (3.2)
Lactic/(acetic + ethanol)	2.5 (0.41)	3.0 (0.42)	1.3 (0.13)	0.3 (0.09)	2.5 (0.57)
Lactic/fermentation acids	0.76 (0.030)	0.78 (0.022)	0.42 (0.041)	0.15 (0.039)	0.77 (0.059)
Ammonia-N (g/kg N)	89 (7.3)	80 (8.9)	71 (8.4)	364 (60.7)	73 (8.8)
pH	4.11 (0.099)	3.95 (0.076)	4.70 (0)	6.00 (0.896)	3.94 (0.119)
<u>Aerobic stability</u>					
Days to pH rise	2.3 (0.52)	3.7 (0.52)	4.3 (3.61)	1.7 (0.52)	4.3 (1.37)
Days to temperature rise	2.0 (0)	2.3 (0.52)	3.3 (1.51)	1.5 (3.21)	3.8 (0.98)
Accumulated temperature rise to day 5 (°C)	69 (21.2)	55 (2.8)	12 (2.1)	8 (0.84)	39 (22.1)
<u>Ex-silo recovery rate</u>					
g DM/kg DM ensiled	983	1042	1056	877	742

**Table 40.** Intake, digestibility, performance, feed conversion efficiency, organ weight and blood metabolites.

	Whole-crop wheat silage				Grass silage	SEM
	Early cut		Late cut			
	Alone	Propionic	Alone	Urea		
Intake (kg/day)						
Silage DM	5.24	5.77	5.76	5.45	4.98	0.196*
Dietary DM	7.83	8.36	8.35	8.05	7.57	0.196*
<i>In vivo</i> dietary DM digestibility (g/kg)	715	715	701	703	796	15.1***
Liveweight						
Start (kg)	440	441	441	441	440	0.8
Finish (kg)	584	594	590	586	610	6.4*
Gain (g/day)	889	944	921	894	1051	41.3*
Carcass weight						
Finish (kg)	309	313	310	302	337	3.8***
Gain (g/day)	575	598	577	529	747	23.6***
Kill-out rate (g carcass/kg livewt.)	529	527	524	515	552	4.2***
Carcass classification						
Conformation score	3.0	3.1	3.0	3.1	2.7	0.10*
Fatness score	4.3	4.4	4.2	4.2	4.4	0.13
Kidney + channel fat wt. (kg)	10.0	10.7	9.5	10.3	12.6	0.65*
Kidneys (kg)	0.98	0.98	1.03	1.03	1.11	0.048
Liver (kg)	6.2	6.5	6.3	6.6	6.8	0.18
Rumen (kg)	12.1	12.7	12.0	12.0	11.9	0.30
Rumen contents (kg)	42.3	45.7	44.3	42.1	31.5	1.59***
Feed conversion efficiency						
Dietary DM intake/livewt. gain	8.95	9.08	9.21	9.30	7.34	0.341***
Dietary DM intake/carcass gain	13.7	14.3	14.8	15.8	10.2	0.594***
Blood metabolites						
Ammonia (mg/l)	0.13	0.07	0.07	0.39	0.13	0.041***
βhydroxybutyrate (mmol/l)	0.25	0.28	0.29	0.32	0.29	0.017*
Glucose (mmol/l)	4.33	4.38	4.25	4.27	4.36	0.087NS
Urea (mmol/l)	3.71	3.81	4.34	6.45	4.86	0.153***

**Experiment 31: Nutritive value of whole crop wheat and grass silages for beef cattle when offered alone or in mixtures**

Previous experiments have shown that when offered to finishing cattle as the sole forage source, whole crop wheat (WCW) silage supported lower growth rates than good quality grass silage (O'Kiely and Moloney, 1995, 1999). A higher content of grain in WCW silage should increase its potential. WCW harvested at 350 g dry matter (DM)/kg or at about 500 gDM/kg plus urea addition supported similar carcass gains (O'Kiely and Moloney, 1999). This experiment quantified the conservation characteristics of good quality grass silage and WCW ensiled at 350 g/kg or 550 g/kg (plus urea), and their nutritive value for beef heifers when offered alone or in mixtures.

