

Improving the efficiency and sustainability of milk production systems through the production and utilisation of high-quality grass silage

Final Report for AgriSearch in Relation to Contract D-89-17

Research team

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EXECUTIVE SUMMARY

- While 'alternative' forages such as whole crop silage and maize silage are included in dairy cow diets to a limited extent in Northern Ireland (NI), grass silage remains the predominant forage within the diet of housed dairy cows. In addition, while silage feeding was previously largely confined to the 'winter period', an increasing proportion of cows in NI are now housed either all year, or for increasing parts of the year. Consequently, reliance on grass silage is increasing.
- Historically the focus on most farms, and within most research programmes, was the production of high quality first cut silage. However, the increasingly confined nature of our production systems means that all silage harvests (1st, 2nd, 3rd and even 4th) make important contributions to the diets of our dairy herds. While the increasing importance of grass silage in dairy cow diets is clear, until this project there had not been a focused grass-silage research programme within NI for approximately 15 years. Recognising the importance of grass silage to the NI dairy sector, this research project involved a targeted programme of research which was designed to address a number of recently identified knowledge gaps. These related to the production of high-quality grass silage in the diets of high yielding dairy cows. The premise of this project was that high quality silage can, and should, play an increasing role in dairy cows diets in Northern Ireland, and that AFBI research can help overcome some of the barriers to improvements in silage quality.
- This project was co-funded by DAERA and AgriSearch. The project comprised 11 work-packages, and the work undertaken within these has been summarised within this nine-chapter report.
- Chapter 1: This study was designed to capture basic information on silage making practices within Northern Ireland, and to identify factors which farmers believe limit improvements in silage quality. One-hundred and seventy-four

dairy farmers were surveyed to identify key silage-making practices, and factors perceived to influence quality of grass silage made on their farms. The majority of farmers (64.9%) harvested grass for silage three times/year, 62.1% normally used a contractor, and 46.5% routinely used a silage additive. Delays to mowing and delays to harvesting due to adverse weather/poor ground conditions were perceived to have a large/very large impact on silage quality (67.9 and 53.2% of farmers, respectively). Inadequate wilting, poor quality swards on owned land, on rented land, and 'contamination' of first cut grass with autumn/winter growth herbage, were all perceived as having a large/very large impact on silage quality (32.2, 26.5, 39.9, 29.9% of farmers, respectively). Over the previous decade 11.0, 41.0 and 36.5% of farmers claimed a small, moderate or large improvement in silage quality, mainly due to earlier cutting of grass and ensiling better quality swards.

Chapter 2: This study was designed to examine changes in silage composition • in Northern Ireland over a 20-year period through the analysis of a dataset comprising 76,452 grass silage samples from commercial farms. These samples had been analysed by the Hillsborough Feed Information Service (HFIS) at the Agri-Food and Biosciences Institute (AFBI) at Hillsborough over a 20-year period (1998 to 2017). The effects of harvest number (1, 2 or 3), farm location (east vs west), and harvest year were examined. The number of silage samples submitted for analysis increased between 1998 and 2012, and then declined after 2013. The predominance of first harvest samples reflects the importance placed by farmers on this harvest, as it is generally offered to the most productive livestock on farms. Most of the differences between harvests 1 - 3 were significant, although small. Silage crude protein increased from harvests 1 to 3, while ammonia nitrogen levels were higher in 3rd harvest silages. Fibre concentrations decreased from harvest 1 to 3, while DM digestibility and D-value (% DM) were higher in 1st compared to 2nd harvest silage. Higher digestibility of 3rd compared to 2nd harvest silages is reflected in lower fibre concentrations in the latter. Silages made in the east of NI generally had higher DM, crude protein, water soluble carbohydrate, digestibility and intake potential, and a lower fibre content, than those made in the west where

weather conditions are generally less favourable. Across the twenty-year period, within each of harvests 1, 2 and 3, silage DM and sugar content increased, while fibre content decreased. Crude protein levels did not change over time, and largely followed the trend in fertiliser nitrogen usage during this period. There was no significant improvement in silage digestibility over the period, while silage intake potential increased by approximately 8% (from 88.8 to 96.1 g/kg W^{0.75}). The absence of an increase in digestibility highlights the need for a renewed focus on improving silage quality.

Chapter 3: The aim of this experiment was to establish the impact of transitioning from Calcium Ammonium Nitrate fertilisers to Stabilised Urea fertilisers. The use of Calcium Ammonium Nitrate (CAN) and Stabilised Urea (SU) fertilisers on herbage yield and composition, and on silage composition, was compared over two successive seasons. Experimental plots (7.5 m²) were established in a randomised block experiment comprising 18 treatments arranged in a 3 x 6 factorial design. Factors comprised the two fertiliser types (plus zero-fertiliser) and 6 harvesting intervals (weeks 2, 3, 4, 5, 6 and 7 post fertiliser application), with each treatment replicated four times $(4 \times 3 \times 6 = 72)$ plots). These 74 plots were replicated within each of 3 silage harvest periods in 2018, and again in 2019. Herbage samples were collected and yields recorded weekly during weeks 2 - 7 post fertiliser application using a destructive harvesting approach. In addition, herbage harvested at week-7 was ensiled in mini-silos and the resulting silages analysed after 100 days. There were no significant differences between responses to CAN and SU in terms of grass dry matter yield and quality parameters (acid detergent fibre, ash, buffering capacity, metabolisable energy, nitrate, nitrogen, or water-soluble carbohydrate). Similarly, there were no differences in silage quality parameters (ammonia nitrogen, pH, crude protein, lactic acid, acetic acid, propionic acid, butyric acid, ethanol, propanol, or dry matter digestibility). There were no significant interactions between CAN and SU treatments and either harvest number or week, for DM yield. Herbage nitrate concentrations were most variable at the third harvest, suggesting a lower nitrogen-use-efficiency at this harvest. This suggests there may be a benefit in reducing fertiliser application recommendations for late-season silage harvests. Overall, the results of this study support the hypothesis that replacing CAN fertiliser with SU will not impact production of grass silage, meaning farmers can confidently adopt SU as a mitigation strategy to reduce farm nitrogenous emissions without reducing levels of sward productivity. Average annual grass DM yields were 15.5 and 16.1 t DM/ha from the CAN and SU fertiliser treatments, respectively, with differences not significant. In addition, the marginal yield gain response to the application of each fertiliser type were not significantly different, namely 21.5 and 23.2 kg of herbage DM for every kg of N applied as SU or CAN respectively, relative to control plots receiving zero nitrogen.

Chapter 4: This study was designed to examine the effects of delayed autumn • closing of ryegrass-based swards on herbage dry matter yield and quality of first cut silage the following season. Three treatments (comprising different closing defoliation dates) were examined in a replicated plot study. Defoliations (harvested by mechanical mower) took place in mid-September, mid-November and mid-January in 2018/2019 and in 2019/2020. The ratio of living: dead grass tissue, herbage yields and resulting silage quality was assessed during the following May at first-cut silage harvest. Ensiled herbage was analysed after 100 days. Silage dry matter yields from plots defoliated in September were higher than from plots defoliated in January in both seasons. Defoliation in January resulted in silage with significantly lower values for ammonia nitrogen compared with defoliation in September in both seasons, with a significant defoliation date x year interaction. Silage metabolisable energy was significantly higher for plots defoliated in November 2018 and January 2019 compared with those defoliated in September 2018. There were no significant differences in percentages of living and dead grass tissue nor significant correlations between percentages of dead tissue and any silage quality parameters. There were statistically significant effects of defoliation timing for acid detergent fibre and volatile corrected dry matter, but not for other silage quality parameters; ash, nitrate, nitrogen, water soluble carbohydrate, pH, crude protein, lactic acid, acetic acid, propionic acid, butyric acid, valeric acid, ethanol, propanol, or dry matter digestibility.

