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# An evaluation of the effects of rate of nitrogen fertilization of grassland on silage fermentation, in-silo losses, effluent production and aerobic stability

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## Abstract

A factorial experiment was carried out to evaluate the effects of level of nitrogen (N) application to grassland and subsequent treatment of the herbage at ensiling with formic acid on the rate of silage fermentation, dry-matter (DM) recovery, aerobic stability and effluent production. Herbage from the primary regrowth of predominantly perennial ryegrass swards received either 72 (LN), 126 (MN) or 180 (HN) kg N ha$^{-1}$. The herbages were harvested and yield estimated after a 50-day regrowth interval. After storage for 24 h in polythene bags, the herbages were ensiled, unwilted, in laboratory silos (6 kg capacity) either untreated (UT) or treated with formic acid (F) at 3 ml (kg herbage)$^{-1}$. Three silos per treatment were opened and sampled on days 1, 3, 6, 15, 50 and 105 after ensiling. The mean herbage yields for the LN, MN and HN swards were 5·2, 6·3 and 6·2 (s.e. = 0·27) t DM ha$^{-1}$ and there was no carryover effect of nitrogen treatment on the yield of a subsequent 22-day regrowth harvested on 2 September. As level of fertilizer N application increased, concentrations in the herbage at mowing of crude protein, nitrate and ash increased, DM decreased and water-soluble carbohydrate and *in vitro* DM digestibility remained unchanged. Increasing the rate of N fertilizer to the sward resulted in higher silage pH ($P<0·001$) and ammonia N concentrations ($P<0·001$) at each time of sampling. Increasing the rate of fertilizer N increased effluent output ($P<0·001$) and DM recovery ($P<0·001$). Aerobic stability was improved ($P<0·001$) by use of high rates of N fertilizer application. Formic acid treatment restricted fermentation and increased effluent output ($P<0·001$) but had no effect ($P>0·05$) on DM recovery. It is concluded that the rate and extent of pH decline was reduced with increasing N application, associated with increased buffering capacity and decreased DM concentrations of the herbage at ensiling. However, increasing the level of N fertilizer applied increased DM recovery and effluent output, and improved aerobic stability of the resulting silages.

## Introduction

In intensive grassland farming, high rates of fertilizer nitrogen (N) are normally applied to increase grass dry-matter (DM) production. This is known to alter the chemical composition of herbage, decreasing water-soluble carbohydrate (WSC) (Jones *et al.*, 1961; Reid, 1966) and DM content (Spraque and Taylor, 1970) and simultaneously increasing crude protein (CP) concentrations (Reid, 1966). Ensiling the crop is therefore likely to be more difficult because of poor fermentation characteristics, and consequently poorly preserved silages may ensue. Formic acid is commonly used as a silage additive under difficult ensiling conditions; when it is applied in sufficient quantities it improves silage fermentation relative to untreated silages (Steen *et al.*, 1989; O'Kiely, 1993; Keady and Steen, 1995; Keady and Murphy, 1996), and correspondingly leads to higher animal performance (Steen *et al.*, 1989; Keady and Steen, 1995). However, recent findings also show that differences in animal performance can occur when silages of similar preservation characteristics are compared (Steen *et al.*, 1989; Keady and Steen, 1995) but, as the fermentation pathways immediately post-ensiling differed due to changes in pH and the concentrations of lactic and acetic acids, the concentrations of the

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more digestible fractions that were preserved in the silages may have been affected. From a review of the literature by Keady (1991), together with the findings of Steen *et al.* (1995), a poor relationship has been shown to exist between intake of silage and final silage fermentation characteristics as measured individually by pH and the concentrations of ammonia N, lactic acid or volatile fatty acids.

There is a shortage of information in the literature on the effects of increased fertilizer N application to grass swards on the rate of silage fermentation, DM recovery, aerobic stability and effluent production and on the interaction of applying formic acid as an additive on these variables. The present study was designed to provide this information. It was carried out in parallel with a further study that was designed to examine the effects of rate of N fertilizer application to swards on subsequent silage intake and the performance of lactating dairy cows (Keady *et al.*, 1995).

## Materials and methods

### *Sward management*

After harvesting the primary growth on 6 June, each of three replicate blocks of a predominantly perennial ryegrass sward (*Lolium perenne*, *Agrostis stolonifera*, *Dactylis glomerata*, *Poa trivialis*, *Trifolium repens* and weed species accounted for 76%, 15%, 4%, 1%, 3% and 1% of sward cover respectively) was divided into three equal plots. Within each block, three N treatments were allocated at random among the plots. Nitrogen was applied on 20 June in the form of calcium ammonium nitrate (275 g N kg$^{-1}$) at either 72 (LN), 126 (MN) or 180 (HN) kg N ha$^{-1}$, using a precision fertilizer applicator.

After the sward was harvested on 9 August, N fertilizer (33 kg N ha$^{-1}$) was applied to the stubble on 11 August. On 2 September the yield of regrowth was measured from six randomly selected plots per original treatment within each block by cutting the herbage to a stubble height of 4 cm using a reciprocating mower. The mean length and width of the plots were 7·87 and 0·97 m respectively. After mowing, the herbage was weighed and sampled for DM determination.