**Materials and methods.** Whole crop winter wheat (WCW; cv. Rialto) was grown as for commercial, high yield grain production. Representative plots were direct-cut precision-chop harvested on 26 July (WCW1) and 12 August (WCW2), weighed, sampled and ensiled in roofed, walled concrete silos. Urea granules were carefully mixed with WCW2 (30kg/t) at the silo immediately prior to ensiling. Grass was precision-chop harvested from a *Lolium perenne* dominant sward on 18 and 19 May and ensiled as above (GS). Add-SafeR (Trouw Nutrition Ltd.) was applied at 1 l/t grass. Fifteen (9 Charolais and 6 Limousin) crossbred heifers (mean (s.d.) starting liveweight 426 (34.3) kg) were allocated in a randomised complete block (initial liveweight and breed) design to each of the three silages offered as the sole forage source, or to mixtures of GS and WCW silage in the proportions 1:2 and 2:1 on a DM basis. Each animal within the 7 treatments was individually offered its respective silage *ad libitum* for 142 days, supplemented with 3.0 kg concentrates (split evenly between morning and afternoon). Blood samples were obtained by venipuncture at 0800 and 1400h on days 41 and 85. Final liveweights and carcass measurements were obtained immediately before and after slaughter, respectively. *In vivo* digestibility was simultaneously assessed during 4 periods using 7 steers per treatment (mean (s.d) liveweight 333 (41.0)kg). The aerobic stability of the three silages was determined at an ambient temperature of 20°C on 5 occasions during the feedout period. Data from the heifers were analysed as a randomised complete block design, and from

the steers as a completely randomised design, with treatment contrasts being separated using the least significant difference procedure.

**Results and Discussion.** The mean (s.d.) composition of WCW1 at ensiling was dry matter (DM) 361(16.0) g/kg and 99(27.0) g water soluble carbohydrate (WSC)/kg DM. The corresponding values for WCW2 were 507 (28.9) g/kg and 21(9.6) g/kg DM. Crop DM yields were 10.4 t/ha for both WCW 1 and 2. The mean (s.d.) proportion of crop DM accounted for by grain DM for WCW 1 and 2 was 189(17.5) and 423(63.7) g/kg, respectively. The recovery rates of edible DM were 910, 887 (including urea added at ensiling) and 860 kg/t DM ensiled for WCW1, WCW2 and GS, respectively.

**Table 41.** Chemical composition of WCW1, WCW2 and grass silages – mean (s.d.).

Silage type	WCW1	WCW2	Grass
Dry matter <sup>1</sup>	381(7.9)	519(31.8)	191(7.0)
C.protein <sup>2</sup>	116(3.0)	186(33.1)	183(5.4)
In vitro DMD <sup>1</sup>	724(15.4)	716(13.8)	732(22.4)
NDF <sup>2</sup>	414(31.0)	492(26.6)	540(21.7)
ADF <sup>2</sup>	225(22.3)	284(21.0)	331(12.8)
Ash <sup>2</sup>	60(5.6)	56(12.5)	99(4.2)
WSC <sup>2</sup>	58(5.7)	25(2.0)	17(2.0)
Lactic acid <sup>2</sup>	60(8.4)	9(2.5)	72(24.1)
NH <sub>3</sub> -N <sup>3</sup>	70(4.6)	514(75.4)	127(16.5)
PH	3.7(0.12)	8.5(0.33)	4.3(0.19)