- **Chapter 5**: More frequent harvesting of grass swards offers an opportunity to ٠ improve the nutritive value of grass silage. This study investigated the effect of offering silage produced within either a three- (3H) or four-harvest (4H) system on dairy cow performance. Eighty dairy cows were allocated to one of the two harvesting frequency treatments at calving, and remained on experiment for 25 weeks. Within each harvesting frequency cows were offered each silage for a pre-determined number of days, in proportion to the DM yield of each harvest. Silages were offered as part of a mixed ration containing 8 kg concentrate/cow per day. The remaining concentrate component of the diet was offered on a feed-to-yield basis, through an out-of-parlour feed system. Herbage yields with the 3H and 4H systems were 13.4 and 12.3 t DM/ha, respectively. Silage produced within the 4H system had higher metabolisable energy and crude protein content than that produced within the 3H system. Cows offered the 4H silage treatment had greater silage DM intake, milk yield and milk protein content, while milk fat content was greater in cows offered 3H silages. Harvesting frequency had no effect on bodyweight or body condition score. From a whole system perspective the 4H system increased land requirements by 19.5%, reduced concentrate requirements by 2.2%, and increased fat plus protein yield by 6.9%. In conclusion, increasing harvesting frequency from three to four harvests per year can improve silage feed value, silage intakes and milk yield.
- Chapter 6: Chapter 5 identified that benefits could be achieved when moving from a 3- to a 4-harvest system. This study examined the effect of offering grass silages harvested from perennial ryegrass-based swards within a three-harvest (3H) or five-harvest (5H) system. Thirty-four mid-lactation dairy cows were offered silages produced according to a 3H or 5H system in a continuous design experiment that ran over a 21-week period. Within each treatment cows were offered silage from each harvest (in harvest number order) for a pre-determined number of days in proportion to the dry matter yield of herbage harvested. Silages were offered ad libitum while a common concentrate was offered to all cows at 12.0 kg per cow/day over the first 15 weeks of the study and thereafter at 8.0 kg per cow/day. Total yield of herbage harvested over the season from

within the 3H and 5H systems were 12.6 and 11.2 t DM/ha, respectively. Across all harvests the mean metabolizable energy and crude protein content of silages were 10.9 MJ/kg DM and 131 g/kg DM for the 3H system, and 11.5 MJ/kg DM and 152 g/kg DM for the 5H system. Silage dry matter intake was greater for cows offered 5H silages compared to 3H silages (14.1 v. 11.7 kg/day, respectively). Cows offered 5H silages had a greater daily milk yield (33.5 v. 31.9 kg) and energy corrected milk yield (34.9 v. 33.9 kg) compared to cows offered 3H silages. Treatment had no effect on milk fat or protein content. Cows offered 5H silages had increased concentration of conjugated linoleic acid (CLA) and n-3 fatty acids. Treatment had no effect on mean bodyweight or body condition score of cows. Concentrations of volatile fatty acids in rumen fluid differed between the two treatments, cows on 3H having higher acetate and butyrate concentrations in rumen fluid compared to those on 5H. In conclusion, silage produced within a five-harvest system had improved nutritional value, while cows on the 5H treatment had higher silage intakes, milk yield and energy corrected milk yields compared to those on the 3H treatment.

Chapter 7: While the benefits of offering very high-quality grass silage has been demonstrated within Chapters 5 and 6, farmers are often concerned about how to supplement these silages. A three-period change-over design study using 24 mid-lactation multiparous Holstein-Friesian dairy COWS, examined supplementation strategies for a high-quality grass silage (dry matter, 418 g/kg; crude protein, 170 g/kg DM; metabolisable energy, 12.1 MJ/kg DM). Four treatments, in a 2 x 2 factorial arrangement, compared concentrate type (Highstarch or High-fibre) and straw inclusion (Straw or No-straw). Concentrates had a starch and neutral detergent fibre content of 373 and 258 g/kg DM, respectively (High-starch), and 237 and 339 g/kg DM, respectively (High-fibre). In the No-straw treatments, silage and concentrates were offered as a total mixed ration in a 57:43 DM ratio. In the Straw treatments, chopped straw was added at 4% of total DM, replacing part of the silage component of the diet. Following this study, the effect of diet on nutrient utilisation efficiency was examined using four cows/treatment. There were no interactions between concentrate type and straw inclusion for any cow performance or digestibility parameters. Silage dry matter intake (DMI) and total DMI were reduced with the High-fibre concentrate, and with straw inclusion. Neither concentrate type nor straw inclusion had a significant effect on milk yield or milk fat content. The High-starch concentrate increased milk protein content, while straw inclusion decreased milk protein content. Treatment had no effect on cow body weight, condition score, faecal scores, digestibility coefficients or nitrogen and energy utilisation efficiency. In conclusion, supplementing a high-quality grass silage with a carefully formulated 'high starch' concentrate improved DMI and milk protein content with no adverse effects on cow performance. Straw inclusion in the diet had no beneficial effects on DMI, milk production or nutrient utilisation efficiency.

Chapter 8: As already examined in Chapter 4, grass which grows during the • autumn may lower the nutritive value of silage produced the following spring. The impact of removing autumn herbage using sheep, on silage yield and quality the following spring, and on performance of cows offered these silages, was investigated in two experiments. Following harvest of third-cut silage in September a grass sward was split into blocks which were either grazed by sheep during November and December or left ungrazed. Herbage was harvested and ensiled the following May and offered to late-lactation Holstein cows in a two-period balanced change-over design feeding experiment comprising two 28-day periods. Across the two experiments dry matter (DM) yield was 0.8 to 1.0 t ha greater in the ungrazed swards. Silage from grazed swards had a higher metabolizable energy content. In Experiment 1, DM intake was unaffected, while cows offered silage from the grazed sward (GS) had a greater milk (0.8 kg/day) and protein yield (0.03 kg/day) than cows offered silage from the ungrazed sward (UGS). In Experiment 2, cows offered GS had greater DM intake (1.5 kg/day) and fat yield (0.15 kg/day) with a tendency for a greater fat plus protein yield compared to UGS. In both experiments milk fat plus protein yield per ha was greater with UGS. In conclusion, winter grazing using sheep improved silage quality with only marginal benefits on cow performance; however, milk solids output per ha was reduced following winter grazing.

Chapter 9: Daily harvesting of fresh grass for housed livestock when its ٠ nutritive value is high (Zero-grazing) is a labour-intensive process which requires a consistent supply of grass at the optimum growth stage. An alternative approach which may save on labour and requires less time spent on grassland management each day, involves harvesting and ensiling herbage every 4 weeks approximately during the growing season when it is at the same nutritive value as herbage used for zero-grazing. This study examined the impact of these two approaches on dairy cow performance. Thirty-six midlactation Holstein-Friesian dairy cows were offered either zero-grazed fresh grass (ZG), or grass silage (SIL) prepared from the same sward harvested at a similar growth stage, over a single season. Fresh grass was harvested daily and offered to ZG cows for a 12-week period. During this period the same sward was harvested once weekly and ensiled in round bales. Following a five-week ensilage period the silage was offered to cows on SIL for a 12-week period. All cows were also offered 8.0 kg concentrate per day. Zero-grazed grass and grass silage had a mean metabolisable energy content of 11.0 and 11.3 MJ/kg DM, respectively. Mean forage DM intake and total DM intake was greater for cows on Z compared to SIL, with intakes during weeks 8 to 12 of the experiment lower with SIL compared to ZG. Cows on ZG had a higher milk yield, milk protein concentration, milk fat plus protein yield and energy corrected milk yield than cows on SIL. With the exception of milk yield (where the difference was primarily observed during weeks 8-12 of lactation), these differences were observed most weeks during the study period. Milk fat concentration was unaffected by treatment. Milk of cows on ZG had higher concentrations of total monounsaturated fatty acids, total polyunsaturated fatty acids but lower concentrations of saturated fatty acids compared to cows on SIL. Diet had no effect on cow body weight or condition score. This study has shown that when harvested from the same sward, milk yield and energy corrected milk yield was improved when cows were offered zero-grazed grass compared to grass silage. This difference in performance was likely due to the lower forage intake observed with the grass silage based diet.