### *Ensiling*

Six areas, each 6·40 × 0·97 m, were randomly selected within each plot. Herbage was cut on 9 August to a stubble height of 5 cm using a reciprocating mower. After mowing, the herbage was weighed and sampled for the determination of chemical composition, and herbage from each plot was bagged individually and stored at ambient temperature. The following day, herbage from each N treatment was bulked together and passed through a precision-chop harvester. The chopped herbage was thoroughly mixed and again sampled for chemical analysis. For each N treatment, 7-kg quantities of herbage were either untreated (UT) or manually treated with 21 ml of formic acid solution (850 g kg$^{-1}$) (F). Aliquots of 6 kg of herbage (excluding additive) from each 7-kg quantity were then ensiled in each of 108 plastic-pipe silos (eighteen per treatment) as described by O'Kiely and Wilson (1991). A 10·5-kg weight was placed on top of the herbage and the silos were stored indoors under ambient conditions until opening. Three replicate silos per treatment were opened and sampled on days 1, 3, 6, 15, 50 and 105 after ensiling.

### *Measurements*

Samples of herbage collected after mowing on 9 August were analysed for buffering capacity, *in vitro* DM digestibility (DMD) and the concentrations of DM, CP, WSC, modified acid detergent fibre (MADF), ash and nitrates. Samples taken at ensiling were assayed for buffering capacity and the concentrations of WSC and nitrates. Herbage harvested from the subsequent 22-day regrowth on 2 September was weighed and analysed for DM concentration. Effluent was weighed and its DM concentration and pH determined when the silos were opened. The silage was weighed and analysed for pH and the concentrations of DM, ammonia N, WSC, ethanol and lactic, acetic and butyric acids, whereas dried (40°C) samples were assayed for CP. Dried silage (40°C) samples from day 105 were also analysed for MADF, neutral detergent fibre (NDF) and DMD. Recovery of DM was calculated as silage DM removed from the silo, expressed as a proportion of total DM (including additive) ensiled. After sampling on day 105, the remaining silage from the three replicates per treatment was mixed and a composite sample was taken for aerobic stability determination, as described by O'Kiely (1993).

### *Chemical analyses*

Grass DM concentration was determined by drying at 98°C for 16 h in an oven with forced air circulation. Samples of grass dried at 40°C for 48 h, were milled (Retsch Mill, F. Kurt Retsch, and Co. KG, Haan, Germany, with 1-mm screen) and analysed for CP [N × 6·25; N determined according to Sweeney (1989) on a LECO FP 428 Nitrogen Analyser, Leco Corporation, Michigan, USA], *in vitro* DMD (Tilley and Terry, 1963; with the modification that the final residue was isolated by filtration rather than by centrifugation), buffering capacity (Playne and McDonald, 1966), ash (in a muffle furnace at 550°C) and MADF (Clancy and Wilson, 1966). Concentrations of WSC were measured on extracted grass juice by the colorimetric method of Wilson (1978) and nitrates on a Ciba-Corning Express 550 Clinical Analyser using a nitrate food analysis kit from Boehringer Mannheim (catalogue no. 905658), after freezing (18°C), chop ping the frozen sample through a mincer (Muller food processor, type MTK 204 special, Saarbrucken, Germany) and thawing.

Silage DM concentration was determined by drying at 40°C for 48 h. Dried, ground samples were used to determine *in vitro* DMD, CP and NDF contents (Van Soest and Wine, 1967). Samples of undried silage (50 g) were soaked in distilled water (150 ml) for a minimum of 2 h and the pH of the mixture measured using a pH electrode. Ammonia-N was estimated colorimetrically by a modification of the phenol–hypochlorite technique (O'Keefe and Sherrington, 1983). Lactic acid concentration was analysed on a Ciba-Corning Express Clinical Analyser using the method of Boehringer Mannheim (catalogue no. 139004) and acetic and butyric acids and ethanol by gas–liquid chromatography (Ranfft, 1973). Concentration of WSC was measured as for grass on expressed silage juice. DM content of effluent was determined using a refractometer and pH using a pH electrode.

### *Statistical analyses*

Yield and composition of herbage at mowing were analysed as a randomized-block design. Chemical composition of the herbage at mowing and ensiling were analysed as a completely randomized designs. DM recoveries, effluent volume and the composition of silages were analysed as a 3 (level of N applied) × 2 (additive vs. no additive) × 6 (day of opening) factorial design. Silage DMD, NDF, MADF content and aerobic stability data were analysed as a 3 × 2 factorial design. Differences between treatments were tested using Student's *t*-test.

## Results

Level of N application had a quadratic ($P<0{\cdot}05$) effect on herbage DM yield on 9 August. Increasing N application (Table 1) from 72 to 126 kg N ha$^{-1}$ increased herbage DM yield. However, application of 180 kg N ha$^{-1}$ had no further effect on DM yields. Herbage yields on 2 September, after the subsequent 22-day regrowth interval, were not affected ($P>0{\cdot}05$) by any carryover effect of the rate of fertilizer N application on 20 June.

The chemical composition of the herbages at mowing and ensiling are presented in Table 2. As rate of N application increased, herbage DM content significantly decreased ($P<0{\cdot}001$) and CP, ash and nitrate concentrations significantly increased ($P<0{\cdot}05$ or greater). Rate of N application had no effect ($P>0{\cdot}05$) on WSC concentration or DMD of the herbages at mowing. However, higher N application rates were associated with lower WSC concentrations at ensiling. In general higher buffering capacities occurred where increased rates of N were applied. Relative to herbage from the LN and MN swards, that from the HN sward had significantly higher MADF concentrations ($P<0{\cdot}01$ or greater) and the HN herbage had significantly higher nitrate ($P<0{\cdot}001$) concentrations than the LN herbage at mowing. Delaying ensiling for a 24-h period after mowing resulted in a higher buffering capacity and lower concentrations of WSC and nitrates.