**Table 42.** Intake, digestibility, growth and feed efficiency

	%GS	%GS with WCW1				%GS with WCW2			s.e.
	100	67	33	0	67	33	0		
Intake <sup>1</sup>	4.81	5.35	6.27	6.33	6.26	6.01	5.96	0.165***	
LWG <sup>2</sup>	866	941	1019	987	1031	968	869	46.5	
CWG <sup>2</sup>	596	684	706	695	710	711	636	24.4**	
KO <sup>3</sup>	534	539	534	534	533	542	537	4.1	
Conf. <sup>4</sup>	2.5	3.1	3.0	2.9	3.1	3.1	2.7	0.10***	
KCF <sup>5</sup>	11.4	9.4	10.7	9.3	8.5	9.0	10.5	0.96	
Rumen <sup>5</sup>	38	38	40	36	39	38	40	1.5	
DMD <sup>6</sup>	742	748	713	712	710	723	717	10.1	
FCE <sup>7</sup>	8.1	7.9	9.1	9.3	8.9	8.7	9.5	0.36*	
FCE <sup>8</sup>	12.6	11.7	12.8	13.1	12.5	12.4	13.7	0.48	

<sup>1</sup>kg silage DM/day; <sup>2</sup>g live or carcass weight gain/day; <sup>3</sup>g cold carcass/kg liveweight; <sup>4</sup>scale of 1= best to 5= worst; <sup>5</sup>Kidney & channel fat or rumen contents(kg); <sup>6</sup>in vivo DM digestibility (g/kg); <sup>7</sup>kg silage DM intake/kg CWG; <sup>8</sup>kg total DM intake/kg CWG

WCW 1 fermented extensively and had a low protein concentration (Table 41). WCW2 had a restricted fermentation and an elevated concentration of both ammonia-N and protein. Intake was lowest for GS (P<0.05) but *in vivo* DMD did not differ among treatments (P>0.05)(Table 42). Mixtures of WCW with GS, or WCW1 alone, supported higher carcass gains than GS alone (P<0.05). Treatment effects on carcass fatness classification, and on the weight of the rumen, liver and kidneys, were not significant (P<0.05).

## Conclusions

The three silages conserved efficiently, producing feedstuffs of good nutritive value. Including WCW1 in the diet, alone or mixed with GS, increased carcass gain. In contrast, WCW2 increased carcass gain when mixed with GS but not when offered as the sole forage source.

**Experiment 32: Co-ensiling potatoes with unwilted or wilted grass**

Traditionally potatoes were cooked before ensiling, but this is now uneconomical. Preliminary experiments confirmed that the low content of water soluble carbohydrates (WSC) in potatoes rendered them liable to preserve badly if ensiled alone. It was necessary to apply formic acid to chopped potatoes to achieve satisfactory preservation. Their relatively high energy value, allied to their low cost when in surplus supply in late spring, makes co-ensiling unchopped potatoes with grass harvested for first-cut silage potentially attractive. The fermenting juices from the grass should help preserve the potatoes, although this might not be as straightforward with wilted grass. This experiment examined the conservation characteristics of whole potatoes co-ensiled with wilted or unwilted first-cut grass, and the simultaneous effects on the grass silages.

**Materials and Methods.** Grass from a *Lolium perenne* dominant sward was precision-chopped unwilted (6 June) or after a 48 h wilt. It was ensiled in 2 m high laboratory silos (50 kg unwilted and 30 kg wilted grass per silo) (O'Kiely, 1991) with the addition of 0, 10, 20 or 30 kg unchopped ware potatoes (King Edward) per silo. Three replicate silos were used per treatment combination. The potatoes were distributed evenly through the grass within the appropriate silos, but were not placed at the top or bottom surfaces. A 20 kg weight was placed on top of the ensiled feedstuffs within each silo. Silos were stored at approximately 15°C for 214 days. Grass and potatoes were weighed and sampled separately both at ensiling and after ensilage. Effluent collected from unwilted (and where appropriate, wilted) grass treatments after 3, 6, 21 and 214 days of ensilage was weighed and sampled. Analysis of variance was conducted for a 2 X 4 factorial completely randomised design.