Chapter 1

A short survey of key silage-making practices on Northern Ireland dairy farms, and farmer perceptions of factors influencing silage quality

Introduction

The nutritional quality of grass silage, the predominant winter forage for ruminant livestock in western areas of Great Britain and Ireland, varies considerably between farms. Analysis of grass silage samples from Northern Ireland (NI) farms between 1998 and 2017 showed that while average dry matter (DM) content increased, digestible organic matter in the DM (DOMD) remained unchanged (Patterson et al., 2021). Given the advances in silage-making machinery and practices since the 1960s (Wilkinson and Rinne, 2018), the degree of improvement in silage nutritive value has been disappointing. To help address this, a short survey of NI dairy farmers was conducted to provide an overview of key silage-making practices, to examine farmer perceptions of factors that influence the quality of grass silage they produce, and to highlight management practices, which if addressed, provide opportunity to improve silage nutritive value.

Material and methods

A survey of farmers was conducted during a two-day 'Dairy Open Event' held at the Greenmount Campus of the NI College of Agriculture, Food and Rural Enterprise (CAFRE) on 24th and 25th January 2018. Of the 814 farmers who attended, 761 were from NI, representing 681 individual dairy farms. On completion of the event tour, attendees were randomly approached by a member of staff (team of six people) from the Agri-Food and Biosciences Institute (AFBI), and if identified as an active NI dairy farmer, were asked to complete a short questionnaire. Over 90% of those approached agreed to participate. The questionnaire took less than ten minutes to complete, with 174 farmers completing the questionnaire. Disclosure of personal details or herd information was not required.

The survey captured information on silage making practices, including 1) Number of main harvests taken annually, 2) Main silage harvesting equipment used (self-propelled forage harvester (SPFH), trailed harvester, self-loading forage wagon (SLFW), wrapped bales), 3) Contractor use (normally, sometimes, never), and 4) Silage additive use (normally, sometimes, never). The survey also recorded the participant's perceptions on a 1 to 5 scale (1=no effect, 2=some effect, 3=moderate effect, 4=large effect and 5=very large effect) of the impact of the following factors on

the quality of grass silage made on their farm: The effect of delayed cutting, 1) Due to poor weather/poor ground conditions, 2) To allow herbage nitrogen levels to fall, 3) To allow swards to bulk-up to reduce harvest costs, 4) Due to the contractor not being available, and 5) The effect of delayed 'lifting' due to poor weather/poor ground conditions, 6) Grass not being allowed to wilt for long enough, 7) Ensiling autumn/winter growth grass along with first cut silage, 8) Ensiling poor quality grass harvested from owned ground, 9) Ensiling poor quality grass harvested from rented ground, 10) Ensiling slurry residues along with grass, 11) Soil contamination of grass during raking-up, 12) Inadequate compaction of herbage, and 13) Insufficient labour available when making silage. Farmers were also asked to identify 'other' factors which have a negative effect on silage quality on their farm, to detail 'contractor payment systems' and if cutting date would change if contractors charged on a yield basis, to explain how they managed autumn/winter growth grass, and if they believed silage quality on their farm had improved over the last 10 years (none, small, moderate or large improvement). Farmers who indicated that there had been an improvement were asked for the main reasons for the improvement. Results were summarised in Microsoft Excel, and response frequencies determined. Responses to 'open' questions were grouped under relevant headings, and numbers under each heading counted.

Results and discussion

Key silage making practices

The majority (64.9%) of farmers questioned take three main harvests of grass for silage annually, while 22.4% take two harvests (Figure 1A). Silage produced within a two-harvest system will normally have a lower digestibility given that the DOMD of perennial ryegrass declines by about 2.5 g/kg per day up to the point of ear emergence, and by approximately 4 g/kg per day thereafter (Green et al., 1971). Silage digestibility is the single most important determinant of silage feeding value, with each 1% increase in silage DM digestibility (DMD) resulting in an additional 0.33 kg of milk (Keady et al., 2013). The potential benefits of more frequent harvesting was recognised in that 12.1% of those questioned take four harvests, while one farmer operated a five-harvest system.

That grass is harvested by a SPFH on 63.2% of the farms surveyed (Figure 1B) implies that most of the grass cut for silage is precision-chopped. Recent research by Tayyab *et al.* (2018, 2019) reporting higher DMI, higher DM digestibility and higher milk yields in cows offered grass silages with very short chop length. While the use of SLFW can help improve labour and improve fuel use efficiency (Frost and Binnie, 2005), SLFW's were used by only 12.6% of the farmers. In comparison to precision chopped silage, DM intakes and milk yields may be lower in cows offered silage harvested using a SLFW, if not adequately chopped (Randby, 2005).

The high reliance on contractor use (62.1% of farmers normally use a contractor: Figure 1C) aligns with the use of a SPFH to harvest grass, and reflects the disincentive of the large capital investment needed to purchase and maintain modern silage-making machinery. This may also reflect the decreasing availability of labour that is common on many dairy farms (O'Donovan *et al.*, 2008). Only 29.3% of farmers never use a contractor.

Despite the known benefits of silage additive use, especially inoculants, across multiple studies (Keady, 1998; Oliveira *et al.*, 2017), less than half (46.6%) of farmers surveyed always used an additive, with 35.1% never using an additive (Figure 1E). The relatively low uptake of additive use may reflect resistance to the additional costs of using an additive, and a lack of confidence in the outcome.

Farmer perceptions of factors influencing grass silage quality

Farmer perceptions on how a range of factors impact on the quality of grass silage made on their farms (expressed as a percentage of all valid responses) are presented in Tables 1 and Table 2.

Weather, herbage nitrogen levels, contractor availability and charging

Although silage-making is more weather resilient than hay-making, 67.8% of the farmers identified delays in mowing grass due to adverse weather or associated poor ground conditions, as having a large/very large impact on the quality of their silage (Table 1). Similarly, over half (53.2%) perceived such conditions as also adversely affecting silage quality through delays to harvesting the mown grass. The impact of weather-related delays on grass cutting were regarded as minor (i.e. some or none) by only 8.6% of farmers. Mowing delays reduce the digestibility of silage produced

(Keady et al., 2013), but delays in harvesting also lead to excessive losses of energyrich organic matter and a concomitant increase in ash content that further reduces digestibility. Delays in harvesting mown herbage also reduce the stability of silages after silo opening (Wilkinson and Davies, 2013).

Delayed application of organic and inorganic nitrogen (N) fertiliser to grass swards destined for silage can lead to excessively high nitrate-N levels in the mown grass, with negative consequences for silage preservation, especially in difficult to ensile herbage (O'Kiely et al., 2001). However, the majority of farmers (over 50%) perceived delaying cutting to allow herbage N levels to fall has having either 'no' or 'some' effect on silage quality (Table 1), with only 6.9% of farmers identifying this as having a very large effect. This problem can largely be avoided by ensuring an adequate interval between N application and cutting.