**Table 1.** Effects of nitrogen fertilizer application on herbage dry-matter yields (t ha$^{-1}$)

| | Nitrogen fertilizer treatment | | | | Significance | |
|---|---|---|---|---|---|---|
| | LN | MN | HN | s.e.d. | Linear | Quadratic |
| Primary regrowth (9 August) | 5·2$^{a}$ | 6·3$^{b}$ | 6·2$^{b}$ | 0·27 | * | * |
| Secondary regrowth (2 September) | 1.5 | 1·4 | 1·4 | 0·17 | NS | NS |

LN, MN and HN = 72, 126 and 180 kg N ha$^{-1}$ respectively.
Means on the same line with the same superscripts do not differ significantly ($P>0{\cdot}05$).
*$P<0{\cdot}05$; NS, not significant.

**Table 2.** Chemical composition of the herbage at mowing and ensiling

| | Nitrogen fertilizer treatment | | | | |
|---|---|---|---|---|---|
| | LN | MN | HN | s.e.d. | Significance |
| Dry matter (g kg$^{-1}$) | | | | | |
| At mowing | $220^{a}$ | $194^{b}$ | $161^{c}$ | 3·1 | *** |
| Buffering capacity [mequiv (kg DM)$^{-1}$] | | | | | |
| At mowing | $483^{a}$ | $518^{b}$ | $508^{ab}$ | 15·9 | *** |
| At ensiling | $588^{a}$ | $608^{a}$ | $650^{b}$ | 12·3 | *** |
| Nitrate [mg (1 aqueous extract)$^{-1}$] | | | | | |
| At mowing | $568^{a}$ | $863^{ab}$ | $2979^{b}$ | 269·0 | *** |
| At ensiling | $163^{a}$ | $587^{b}$ | $1513^{c}$ | 72·1 | *** |
| *Composition of dry matter* (g (kg DM)$^{-1}$) | | | | | |
| Crude protein | | | | | |
| At mowing | $131^{a}$ | $154^{b}$ | $187^{c}$ | 3·2 | *** |
| WSC | | | | | |
| At mowing | 94·2 | 97·7 | 90·1 | 5·54 | NS |
| At ensiling | $88{\cdot}9^{a}$ | $83{\cdot}1^{ab}$ | $77{\cdot}5^{b}$ | 4·26 | ($P = 0{\cdot}053$) |
| NDF | | | | | |
| At mowing | $512^{a}$ | $531^{b}$ | $535^{b}$ | 6·6 | ** |
| MADF | | | | | |
| At mowing | $284^{a}$ | $289^{a}$ | $302^{b}$ | 4·2 | ** |
| Ash | | | | | |
| At mowing | $89^{a}$ | $94^{b}$ | $101^{c}$ | 2·0 | *** |
| DMD (*in vitro*) | | | | | |
| At mowing | 728 | 728 | 729 | 6·5 | NS |

Treatment abbreviations as in Table 1.
Means on the same line with the same superscript do not differ significantly ($P>0{\cdot}05$).
**$P<0,01$; ***$P<0{\cdot}001$; NS, not significant.

The effects of rate of N fertilizer application, additive and stage of ensiling on silage composition are presented in Tables 3, 4 and 5. The main effect of N fertilizer application rate was not significant ($P>0{\cdot}05$) for *in vitro* DMD, ratio lactic acid–(acetic acid plus ethanol) (L/AE) or the concentration of NDF. The main effect of additive treatment was not significant ($P>0{\cdot}05$) for *in vitro* DMD or the concentrations of CP or MADF. Otherwise the main effects of N rate and additive type were significant for the other variables measured. The concentrations of butyric and propionic acids were below detectable limits.

As the rate of N applied to the sward increased, DM concentration of the silages was significantly decreased ($P<0{\cdot}001$) on days 1, 3, 6, 15 and 105. However, on day 50, the DM of the MN silages was not significantly different ($P>0{\cdot}05$) from either the LN or HN silages. Formic acid treatment increased ($P<0{\cdot}05$) silage DM concentrations on day 6 but did not alter it ($P>0{\cdot}05$) on days 1, 3, 15, 50 or 105.

Silage CP concentration was significantly increased ($P<0{\cdot}001$) at each time of sampling as rate of N applied to the sward increased. Formic acid treatment tended to decrease ($P>0{\cdot}05$) the DMD in the LN and MN silages but tended to increase it ($P>0{\cdot}05$) in the HN silages. Formic acid treatment increased ($P<0{\cdot}05$) the concentration of NDF. The concentration of MADF was higher ($P<0{\cdot}05$) for the HN silages relative to the LN and MN silages.

The untreated LN silages had significantly lower pH values relative to the MN silages ($P<0{\cdot}05$ or greater) on days 1 and 3 and relative to the HN silages ($P<0{\cdot}05$ or greater) at each time of sampling. The LN, F silages had significantly lower pH ($P<0{\cdot}01$ or greater) than the MN, F and HN, F silages on days 1, 3 and 6 and significantly higher pH ($P<0{\cdot}05$ or greater) than the HN, F silages on days 15, 50 and 105. The pH of the MN, F and HN, F silages did not differ significantly ($P>0{\cdot}05$) at any time of sampling.