**Results and Discussion.** Unwilted grass at ensiling had a mean (s.d.) composition of: dry matter (DM) 136 (5.2) g/kg, DM digestibility (DMD) 676 (12.8) g/kg, ash 95 (4.9) g/kg DM, crude protein 184 (7.9) g/kg DM, buffering capacity 596 (15.5) mEq./kg DM, water soluble carbohydrates (WSC) 5 (2.0) g/l and nitrates 832 (176.1) mg/kg DM. The corresponding values for wilted grass were: 270 (3.9) g/kg, 634 (7.5) g/kg, 158 (3.6) g/kg DM, 168 (6.3) g/kg DM, 498 (22.0) mEq./kg DM and 11 (7.0) g/l. The mean (s.d.) composition of the potatoes at ensiling was DM 231 (5.9) g/kg, DMD 797 (52.7) g/kg, ash 66 (1.5) g/kg DM, crude protein 80 (4.3) g/kg DM and buffering capacity 290 (11.9) mEq./kg DM. The mean pH values of effluent collected from the unwilted grass treatments on days 3, 6, 21 and 214 of ensilage were 4.03, 3.90, 3.80 and 4.73, respectively.

**Table 43.** Main effect of rate of potato inclusion on the conservation characteristics of grass and potatoes.

	Rate on inclusion of potatoes				s.e. & sig.
	None	Low	Med.	High	
<b>Grass silage</b>					
Dry matter <sup>1</sup>	195	173	160	162	2.6***
OMD <sup>1</sup>	627	614	613	611	6.1 NS
Ash <sup>2</sup>	112	122	134	139	3.2***
C.protein <sup>2</sup>	149	132	131	144	2.4***
Ph	4.75	5.15	5.22	5.12	0.098*
Lactic acid <sup>2</sup>	38	5	2	1	2.4***
Acetic acid <sup>2</sup>	59	44	40	41	3.5**
Butyric acid <sup>2</sup>	10	37	47	54	2.8***
Ammonia-N <sup>3</sup>	136	430	456	364	23.7***
WSC <sup>2</sup>	12	10	9	8	0.4***
Silage DMr <sup>4</sup>	837	791	807	806	11.8 NS
Effluent <sup>5</sup>	115	106	213	364	27.9***
<b>Potatoes</b>					
Dry matter <sup>1</sup>		307	315	356	9.6**
OMD <sup>1</sup>		919	927	912	5.0 NS
C.protein <sup>2</sup>		54	54	50	1.5 NS
Acetic acid <sup>2</sup>		18	13	10	1.2**
Butyric acid <sup>2</sup>		19	20	18	1.5 NS
Ammonia-N <sup>3</sup>		510	483	385	27.9*
Potato DMr <sup>4</sup>		857	766	759	30.2 NS

<sup>1</sup>g/kg, <sup>2</sup>g/kg DM, <sup>3</sup>g/kgN, <sup>4</sup>gDM recovered/kg grass (or potatoes) DM ensiled, <sup>5</sup>g/kg grass ensiled

The 48 h wilt increased DM concentration. The low concentrations of WSC and the high buffering capacities and ash concentration (wilted grass) predisposed the forages to clostridial-type fermentations (Table 43). Ensiling reduced grass crude protein concentration and digestibility. Under these undesirable conditions, inclusion of potatoes reduced ( $P<0.05$ ) the concentration of DM, lactic and acetic acids and WSC in the grass silage, while increasing ( $P<0.05$ ) ash, butyric acid and ammonia-N concentrations, pH and effluent production. Following ensilage, the DM concentration and digestibility of potatoes were increased, suggesting considerable losses of liquid and/or losses of volatile constituents during oven drying, and possibly the solubalisation of constituents previously not digested by the *in vitro* digestibility assay. The potatoes had a low concentration of lactic acid (<1 g/kg DM) but high concentrations of butyric acid and ammonia-N. The recovery rates of the potatoes following ensilage indicated rates of DM loss comparable to those of the co-ensiled grass.

**Conclusions.** The prevailing ensiling conditions predisposed unwilted and wilted grass to clostridial fermentation. Under these conditions, inclusion of potatoes accentuated the difficulty and promoted a more extreme clostridial fermentation. The potatoes themselves preserved badly, suffered large quantitative losses, and contributed (possibly both directly and indirectly) at higher rates of inclusion to increased production of effluent.

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