A majority of the farmers (64%) considered contractor unavailability to have little impact on the quality of the silage made on their farm (Table 1). This may reflect the relatively large number of contractors now operating in NI, the scale of the machinery used, and the flexibility and willingness of contractors to work 'long-days' to service customer's needs. In contrast, 23.2% of farmers surveyed acknowledged that delaying cutting (to allow swards to 'bulk-up' and reduce contractor harvesting costs) was having a large/very large impact on silage quality on their farms. A follow-up question identified that 89% of the farmers were charged on a per hectare basis and 2.4% on the basis of 'hours-worked' (Figure 1D). It appears that many farmers rationalise that cost savings (per tonne of herbage harvested) made by harvesting a greater yield of crop can offset the disadvantages of feeding a silage of lower quality. Nevertheless, 64% of the farmers indicated that they would consider cutting their grass earlier if their contractor offered a yield-based charging option. Technology already exists on many modern harvesters to assess yield, and it is likely that a move to a yield-based charging approach could contribute to greatly improving the quality of silage made on many NI dairy farms.

Ensilage practices

The benefits of rapid wilting are well known, with Keady (2013) concluding that rapid wilting of herbage from 16% to 32% DM increased silage DM intake by 17% and milk solids output by 3%. While the mean DM content of first cut grass silages in NI

increased by 6.6% units between 1998 and 2017 (Patterson et al., 2021), in the current survey 32.2 of farmers perceived inadequate wilting of mown grass to have a large/very large impact on the quality of the silage made on their farms (Table 2).

The impact on silage quality arising from 'contamination' of primary growth herbage with herbage that has grown in the same sward during the previous autumn/winter does not appear to have been examined experimentally. Nevertheless, 29.9% of farmers perceived that this 'autumn/winter growth' herbage had a large or very large detrimental impact on the quality of their first-cut silage. In response to a follow-up question, almost 84% of the farmers stated that they try to remove this grass, the majority (76.7%) by grazing with sheep, with the sheep normally removed from fields before the end of December.

Perennial ryegrass, the predominant sown species on NI dairy farms, has a higher digestibility than 'weed grasses' which frequently infest older swards (Frame, 1989). Recognising this, 26.5% of farmers indicated that poor quality swards on 'owned ground' had a large/very large impact on silage quality, increasing to 39.9% on rented ground. Pasture reseeding rates in NI are in general low, especially on rented land, a reflection of the 11-month 'conacre' system in place, which disincentives reseeding.

Soil and slurry contamination of grass can have a detrimental effect on silage quality through changes to forage mineral composition and the presence of bacteria which negatively affect silage fermentation (McDonald et al., 1991). While these were not considered to be major issues by the majority of farmers surveyed (Table 2), 23% of farmers perceived that ensiling slurry residues with grass had a large/very large effect on silage quality on their farms, while the equivalent figure for soil contamination was 24.1%. Both problems can be avoided by good management.

Relatively few (18.9%) farmers perceived inadequate compaction of grass in the silo as having a large or very large effect on silage quality. While silos are filled much faster today than in the past, the risk of inadequate silo compaction is likely reduced by use of heavier machinery at filling, and by the greater depth of modern silos.

Given the reliance on contractor use on the majority of farms surveyed, it was unsurprising that insufficient labour was regarded by 66.1% of the farmers as having no/some, effect on their silage quality. On farms where contractors are not used, inadequate labour can lead to problems at busy times, and 19% of farmers perceived inadequate labour at silage making to have a large/very large effect on silage quality.

In addition to the 13 factors highlighted in Tables 1 and 2, farmers were asked to suggest 'other' factors having a negative effect on silage quality on their farms. Of the 31 valid responses obtained (ie issues not already covered by the 13 factors highlighted above), issues relating to 'soil nutrients' were highlighted by 32% of farmers, inadequate sunshine/low herbage sugar levels by 23% of farmers, and issues related to sealing silos/aerobic stability by 13% of farmers.

Changes in silage quality over the previous 10 years

Of farmers questioned, 11.6% believed there had been no improvement in silage quality on their farms over the past 10 years, while 11.0, 41.0 and 36.5% believed there had been a small, moderate or large improvement, respectively. 'Earlier/more frequent cutting of grass' (37.1%) and 'reseeding/improved varieties/weed control' (22.4%) were listed as the predominant reasons for improved silage quality. The most common 'other' reasons given included 'improved knowledge/improved management practices' (11.4%), 'wilting/tedding' (5.7%), 'improved machinery/own machinery' (3.8%), 'improved soil nutrition' (3.3%), and 'grazing by sheep over the winter' (2.4%). These findings clearly highlight the potential of earlier and more frequent cutting, and improving sward quality, to improve silage nutritive value within NI.

It is recognised that those who participated in the survey were a 'self-selected' group of dairy farmers, their participation reflecting their attendance at a technology transfer event. While it is possible that this group differed from the NI dairy farmer population as whole, author experience is that such events attract a diverse range of farmers. Nevertheless, while the results must be caveated within this scenario, this simple to enact survey provided valuable information on current silage making practices in NI, and farmers perceptions of key factors impacting on silage quality.

Conclusions

Weather mediated delays to cutting and harvesting grass were identified as having the greatest impact on silage nutritive value. However, many issues which can be

overcome through improved management practices were identified by a substantial number of farmers as having various degrees of negative impacts on silage quality on their farms. Improvements in silage quality which have occurred on most farms over the last ten years were attributed primarily to the earlier cutting of grass and the ensiling of herbage from better quality swards.

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Table 1. Farmer perceptions (% of farmers) of the impact of factors influencing thetiming of cutting and harvesting of grass on the quality of grass silage made on theirfarms

	*Impact				
	1	2	3	4	5
Delaying cutting due to poor weather or poor ground conditions	2.3	6.3	23.6	33.3	34.5
Delaying cutting to allow herbage nitrogen levels to fall	35.6	21.3	22.4	13.8	6.9
Delaying cutting (allow swards to bulk-up to reduce harvest cost)	42.8	23.1	11.0	11.6	11.6
Delaying cutting due to the contractor not being available	44.2	20.1	11.0	14.9	9.7
Delaying 'lifting' due to poor weather or poor ground conditions	13.3	11.0	22.5	31.8	21.4

*Impact = none (1); some (2); moderate (3); large (4) and very large (5).

Table 2. Farmer's perceptions (% of farmers) of the impact of a range of ensilage

 practices on the quality of the grass silage made on their farms

	*Impact				
	1	2	3	4	5
Grass not being allowed to wilt for long enough	13.2	18.4	36.2	23.6	8.6
'Contamination' of 1 st cut herbage at ensilage with grass that grows during late autumn/winter	36.8	12.6	20.7	18.4	11.5
Ensiling poor quality grass harvested from owned ground	39.1	17.2	17.2	18.4	8.1
Ensiling poor quality grass harvested from rented ground	31.2	12.7	16.2	28.3	11.6
Ensiling slurry residues along with grass	53.5	14.9	8.6	11.5	11.5
Soil contamination of grass during raking up	34.5	23.9	15.5	12.6	11.5
Inadequate compaction of herbage (due to silo filled too quickly)	44.4	19.5	17.2	12.4	6.5
Insufficient labour being available when making silage	42.5	23.6	15.5	13.2	5.2

*Impact = none (1); some (2); moderate (3); large (4) and very large (5)



Figure 1 Survey outcomes in relation to: (A) Number of main harvest taken per year, (B) Percentage of farmers using different harvesting systems, (C) Percentage of farmers using a contractor, (D) Percentage of farmers subjected to different contractor charging systems, (E) Percentage of farmers who normally use an additive, and (F) Percentage of farmers according to Perceived improvement in silage quality on their farm over the last 10 years.