As the rate of N applied to the sward increased, ammonia N concentrations in the resulting silages increased ($P<0{\cdot}01$ or greater) on days 3, 6, 15, 50 and 105. However, on day 1 the ammonia N con-

**Table 3.** Effects of level of nitrogen fertilizer application, additive treatment and stage of ensilage on silage composition

| Nitrogen fertilizer treatment Additive (Add) | | LN | | MN | | HN | | | Significance | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Time (days) | Control | Formic acid | Control | Formic acid | Control | Formic acid | s.e.d. | Level of N fertilizer (N) | Additive (Add) | Time (T) | N × Add | N × T | Add × T | N × Add × T |
| Dry matter (g kg$^{-1}$) | 1 | 206 | 206 | 188 | 191 | 163 | 161 | | | | | | | | |
| | 3 | 208 | 213 | 190 | 187 | 161 | 168 | | | | | | | | |
| | 6 | 197 | 204 | 179 | 184 | 153 | 166 | 5·5† | *** | ** | *** | NS | *** | NS | NS |
| | 15 | 197 | 200 | 183 | 191 | 161 | 166 | | | | | | | | |
| | 50 | 190 | 182 | 179 | 188 | 160 | 164 | | | | | | | | |
| | 105 | 196 | 193 | 187 | 183 | 161 | 175 | | | | | | | | |
| Crude protein [g (kg DM)$^{-1}$] | 1 | 128 | 127 | 145 | 143 | 180 | 189 | | | | | | | | |
| | 3 | 120 | 118 | 141 | 142 | 177 | 182 | | | | | | | | |
| | 6 | 124 | 126 | 144 | 148 | 183 | 182 | 4·3† | *** | NS | *** | NS | ** | NS | NS |
| | 15 | 126 | 126 | 143 | 139 | 172 | 182 | | | | | | | | |
| | 50 | 127 | 133 | 145 | 143 | 178 | 187 | | | | | | | | |
| | 105 | 142 | 141 | 155 | 155 | 195 | 185 | | | | | | | | |
| *In vitro* DMD [g (kg DM)$^{-1}$] | 105 | 717 | 700 | 714 | 701 | 687 | 705 | 9·4‡ | NS | NS | | * | | | |
| NDF [g (kg DM)$^{-1}$] | 105 | 515 | 548 | 511 | 531 | 541 | 541 | 12·5‡ | NS | * | | NS | | | |
| MADF [g (kg DM)$^{-1}$] | 105 | 298 | 315 | 303 | 306 | 330 | 316 | 8·0‡ | * | NS | | NS | | | |

Treatment abbreviations as in Table 1.
†s.e.d. for the three-way interaction of nitrogen × additive × time.
‡s.e.d. for the two-way interaction of nitrogen × additive.
*$P<0{\cdot}05$; **$P<0{\cdot}01$; ***$P<0{\cdot}001$; NS, non significant

**Table 4.** Effects of level of nitrogen fertilizer application, additive treatment and stage of ensilage on silage composition

| Nitrogen fertilizer treatment<br>Additive (Add) | | LN | | MN | | HN | | | Significance | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Time (days) | Control | Formic acid | Control | Formic acid | Control | Formic acid | s.e.d. | Level of N fertilizer (N) | Additive (Add) | Time (T) | N × Add | N × T | Add × T | N × Add × T |
| pH | 1 | 4·73 | 4·47 | 4·83 | 4·60 | 5·20 | 4·63 | | | | | | | | |
| | 3 | 4·57 | 4·47 | 4·70 | 4·63 | 5·00 | 4·67 | | | | | | | | |
| | 6 | 4·40 | 4·40 | 4·43 | 4·57 | 4·50 | 4·57 | 0·042† | *** | *** | *** | *** | *** | *** | *** |
| | 15 | 4·00 | 4·13 | 4·00 | 4·00 | 4·23 | 3·93 | | | | | | | | |
| | 50 | 3·93 | 4·13 | 3·90 | 3·97 | 4·17 | 3·90 | | | | | | | | |
| | 105 | 4·10 | 4·20 | 4·10 | 4·13 | 4·37 | 4·10 | | | | | | | | |
| Ammonia nitrogen [g (kg N)$^{-1}$] | 1 | 42 | 20 | 49 | 31 | 81 | 56 | | | | | | | | |
| | 3 | 38 | 21 | 49 | 56 | 87 | 56 | | | | | | | | |
| | 6 | 37 | 27 | 55 | 45 | 92 | 70 | 6·5† | *** | *** | *** | *** | * | NS | NS |
| | 15 | 55 | 45 | 71 | 61 | 126 | 86 | | | | | | | | |
| | 50 | 59 | 42 | 69 | 57 | 114 | 85 | | | | | | | | |
| | 105 | 56 | 45 | 72 | 67 | 125 | 93 | | | | | | | | |
| Water-soluble carbohydrate g (kg DM)$^{-1}$ | 1 | 71 | 98 | 70 | 94 | 53 | 69 | | | | | | | | |
| | 3 | 52 | 85 | 41 | 81 | 29 | 63 | | | | | | | | |
| | 6 | 51 | 90 | 28 | 80 | 20 | 67 | 4·6† | *** | *** | *** | *** | *** | *** | NS |
| | 15 | 11 | 35 | 10 | 40 | 5 | 22 | | | | | | | | |
| | 50 | 13 | 19 | 12 | 33 | 5 | 19 | | | | | | | | |
| | 105 | 12 | 22 | 13 | 43 | 12 | 36 | | | | | | | | |

Treatment abbreviations and significance levels as in Tables 1 and 3 respectively.
†s.e.d. for the three-way interaction of nitrogen × additive × time.