Chapter 2

Review of grass silage quality on Northern Ireland farms between 1998 and 2017

INTRODUCTION

The ruminant livestock sector in Northern Ireland (NI) is largely grassland based, with 96% of all agricultural land area classified as grassland (DAERA, 2018). Ruminant livestock traditionally graze outdoors from March/April until September/October, and are housed and offered grass silage based diets for the remainder of the year. However, in recent years there has been an increase in the number of NI farms where livestock, especially dairy cows, are either completely housed all year, or housed at night for extended periods throughout the year. This follows the trend observed within Great Britain (March et al., 2014). Given the small area of maize grown for silage in NI, grass silage looks set to remain the predominant conserved forage for the ruminant livestock sector, which is reflected in the fact that grass silage was produced on 37% (298 480 ha) of the total grassland area in 2017 (DAERA, 2018).

Many factors affect grass silage quality, including sward composition, weather conditions, soil type, cutting date, additive use, speed of silo filling and degree of compaction, type of cover, ammonia and fibre content, and feed-out rate post opening (Frame & Laidlaw, 2011). Grass silage quality is normally defined as the combination of chemical composition, fermentation characteristics and nutritive value, and 'silage quality' has a direct impact on subsequent animal performance. In a review, Keady et al. (2013) found that for each 10 g/kg increase in silage digestible organic matter in the dry matter (DOMD or D-value), dry matter intake (DMI) and milk yield increased by 0.22 kg/day and 0.33 kg/day, respectively, while carcass gain in beef cattle and finishing lambs increased by 23.8 g/d and 9.3 g/d, respectively. Similarly, Steen et al. (1998) reported that the intake of beef cattle increased by 15 g/kg of the mean intake, for each 10 g/kg increase in silage apparent digestibility. Furthermore, these authors identified that silage intake is closely related to factors which influence the extent of digestion, and the rate of passage of material through the animal, as indicated by the strong relationships with in vivo apparent digestibility, rumen degradability, fibre concentration and N fractions of the silage.

Changes in the quality of grass silage produced on NI farms have been reviewed periodically over the last 50 years. For example, Jackson et al. (1974) and Unsworth (1981) summarised the quality of silage produced between 1967 – 1972 and between 1973 – 1979, respectively. In general, over the period covered by these reviews there

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were no consistent trends in silage DM (dry matter) content, fibre content and digestibility. There was however a marked increase in silage CP (crude protein) content during the period between 1973-1979, despite similar levels of fertiliser N use during that period, with Unsworth (1981) explaining this trend by a general shift to earlier cutting dates and the adoption of more frequent cutting regimes during those years. Unsworth (1981) also suggested that differences in chemical composition of silages between years could be ascribed to variations in the climatic conditions, and it should be noted that the periods covered within each of these reviews were relatively short, typically 5 - 7 years.

However, significant changes in silage making practices and technologies have taken place since silage quality in NI was last reviewed, with some of these changes reviewed by Wilkinson & Rinne (2018). Consequently, silages produced today might be expected to be of a very different quality compared to silages reviewed by Unsworth (1981), and indeed silage produced two decades ago. Furthermore, silage analytical techniques have also changed considerably over the years, with the use of 'wet chemistry' now largely superceded by Near Infrared Reflectance Spectroscopy (NIRS) which is routinely used to predict silage composition, fermentation characteristics, digestibility and intake potential (Park et al., 1998).

Thus the current study was designed to examine changes in the quality of grass silage produced on NI farms over a 20 year period, from 1998 to 2017. This involved the analysis of a dataset comprising silage samples from commercial farms which were analysed by the Hillsborough Feed Information Service (HFIS) at the Agri-Food and Biosciences Institute (AFBI) Hillsborough over this period. This paper is timely given that this commercial silage analysis service was scaled down substantially in 2018. The primary aim of the study is to identify long term changes in silage quality between 1998 and 2017, and to assess the effect of harvest number and farm location on silage quality parameters during the same period.

MATERIALS AND METHODS

During the 20 year period between 1998 and 2017, a total of 78,958 grass silage samples from commercial farms across NI were submitted to the HFIS laboratory at

AFBI Hillsborough. Each silage sample had information available describing year of harvest (1998–2017), harvest number (1, 2, 3, 4 and 5), and county of origin based on the farm address (Antrim, Armagh, Down, Fermanagh, Londonderry and Tyrone). Fresh silage samples had been scanned within 24 hours of receipt using Near Infrared Reflectance Spectroscopy (NIRS), as described by Park et al. (1998). The NIRS spectra generated were then used to predict the chemical composition (DM, CP, pH, neutral detergent fibre (NDF), acid detergent fibre (ADF), water soluble carbohydrate (WSC) and ash), fermentation characteristics (lactic acid (LA), volatile fatty acids (VFA) and ammonia nitrogen (NH₃-N)) and 'nutritive values' of these silages (dry matter digestibility (DMD), D-value, dairy intake potential and beef intake potential), using a series of prediction equations. These prediction equations were developed at AFBI based on the analysis of 136 grass silages of differing qualities obtained from local farms, as described in detail by Steen et al. (1998). In summary, these samples were analysed for a wide range of parameters using wet chemical analysis, their rumen degradability, digestibility and intake potential measured, and samples scanned using NIRS. Chemical composition data and data from the animal trials were then used to create the NIRS calibration equations for grass silage. Having developed these equations, the laboratory at AFBI Hillsborough maintains the Master NIRS instrument for Proficiency Ring Testing of the Forage Analysis Assurance (FAA) Group for grass silage in the United Kingdom (UK) and Ireland.

Of the 78,958 results available within the data base, 2507 results were excluded for a number of reasons: unknown harvest number (n = 2159); fourth and fifth harvest samples (n = 257); samples with DM content >60% (n = 90); and samples with an ammonia concentration >100% of total N (n=1). This left a total of 76,452 silage samples for inclusion within the analysis. Table 1 summarises the number of samples included within the analysis from each harvest year, for each of harvests 1, 2 and 3, by geographic location within NI. Counties Antrim, Down and Armagh were ascribed as 'East' and counties Fermanagh, Londonderry and Tyrone were ascribed as 'West'. Mean monthly weather data (temperature and rainfall) were extracted from the Meterological Office database (https://www.metoffice.gov.uk/climate/uk/summaries/datasets) for all weather stations (n = 120) across NI for the period 1998 - 2017Data describing total quantities fertilizer nitrogen delivered in NI for agriculture and horticulture use over the period 1998 - 2017

were also obtained (<u>https://www.daera-ni.gov.uk/publications/fertiliser-statistics-2009-</u> 2019) and is presented in Appendix 1.

Silage quality variables over the 20 year period were examined for linear effects using an unbalanced ANOVA with a factorial arrangement of Year, Location and Harvest fitted as the treatment factors. If any of the treatment effects were significant (P<0.05) then Fisher's LSD test was used to assess the pairwise differences between individual levels of that effect. In addition, simple linear regression analysis was conducted within each harvest to examine if silage quality changed over the 20 year period. All data were analysed using GenStat (16th edition; VSN International Limited, Oxford, UK).

RESULTS AND DISCUSSION

The general increase in the number of silage samples submitted to AFBI for analysis between 1998 and 2012 (Table 1) is likely to reflect an increasing level of confidence that farmers had in the service, and a general move by farmers to more closely align rations offered to silage quality. The decline in the number of samples submitted after 2013 was largely due to the increasing availability of similar analytical services within the commercial sector. The large number of first harvest samples analysed, relative to second harvest, demonstrates the importance placed by farmers on first harvest silage, with this likely to be the forage offered to the most productive livestock on farms over the winter. The small number of third harvest samples analysed is likely to reflect that fact that many farmers (especially dry stock farmers) still operate a two-harvest system, and that third harvest silage is normally offered to 'lower production' livestock.

	E	astern countie	28	W			
Year	1 st harvest	2 nd harvest	3 rd harvest	1 st harvest	2 nd harvest	3 rd harvest	Annual total
1998	768	376	47	710	314	13	2228
1999	700	450	48	632	356	15	2220
2000	931	507	74	698	374	32	2616
2000	829	517	89	603	340	32 42	2010
2001	1642	612	64	1283	469	26	4096
2002	1342	673	87	1110	508	28	3748
2004	1264	785	150	1232	690	64	4185
2005	1343	677	137	1167	594	61	3979
2006	1250	740	160	1049	691	64	3954
2007	1363	848	137	1037	490	49	3924
2008	1488	1007	202	1156	622	32	4507
2009	1571	922	154	1193	617	41	4498
2010	1469	1024	217	1087	698	68	4563
2011	1593	1028	283	1376	702	62	5044
2012	1714	1179	273	1780	886	151	5983
2013	1553	1032	348	1363	819	177	5292
2014	1197	661	234	1149	558	122	3921
2015	869	513	147	846	408	56	2839
2016	894	581	167	939	470	64	3115
2017	998	624	189	936	451	68	3266
Total	24850	14756	3207	21346	11057	1236	76452

Table 1. The number of silage samples analysed by AFBI each year between 1998 and 2007, subdivided by harvest number (1st, 2nd and 3rd), within eastern and western counties of Northern Ireland (Eastern counties, Antrim, Down and Armagh: Western counties, Londonderry, Tyrone and Fermanagh)

Comparison of 1st, 2nd and 3rd harvests

When examining the effects of harvest number on silage quality (Table 2), it is important to recognise that the number of 3^{rd} harvest samples analysed was relatively small, and that farmers submitting these samples may represent a 'self-selecting' group who may make better quality third cut silages than the average farmer. First harvest silages had a higher DM content (260 g/kg, *P*<0.001) than both 2nd and 3rd harvest silages, likely reflecting a general trend for more favourable weather and

ground conditions at the time of first harvest (Table 2). Nevertheless, from a practical point of view, differences in DM content between harvests were surprisingly small. The increase in silage CP content from 1st harvest through to 3rd harvest (118, 121 and 140 g/kg DM, respectively: P<0.001) likely reflects the increasingly vegetative stage of herbage harvested as the season progresses, with this reflected in the decreasing NDF and ADF content of the silages. Increasing ash concentrations with later harvests (P<0.001) may reflect increasing soil contamination of crops, or soil contamination being less 'diluted' within lighter crops later in the season, or simply differences in herbage mineral content at the time of harvest due to differences in plant physiology. The higher NH₃-N levels in 3rd harvest silages (107 g/kg compared with 103 and 102 g/kg for 1st and 2nd harvests, respectively) suggest increased levels of proteolysis of plant protein by plant and microbial enzymes in these later harvests. The high lactic acid concentrations observed across all harvests indicate lactic acid based fermentations dominate within the data set, with concentrations highest in 3rd harvest silages. In contrast, volatile fatty acids (VFA) concentrations were higher at first harvest (P<0.001: 27.2 g/kg DM) than at either of harvests 2 or 3 (23.3 and 22.9 g/kg DM, respectively). Differences in pH at harvests 1 and 2 reflect differences in lactic acid concentrations at these two harvests. That DM digestibility and D-value (% DM) were higher in 1st compared to 2nd harvest silage is not unexpected, although this difference arose despite only small differences in fibre content between these two harvests. Nevertheless, the higher digestibility of 3rd compared to 2nd harvest silages is reflected in lower fibre concentrations with the latter. Both dairy and beef intake potential followed similar trends to digestibility, which is not surprising as the latter a key driver of intake (Steen et al., 1998). In general, while there were many significant differences in guality between silage samples submitted for analysis from 1st, 2nd and 3rd harvests, perhaps surprisingly, many of the differences observed between harvests were numerically small. For example, the similar digestibility and intake potentials of 1st and 3rd harvest silages would suggest that similar levels of performance might be achieved when these two silage were offered, although in practice this is unlikely to be the case.

Table 2 Effect of silage harvest (1st, 2nd and 3rd) and farm location (eastern and western counties) on silage quality in Northern Ireland between 1998 and 2017

	Harvest					Location				
	1 st harvest	2 nd harvest	3 rd harvest	SEM	<i>p</i> -value	Eastern counties	Western counties	SEM	<i>p</i> -value	
Dry matter (g kg ⁻¹)	260 ^b	256 ^a	257 ^a	0.4	< 0.001	270	244	0.2	< 0.001	
Crude protein (g kg ⁻¹ DM)	118 ^a	121 ^b	140 ^c	0.1	< 0.001	122	118	0.1	< 0.001	
Neutral detergent fibre (g kg ⁻¹ DM)	509 ^c	503 ^b	477 ^a	0.3	< 0.001	496	516	0.1	< 0.001	
Acid detergent fibre (g kg ⁻¹ DM)	329 ^c	327 ^b	312 ^a	0.2	< 0.001	323	333	0.1	< 0.001	
Water soluble carbohydrate ($g kg^{-1} DM$)	24.1 ^b	23.7 ^a	25.2 ^c	0.11	< 0.001	26.8	20.6	0.05	< 0.001	
Ash $(g kg^{-1} DM)$	76.5 ^a	80.9 ^b	89.2 ^c	0.06	< 0.001	80.0	77.0	0.02	< 0.001	
NH_3-N (g kg ⁻¹ total N)	103 ^b	102 ^a	107 ^c	0.2	< 0.001	101	106	0.1	< 0.001	
pН	4.03 ^b	3.97 ^a	4.05 ^c	0.04	< 0.001	4.01	3.97	7.07x10 ⁻⁴	0.008	
Lactic acid (g kg ⁻¹ DM)	68.3 ^a	71.6 ^b	77.1°	0.19	< 0.001	71.6	67.8	0.08	< 0.001	
Volatile fatty acids (g kg ⁻¹ DM)	27.2 ^b	23.3 ^a	22.9a	0.09	< 0.001	23.7	28.2	0.04	< 0.001	
Dry matter digestibility (% DM)	70.3 ^c	68.5 ^b	69.7 ^a	0.03	< 0.001	69.9	69.3	0.01	< 0.001	
D-value (% DM)	67.2 ^c	65.6 ^a	66.4 ^b	0.03	< 0.001	66.7	66.5	0.01	< 0.001	
Dairy intake potential (g kg $W^{0.75}$)	94.2 ^c	91.3 ^a	93.0 ^b	0.06	< 0.001	94.7	91.2	0.02	< 0.001	
Beef intake potential (g kg $W^{0.75}$)	78.6 ^c	74.7 ^a	76.0 ^b	0.06	< 0.001	78.6	75.4	0.02	< 0.001	

Means with the same superscript within 'Harvest' do not differ significantly (p>0.05)