**Table 5.** Effects of level of nitrogen fertilizer application, additive treatment and stage of ensilage on silage composition

| Nitrogen fertilizer treatment Additive (Add) | | LN | | MN | | HN | | | Significance | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Time (days) | Control | Formic acid | Control | Formic acid | Control | Formic acid | s.e.d. | Level of N fertilizer (N) | Additive (Add) | Time (T) | N × Add | N × T | Add × T | N × Add × T |
| Lactic acid [g (kg DM)$^{-1}$] | 1 | 56 | 28 | 61 | 25 | 52 | 28 | | | | | | | | |
| | 3 | 76 | 40 | 55 | 20 | 57 | 30 | | | | | | | | |
| | 6 | 72 | 36 | 83 | 39 | 106 | 46 | 5·8† | *** | *** | *** | *** | *** | *** | ** |
| | 15 | 119 | 77 | 147 | 88 | 154 | 112 | | | | | | | | |
| | 50 | 156 | 99 | 173 | 111 | 165 | 136 | | | | | | | | |
| | 105 | 125 | 71 | 142 | 77 | 143 | 100 | | | | | | | | |
| Ethanol [g (kg DM)$^{-1}$] | 1 | 6 | 3 | 5 | 3 | 3 | 3 | | | | | | | | |
| | 3 | 6 | 3 | 4 | 3 | 3 | 3 | | | | | | | | |
| | 6 | 7 | 4 | 5 | 3 | 5 | 3 | 3·5† | *** | *** | *** | *** | *** | *** | *** |
| | 15 | 17 | 32 | 8 | 4 | 9 | 9 | | | | | | | | |
| | 50 | 20 | 56 | 7 | 13 | 8 | 7 | | | | | | | | |
| | 105 | 15 | 46 | 8 | 19 | 10 | 5 | | | | | | | | |
| Acetic acid [g (kg DM)$^{-1}$] | 1 | 8 | 0 | 11 | 1 | 12 | 4 | | | | | | | | |
| | 3 | 11 | 2 | 13 | 2 | 18 | 7 | | | | | | | | |
| | 6 | 13 | 4 | 18 | 5 | 23 | 7 | 1·4† | *** | *** | *** | NS | NS | * | NS |
| | 15 | 20 | 10 | 22 | 11 | 24 | 13 | | | | | | | | |
| | 50 | 24 | 14 | 25 | 14 | 28 | 16 | | | | | | | | |
| | 105 | 24 | 12 | 26 | 14 | 30 | 16 | | | | | | | | |
| L/AE | 1 | 3·5 | 10·3 | 4·0 | 7·4 | 3·4 | 5·5 | | | | | | | | |
| | 3 | 4·5 | 11·4 | 3·2 | 6·0 | 2·7 | 2·9 | | | | | | | | |
| | 6 | 3·7 | 4·2 | 3·6 | 4·6 | 3·9 | 4·7 | 1·13† | NS | *** | *** | NS | *** | *** | *** |
| | 15 | 3·2 | 1·8 | 5·0 | 6·0 | 4·7 | 5·4 | | | | | | | | |
| | 50 | 3·6 | 1·4 | 5·3 | 4·3 | 4·6 | 6·3 | | | | | | | | |
| | 105 | 3·2 | 1·2 | 4·3 | 2·9 | 3·6 | 4·7 | | | | | | | | |

†s.e.d. for the three-way interaction of nitrogen × additive × time.
‡L/AE, ratio lactic acid – (acetic acid + ethanol).
Treatment abbreviations and significance levels as in Tables 1 and 3 respectively.

**Table 6.** Effects of level of nitrogen fertilizer application and additive treatment on silage effluent, dry-matter recovery and aerobic stability characteristics

| Nitrogen fertilizer treatment | | LN | | MN | | HN | | | Significance | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Additive (Add) | Time (days) | Control | Formic acid | Control | Formic acid | Control | Formic acid | s.e.d. | Level of N fertilizer (N) | Additive (Add) | Time (T) | N × Add | N × T | Add × T | N × Add × T |
| Dry-matter recovery [g (kg DM ensiled)$^{-1}$] | 1 | 934 | 937 | 966 | 984 | 976 | 972 | | | | | | | | |
| | 3 | 940 | 961 | 976 | 963 | 953 | 967 | | | | | | | | |
| | 6 | 900 | 927 | 915 | 943 | 919 | 961 | 28·7† | *** | NS | *** | NS | NS | NS | NS |
| | 15 | 890 | 898 | 921 | 952 | 924 | 942 | | | | | | | | |
| | 50 | 857 | 815 | 884 | 924 | 891 | 907 | | | | | | | | |
| | 105 | 877 | 850 | 930 | 897 | 903 | 959 | | | | | | | | |
| Effluent volume [g (kg ensiled)$^{-1}$] | 1 | 0 | 0 | 0 | 0 | 5 | 6 | | | | | | | | |
| | 3 | 0 | 0 | 0 | 0 | 22 | 34 | | | | | | | | |
| | 6 | 0 | 0 | 0 | 2 | 28 | 61 | 7·8† | *** | *** | *** | ** | *** | NS | NS |
| | 15 | 0 | 0 | 15 | 29 | 57 | 68 | | | | | | | | |
| | 50 | 0 | 6 | 21 | 36 | 79 | 97 | | | | | | | | |
| | 105 | 8 | 14 | 28 | 42 | 81 | 108 | | | | | | | | |
| Effluent losses [g DM (kg ensiled)$^{-1}$] | 1 | 0·0 | 0·0 | 0·0 | 0·0 | 0·1 | 0·2 | | | | | | | | |
| | 3 | 0·0 | 0·0 | 0·0 | 0·0 | 0·6 | 1·2 | | | | | | | | |
| | 6 | 0·0 | 0·0 | 0·0 | 0·1 | 1·0 | 2·4 | 0·32† | *** | *** | *** | *** | *** | ** | * |
| | 15 | 0·0 | 0·0 | 0·8 | 1·8 | 2·1 | 2·6 | | | | | | | | |
| | 50 | 0·0 | 0·3 | 1·2 | 2·2 | 3·2 | 3·9 | | | | | | | | |
| | 105 | 0·5 | 0·8 | 1·8 | 2·6 | 3·2 | 5·4 | | | | | | | | |
| Aerobic stability | | | | | | | | | | | | | | | |
| Maximum pH increase | 105 | 3·60 | 4·45 | 4·50 | 4·45 | 4·65 | 0·65 | 0·387‡ | *** | ** | *** | | | | |
| Days to maximum temperature rise | 105 | 6·5 | 7·0 | 7·0 | 8·0 | 7·0 | 8·0 | 0·29‡ | * | ** | NS | | | | |
| Maximum temperature rise (°C) | 105 | 33·5 | 35·5 | 30·0 | 29·0 | 31·5 | 17·0 | 4·57‡ | NS | NS | NS | | | | |
| Accumulative daily temperature increase to day 5 (°C) | 105 | 49·0 | 30·0 | 32·5 | 32·5 | 21·0 | 13·5 | 4·23‡ | *** | * | NS | | | | |

†s.e.d. for the three-way interactions of nitrogen × additive × time.
‡s.e.d. for the two-way interactions of nitrogen × additive.
Treatment abbreviations and significance levels as in Tables 1 and 3 respectively.

centration of the MN silages was not significantly different ($P > 0{\cdot}05$) from either the LN or HN silages. Formic acid treatment decreased ($P < 0{\cdot}001$) ammonia N concentrations at each time of sampling relative to the untreated silages.

The LN silages had significantly higher WSC concentrations on days 3 and 6 ($P < 0{\cdot}05$ or greater) relative to the MN silages and on days 1, 3, 6 and 15 ($P < 0{\cdot}01$ or greater) relative to the HN silages. However, on day 105 post-ensiling the MN and HN silages had higher concentrations ($P < 0{\cdot}05$ or greater). Formic acid treatment significantly increased ($P < 0{\cdot}001$) WSC concentrations at each time of sampling. Lactic acid concentrations of the LN, UT silages were significantly higher ($P < 0{\cdot}01$ or greater) on day 3 and significantly lower ($P < 0{\cdot}01$ or greater) on days 15 and 105 relative to the MN, UT and HN, UT silages. Similarly, lactic acid concentrations of the LN, F silages were significantly higher ($P < 0{\cdot}001$) on day 3 relative to the MN, F silages and significantly lower ($P < 0{\cdot}001$) on days 15 and 105 relative to the HN, F silages. Formic acid treatment significantly decreased ($P < 0{\cdot}001$) lactic acid concentrations at each time of sampling.

Ethanol concentrations increased as ensilage progressed and the concentrations in the LN silages were significantly higher ($P < 0{\cdot}001$) than the MN and HN silages on days 15, 50 and 105. Formic acid treatment increased ethanol concentrations in the LN silages on days 15, 50 and 105 ($P < 0{\cdot}001$) and in the MN silages on day 105 ($P < 0{\cdot}01$), the magnitude of the increase being greater for the LN treatment. Formic acid treatment resulted in significantly lower concentrations of acetic acid ($P < 0{\cdot}001$) at each time of sampling. As the rate of N applied to the sward increased, acetic acid concentrations increased ($P < 0{\cdot}001$).

Rate of N application to the swards had no effect ($P > 0{\cdot}05$) on the L/AE ratio in the UT silages. However, on days 1 and 3 increasing the rate of N applied to the swards resulted in a decrease ($P < 0{\cdot}05$ or greater) in the L/AE ratio of the formic acid-treated silages. The LN, F silage had lower L/AE ratios ($P < 0{\cdot}01$ or greater) than the MN, F and HN, F silages on days 15 and 50 and significantly lower L/AE ratios ($P < 0{\cdot}01$) than the HN, F silage on day 105.

The effects of N application rate and additive treatment on DM recovery, effluent production and aerobic stability are presented in Table 6. The MN and HN silages increased DM recovery ($P < 0{\cdot}001$) relative to the LN silage. Formic acid treatment did not alter ($P > 0{\cdot}05$) DM recovery.

Increasing the rate of N applied to the sward increased ($P < 0{\cdot}001$) effluent output from the resulting silages. The HN silages had significantly higher levels ($P < 0{\cdot}001$) of effluent output on days 3, 6, 15, 50 and 105, whereas the MN silages had higher ($P < 0{\cdot}001$) effluent outputs relative to the LN silages on days 15, 50 and 105. Formic acid treatment increased ($P < 0{\cdot}05$ or greater) effluent output on days 6, 50 and 105 relative to the untreated silages. As the level of N applied to the swards increased, effluent DM losses increased ($P < 0{\cdot}001$) on days 15, 50 and 105.