D-value, Digestible Organic Matter in dry matter; W^{0.75}, metabolic liveweight

Impact of location (east versus west) on silage quality

Farmers perceptions of the importance of weather on silage quality was highlighted in a recent survey by Ferris et al. (2018), in which 68% of farmers indicated that delayed cutting of grass due to poor weather or ground conditions had either a large or very large effect on silage quality on their farms. Similarly, in the same survey, 53% of farmers indicated that delayed lifting of grass crops due to poor weather or ground conditions had either a large or very large effect on silage quality on their farms. Many of the differences which exist between the composition of silages analysed from eastern and western counties are likely due to differences in weather patterns between these two regions of NI. For example, mean NI Meteorological office data between 1998 and 2017 shows that average annual rainfall was 1065 mm and 1224 mm in eastern and western counties, respectively. Furthermore, total rainfall during the 'silage making period', namely May to September, was 401 and 524 mm respectively in eastern and western counties, while average temperatures during the same period were 13.0°C and 12.6°C respectively. The higher (*P*<0.001) DM concentration of silage samples from eastern compared to western counties (268 vs 244 g/kg, respectively) likely reflects lower rainfall and increased sunshine hours with the former. Differences in CP levels are likely to reflect the use of lower applications of N fertilizer in the west, a reflection of the generally less intensive nature of agriculture in that part of NI. While silage CP can also reflect crop maturity stage at harvest, the similar digestibility of silage in the east and west suggests that crops were harvested at similar maturities. The higher fibre (NDF and ADF) levels in silages from the west, compared to the east, were not reflected in differences in silage digestibility. The higher WSC levels in samples from the east likely reflect their higher DM content, and an associated less extensive fermentation, with this reflected in their higher (P<0.008), although numerically similar, pH. Higher NH₃-N levels in silages from the western part of NI suggest higher levels of proteolysis of plant proteins, with this again likely driven by differences in silage DM content. Silage digestibility (DMD and D-value), although very similar between eastern and western counties, was higher in samples from the former. The higher intake potential (P<0.001) of silages made in eastern counties is likely to be largely a reflection of the difference in DM content, with DM another key driver of intake potential (Steen et al., 1998).

Changes in silage quality between 1998 and 2017

In this study silage quality is considered to encompass chemical composition, fermentation characteristics and nutritive value.

Chemical composition

Within each of the three harvests, silage DM content increased (p<0.001) over the 20 year period (Figure 1a), and while there was considerable year-to-year variation in silage DM content, the mean rates of increase in DM were 3.39, 2.0 and 2.2 g/year for harvests 1, 2 and 3, respectively. Indeed, this appears to be part of a longer term trend, with Figure 2a demonstrating that mean silage DM content during the first 10 years of this survey period (1998 – 2007) was higher than the mean DM reported by Unsworth (1981) between 1973 – 1979. Nevertheless, DM reported by the latter author did not differ from that reported by Jackson et al. (1974) between 1967 – 1972. Changing climatic conditions may have contributed to this increase in DM content, with sunshine hours being the main weather factor influencing the rate of wilting of herbage, and consequently silage DM content (Wright, 1997). Similarly, mechanical treatments such as conditioning and spreading the cut swath in good weather conditions also enhance field wilting (Frame and Laidlaw, 2011). However, it is likely that changes in silage making technology was the primary driver of the increasing DM contents observed. The use of mower conditioners, mowers which spread the cut sward over most of the mown area, grass tedders, and grass rakes which allow mown herbage to be raked up quickly, have all facilitated the adoption of rapid wilting techniques, thus allowing farmers to maximize the opportunity offered by short periods of good weather. While ensiling herbage with higher DM contents will reduce effluent losses and improve fermentation characteristics, silage made using rapid wilting techniques has been shown to have a higher intake and to improve animal performance (Yan et al., 1996 & 1998).

There was no significant change in silage CP content over the 20-year period examined within the study, although third harvest silages had a consistently higher CP than either 1st or 2nd harvest silage each year (Figure 1b). However, mean protein levels over the twenty year period covered by the data set were actually lower than those in samples analysed between 1967 – 1972 (mean of 135 g/kg DM) and 1973 – 1979 (mean of 144 g/kg DM)(Figure 2b). Herbage CP content is largely determined by the maturity of the herbage at harvest, and by applications of both organic and inorganic N. The latter likely explains the decline in silage protein levels between 1979-1997 being 101 000 tonnes/year, compared with an average value of 848 000 tonnes/year between 1998-2015 (DAERA 2020). Within the timeframe of the current dataset, the introduction of the Nitrates Action Programme (NAP) in NI (DAERA, 2008), as required by the EU Nitrates Directive, led to a reduction in fertilizer

N applications to grassland. This is highlighted in Appendix 1, which shows the total quantities of fertilizer N delivered in NI between 1998 and 2017 (DAERA, 2020). While there was much variation in silage crude protein concentrations from year to year, there was a trend, especially in the latter part of the data set, for silage CP levels to follow the trends in fertilizer N deliveries. The impact of the reduction in silage protein levels, relative to historical levels, has mixed implications for ruminant nutrition. For example, protein is an essential nutrient for livestock production, and lower protein levels in silages may necessitate increased levels of protein supplementation via concentrates. However, silage protein is readily degradable in the rumen, and if the ammonia arising from its breakdown is not captured efficiently by rumen microbes, much will be excreted in manure. Thus lower protein silages may actually result in improved N use efficiency in ruminants, albeit with additional costs associated with concentrate purchases.

Both NDF and ADF level in the silage samples analysed showed a significant decline over the 20 year study period (Figure 1c & 1d). The likely explanation for this is a move by farmers to harvest herbage either earlier, or more frequently so as to increase silage digestibility, as demonstrated by Kuoppala et al. (2008) and Randby et al. (2012). However, it is also possible that the increased use of additives that contain enzymes such as cellulose, may have contributed to a fall in fibre concentrations.

The WSC concentration of silage samples increased (*P*>0.001) over the 20 year period within all harvests (Figure 1e). This increase in residual WSC levels is likely to reflect a less extensive fermentation as a consequence of the increase in DM concentration of the herbage ensiled, as discussed in <u>McDonald et al. (1991</u>). While higher residual WSC levels may provide a rapidly available energy source for rumen microbes, they can also leave the silage more susceptible to secondary fermentation following silo opening, with an associated loss of nutritive value.

While ash is derived from the inorganic constituents of silage, it can also be indicative of soil contamination. High ash levels as a result of soil contamination (> 100 g/kg DM) can lead to a poor fermentation, reduce intakes and poorer animal performance (AHDB, 2012). Ash concentrations in NI silages have remained relatively unchanged over the 20 year period, with levels generally within the range of 75 – 90 g/kg DM (Figure 1f). There is anecdotal evidence that grass rakes, which have been increasingly used by contractors over the 20

years experimental period to 'row up' grass for lifting, can increase soil contamination. While this may be an issue if rakes are set too 'low', the absence of an increase in ash concentrations suggests that this has not be a significant issue.

(b)




Figure 1. Changes in the (a) dry matter (DM), (b) crude protein (CP), (c) acid detergent fibre (ADF), (d) neutral detergent fibre (NDF), (e) water soluble carbohydrate (WSC) and (f) ash content of first (solid line), second (dashed line) and third harvests (dotted line) of grass silages made on Northern Ireland farms and analysed at AFBI between 1998 and 2017. Data with *, ** and *** indicate the relationship was significant at the *p*<0.05, *p*<0.01 or *p*<0.001 level, respectively, or ns = non-significant



Figure 2. Long term trends in dry matter content (a) and crude protein content (b) of grass silage samples from Northern Ireland farms analysed by AFBI between 1967–1972 (Jackson *et al.*, 1974), 1973-1979 (Unsworth, 1981) and 1998-2007 & 2008-2017 (current data set)

Fermentation characteristics

Lactic acid concentrations declined over the 20 year period in each of harvests 1 (P<0.05), 2 (P<0.05) and 3 (P<0.001), with total VFA concentrations also declining (P<0.001) (Figure 3a and b, respectively). These effects suggest a shift towards more restricted fermentations within NI silages, in line with the increasing residual WSC

concentrations observed, and this is likely a consequence of the increasing DM concentration of the herbage ensiled. In view of the trends in lactic acid and VFA concentrations, it was surprising that silage pH did not change (*P*>0.05) over the 20 year period (Figure 3c). In higher DM silages with a restricted fermentation, Coblentz & Akins (2018) reported that lower levels of fermentation acids were associated with a higher final pH. It is of course true that achieving a low pH is less critical with higher DM silages.