Nitrogen application rate and additive treatment had significant effects on changes in pH and temperature under aerobic conditions. Days to maximum temperature rise after sampling the silages on day 105 were significantly higher ($P < 0{\cdot}05$) for the MN and HN silages relative to the LN silages. Temperature increase to day 5 was lower ($P < 0{\cdot}001$) for the HN relative to the LN silages. Maximum pH increase was lower ($P < 0{\cdot}05$) for the HN relative to the LN silages. For the LN and MN silages, F treatment did not alter ($P > 0{\cdot}05$) maximum pH increase, however, for the HN silages it decreased ($P < 0{\cdot}05$) it. Formic acid treatment increased ($P < 0{\cdot}01$) days to maximum temperature and decreased ($P < 0{\cdot}05$) accumulative daily temperature rise to day 5. Nitrogen application rate or additive treatment did not alter ($P > 0{\cdot}05$) maximum temperature.

## Discussion

The main objective of the present study was to examine the effects of N application on the silage fermentation process from days 0 to 105, as well as on DM recovery, effluent production and aerobic stability. However, it also provided the opportunity to examine the effects of applying different rates of N on herbage DM production of the primary regrowth from established swards. The mean increase in grass DM yield was 21 kg DM (kg N)$^{-1}$ applied between 72 and 126 kg N ha$^{-1}$ and –2 kg DM (kg N)$^{-1}$ between 126 and 170 kg N ha$^{-1}$. Mean annual responses for swards that had received 300 kg N ha$^{-1}$ year$^{-1}$ (relative to no N) of 23 kg DM (kg N)$^{-1}$ (Morrison *et al.*, 1980) and 20 kg DM (kg N)$^{-1}$ (Hopkins *et al.*, 1990), averaged over twenty-one and sixteen sites within Britain respectively, have been reported, which is similar to the response in the present study of 21 kg DM kg$^{-1}$ N applied between 72 and 126 kg N ha$^{-1}$.

Prins and van Burg (1977) have shown that the differences in carryover effect to a subsequent regrowth, where a range of rates of N were applied to the previous growth of a sward, decrease if N is applied to the regrowth. The apparent absence of such a residual effect on the yield of the secondary regrowth in the present study was probably obscured by the application of N immediately after harvesting the herbage for ensiling.

Achieving a rapid lactic acid-dominant fermentation becomes more difficult as herbage DM and WSC concentrations decrease and as buffering capacity increases. In the present study increasing the rate of N applied to the sward produced herbage composition characteristics indicative of more difficult ensiling conditions, and furthermore led to increased nitrate and ash concentrations. Although increased rates of N fertilizer had no effect on herbage WSC concentrations at mowing, they reduced WSC concentrations by the time of ensiling, in agreement with the results of Wilson (1969), Wilson and Flynn (1979) and O'Kiely *et al.* (1994), who reported that increasing rates of N application consistently reduced herbage WSC concentrations. The absence of an effect of rate of N fertilizer on *in vitro* DMD is in agreement with the results of Wilson (1969) and Wilson and Flynn (1979). However, O'Kiely *et al.* (1994) reported an increase in *in vitro* DMD as a result of increased N fertilizer applications, thought to be due in part to higher leaf–stem ratios associated with increased N application. The increased MADF concentrations from higher applications of N do not support the simultaneous absence of an effect on *in vitro* DMD, and consequently support the findings of Hopkins *et al.* (1990) that MADF may not accurately reflect differences in digestibility within grass species. The increase in buffering capacity and decrease in WSC concentrations between mowing and ensiling may be explained by some fermentation occurring during storage before ensiling, whereas the corresponding decrease in nitrate concentrations may have been due to reductions of nitrate to ammonia or oxides of N during this period.

Although the silages exhibited the chemical characteristics of a satisfactory fermentation by day 50, indicating the presence of sufficient WSC to support a lactic acid-dominant fermentation within the prevailing conditions, the initial rate of decline in pH was slow, taking somewhere between 6 and 15 days to reach a pH of 4·23 or less. This contrasts with the results of Keady and Steen (1994; 1995) and Keady *et al.* (1994), who ensiled herbages of low DM (146–158 g $kg^{-1}$) and WSC [78–88 g $(kg\ DM)^{-1}$] concentrations, and found a more rapid rate of decline in pH compared with that in the present study (reaching a pH of 4·2 or less by day 5 after ensiling). The slower decline in pH in the present work was possibly due in part to the relatively high buffering capacity of the grass at ensiling. This is supported by the high concentration of lactic acid required to decrease pH to 4·0 during fermentation. Furthermore, the unfavourable changes to some of the chemical indices of grass ensilability (WSC and buffering capacity) that occurred during the pre-ensiling overnight storage, together with the possible domination of the microflora by aerobes, may also have decreased the initial rate of pH decline.

In the first days of ensiling, pH values were lower as the rate of N fertilizer application decreased. Because the concentrations of lactic acid were not greatly influenced by the level of N application, the lower pH values appear to reflect lower buffering capacities, as well as lower ammonia concentrations, which would lead to less elevated pH. The suggested lower buffering capacities in the early stages of ensiling would reflect corresponding differences in the herbage, together with a slower increase in buffering capacity, during the early stages of ensiling in parallel with the lower initial concentrations of acetic acid.

After the first days of ensiling, lactic acid concentrations increased markedly, and at a faster rate where higher rates of N fertilizer had been applied. In the absence of formic acid treatment, low residual WSC concentrations occurred by day 15 and the concentrations of lactic acid subsequently decreased between days 50 and 105 of ensilage. These latter effects, paralleled by increases in acetic and ammonia concentrations and pH, suggest that had ensiling continued beyond 105 days the overall fermentation profile would have deteriorated markedly. The data suggest that these effects would have been greater where the highest rate of N fertilizer had been applied.

The poorer final fermentation (day 105) of silages due to increased N application, as measured by ammonia N and acetate concentrations, is similar to the results of Stevens *et al.* (1992), who ensiled three harvests of herbage from swards that had received varying levels of fertilizer N. However, Wilson (1969) ensiled herbage from swards that had received varying levels of N fertilizer and observed that in only one out of three harvests were ammonia N concentrations increased as a result of increased N fertilizer application.

The more rapid initial decline in silage pH immediately after ensiling, but subsequent slower decrease due to F treatment, is in agreement with the results of Keady and Steen (1994; 1995). Similarly Keady *et al.* (1994) and Keady and Murphy (1996) reported that, although formic acid treatment resulted in a lower pH immediately after application, it delayed subsequent pH decline, such that the untreated silage obtained a pH of 4·2 or less at an earlier stage during the fermentation period. Owing to the antimicrobial properties of formic acid (Woolford, 1975), this treatment restricted silage fermentation as indicated by the lower concentrations of fermentation products (lactic, acetic, butyric and propionic acids) (O'Kiely, 1993; Keady *et al.*, 1994; 1996; Keady and Steen, 1994; 1995) and improved silage fermentation as measured by reductions in acetic acid and ammonia N concentrations, which is in agreement with O'Kiely (1993), Keady *et al.* (1994), Keady and Steen (1994; 1995), Keady *et al.* (1996) and Keady and Murphy (1996). The concentrations of ethanol increased markedly after day 6 of ensiling where formic acid was applied to the herbage that had received the low rate of N fertilizer application. Elevated concentrations of ethanol have previously been obtained with formic acid treatment and have been attributed primarily to yeast activity (McDonald *et al.*, 1991). The interaction with N fertilizer and additive treatment on ethanol production in the present experiment was possibly a result of the increased buffering capacity of the herbage at ensiling owing to increased N fertilization; this was subsequently followed by higher lactic and acetic acid production as a consequence of which less WSC was left for ethanol production by yeast.

The weight that was placed on top of the ensiled herbage in the present study should have exerted a vertical pressure approximately equivalent to that exerted by 1 m of unwilted silage. Using the equation of Weissbach and Peters (1983), it is estimated that in 1·5-m-high silos, effluent output would be 157, 82 and 23 l (t grass)$^{-1}$ for herbage having DM concentrations of 160, 190 and 220 g kg$^{-1}$ at ensiling respectively. The lower effluent volumes recorded in the present study suggest an underestimation of absolute effluent production when using the current laboratory silo system compared with predictions for conventional farm silos. This was probably due to increased friction resulting from the greater herbage surface area per unit mass being in contact with the laboratory silos decreasing effluent flow. Increased effluent production caused by increased rate of N fertilizer can be explained by the effects of N fertilizer on grass DM concentration and the relationship (Castle *et al.*, 1973; Weissbach and Peters, 1983) between the latter and effluent production. The results agree with those of Wilson (1969) and Jones (1993), both of whom reported increased effluent production as a result of increased N fertilizer applications to silage swards.

The increased silage effluent production due to formic acid treatment in the present study is similar to the results of Pedersen *et al.* (1973) and O'Kiely (1993). Both Winters *et al.* (1987) and McDonald *et al.* (1991) suggested that this could be due to a greater leakage of cell contents as a result of the ability of formic acid to penetrate beyond the epicuticular layer into the plant tissue and disrupt mesophyll cell membranes.

Recovery of ensiled DM from the silos was high, and the effects of additive treatment was small. These high rates of recovery could be attributed to low effluent losses and the absence of surface waste. The increased loss of DM in the effluent, as a result of increased N fertilizer application, is expected because of larger volumes of effluent produced. However, total in-silo losses differed by 67·5 g kg DM$^{-1}$ ensiled for the LN and HN treatments; 3·85 g of this can be accounted for by effluent losses. Volatile nutrients are lost from silage during oven drying, possibly leading to an overestimation of in-silo losses. This effect could have been greater for the LN silages, which had high ethanol concentrations. The higher in-silo losses of the LN treatment are probably primarily due to ethanol production, which is accompanied by the production of $CO_2$, and is responsible for high DM losses (McDonald *et al.*, 1991).

The improvements in aerobic stability due to formic acid treatment are in agreement with the observations of O'Kiely (1993) and Keady and Steen (1994; 1995) when both the untreated and formic acid treatments were well preserved. The importance of yeast in aerobic deterioration of silages is well documented (Woolford *et al.*, 1982; Lindgren *et al.*, 1985). On exposure to air the silages from the LN sward were less stable; this is probably associated with these silages having the highest ethanol concentrations, which suggests higher yeast activity. In addition these silages had lower concentrations of ammonia N relative to the other treatments. Ammonia has been shown to have strong antifungal activity (Bothast *et al.*, 1975).

In conclusion, the results from the present study indicate that increasing the rate of N fertilizer applied to the sward had a quadratic effect on herbage yield but no carryover effect on the yields of a subsequent regrowth. The rate and extent of pH

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