While NH₃-N concentrations (as a proportion of total N), tended to increase over the 20 year period, this effect was only significant (*P*<*0.001*) for 3rd harvest silages (Figure 3d). Increasing NH₃-N concentrations suggest increasing levels of plant proteolysis, with DM and pH being the most important factors affecting this process in silages (Muck et al., 1996). However, proteolysis tends to be more extensive in wetter silages, and given the increasing DM concentrations observed across the twenty year period in this study, the trends in ammonia N concentrations may be as a result of a slower fall in pH with higher DM silage, with an associated increase in proteolysis.



Figure 3. Changes in the (a) lactic acid, (b) volatile fatty acids (VFA), (c) pH and (d) NH₃-N content, of first (solid line), second (dashed line) and third harvests (dotted line) of grass silages made on Northern Ireland farms and analysed at AFBI between 1998 and 2017. Data with *, ** and *** indicate the relationship was significant at the p<0.05, p<0.01 or p<0.001 level, respectively, or ns = non-significant.

Intake potential and digestibility

Despite the upward trend in DMD in 1st harvest silages (Figure 4a) and D-value (Figure 4b), these effects were not significant. Furthermore the DMD and D-value of 2nd and 3rd harvests tended to decrease over time, although this was only significantly with 3rd harvest silage (*P*<0.05). Given the significant reductions in silage ADF and NDF contents in all harvests over the 20 year period, an increase in silage digestibility might have been expected, albeit digestibility is also affected by CP concentration which did not change over the same period. The absence of any measurable improvement in silage digestibility is of significant concern given that digestibility is considered to be one of the most important determinants of silage feeding value and performance of animals offered grass silage (Keady et al., 2013). Silage digestibility is affected by the quality of the herbages ensiled and plant maturity at harvest. While plant breeding has resulted in incremental improvements in both yield and digestibility of perennial ryegrass varieties in recent

decades, the low rate of reseeding in NI (approximately 3.5% of the NI grassland area per year) has likely limited opportunities to benefit from these new varieties. While most farmers recognize that mowing herbage at a less mature stage will result in silage with a higher digestibility, many factors prevent this from happening, including adverse weather and/or ground conditions, high herbage nitrate level, and unavailability of contractors (Ferris et al., 2019). In addition, the majority of contractors still charge farmers on an area basis, and not on the basis of herbage yield, thus incentivising farmers to delay harvesting to increase yields, and as such reduce contractor charges per tonne of herbage ensiled (Ferris et al., 2019). Nevertheless, the same survey indicated that for farmers who believed silage quality had improved on their farms over the previous decade, 37% attributed this to 'earlier/more frequent cutting of grass', while 22% attributed this to 'reseeding/improved varieties/weed control'.

The calculated intake potential of silages for dairy cows increased significantly (*P*<0.001: Figure 4c) within all three harvests between 1998 and 2017, by approximately 8%. In contrast, the calculated intake potential of silages for beef cattle (Figure 4d) increased only within 1st harvest silages (*P*<0.05). That different intake responses are derived from the same data set is due to the adoption of different intake predictions for lactating dairy cows and growing beef cattle. For example, the intake potential for beef cattle is derived by placing weightings on a number of parameters derived from the NIRS analysis of silage, including DM, CP, NH₃-N, and DOMD (Steen et al., 1998). In contrast, the intake potential of silage for dairy cows is derived from models which include a correction for supplementary concentrates, a milk yield adjustment factor to standardize milk yields, with these models converting a predicted intake potential for beef cattle to one for dairy cows (McNamee et al., 2005). In the case of dairy-intake potential, the increase in silage DM content, and the reduction in fibre content over the 20 years period are two of the key drivers for the increase in intake potential observed, likely from a combination of earlier harvesting and a move toward rapid wilting of crops pre-ensiling.



Figure 4. Changes in the (a) dry matter digestibility (DMD), (b) D-value, (c) dairy intake potential and (d) beef intake potential of first (solid line), second (dashed line) and third harvests (dotted line) of grass silages made on Northern Ireland farms and analysed at AFBI between 1998 and 2017. Data with *, ** and *** indicate the relationship was significant at the p<0.05, p<0.01 or p<0.001 level, respectively, or ns = non-significant

CONCLUSION

This unique database allows for a long term examination of trends in the quality of grass silage produced on NI dairy farms, both between harvests, between eastern and western regions, and over a 20 year time period. While crude protein increased from harvests 1 to 3, and fibre concentrations decreased, in general, most of the differences between harvests, although significant, were small and of little practical importance. In general, differences between silage made in the east and west of NI reflect differences in climatic conditions between these two regions. Over the 20 year period, silage DM content increased, most likely reflecting the adoption of rapid wilting techniques, with this accompanied by higher residual sugar levels, and decreasing lactic acid levels. While fibre concentrations decreased over the 20 year period, this was not accompanied by an increase in silage DMD, a disappointing observation. Given that grass silage remains

the predominant forage for housed ruminant livestock in NI, and the absence of significant improvements in parameters such as DMD, a renewed focus on improving silage quality is required.

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Chapter 3

Comparison of the effects of Calcium Ammonium Nitrate and Stabilised Urea fertilisers on grass yields and silage quality

Introduction

Managed grasslands (excluding rough-grazing areas) cover approximately 79% (818,000 ha) of the farmed agricultural land area in Northern Ireland and underpin the sustainability of ruminant livestock production in the province (DAERA, 2021a). The predominant forage species in Northern Irish grassland pastures is perennial ryegrass (*Lolium perenne L.*, PRG) which provides substantial annual yields of a high quality, reliable and cost-effective home-grown forage source as either grazed grass or conserved forage (largely as grass silage) (Patterson et al 2021). The agricultural productivity of PRG-dominated grasslands requires significant quantities of nitrogen fertiliser each year, and it is estimated that around 342,000 tonnes of fertiliser were purchased in Northern Ireland in 2020 containing 86,700 tonnes of N (DAERA, 2020b). A significant quantity of this would have been applied to grasslands, with farmers in Northern Ireland permitted to utilise up to 272 kg N/ha/year for grass production on dairy farms and 222 kg N/ha/year on other livestock farms as per the Nutrient Action Programme (DAERA, 2022). The nitrogen limits are the maximum nitrogen application rates for the whole area of grassland, and not individual fields. There is no specific nitrogen limit for silage.

N Fertiliser, Ammonia (NH₃) and Nitrous Oxide (N₂O)

The importance of high-quality grass production in Northern Ireland for feeding livestock is clear, however, the application of N fertilisers to agricultural soils in order to support high levels of production is a major source of N₂O emissions (Roche et al., 2016). Compared to its global pre-industrial value of around 270 ppb the concentration of N₂O was approximately 20% higher by 2011 as measured at the Mace Head research station (part of the global greenhouse gas (GHG) observation network) in Ireland (Dwyer, 2012). Between 2011 and 2019, N₂O concentrations increased a further 2.5% from 324 ppb to 332ppb (IPCC, 2021). A further source of nitrogenous emissions from agricultural land is ammonia (NH₃), which is a source of particulate pollution hazardous to human health (DAERA, 2021b).

The 'Making Ammonia Visible' report (DAERA, 2017) stated that 91% of all ammonia (NH₃) emissions in Northern Ireland come from agriculture, and 76% of total nitrous oxide (N₂O) emissions are attributed to agriculture (DAERA, 2021b). It has subsequently been recommended (DAERA, 2017) stabilised urea (SU) fertilisers (also known as treated urea or protected urea) are used, which are less vulnerable to volatilisation and de-nitrification,

in place of using CAN or straight urea products. The latter loses more NH₃ through volatilisation than CAN (Forrestal et al., 2016) but CAN emits more N₂O, which is a more potent greenhouse gas (GHG than unamended urea (Dampney, Chadwick, Smith, & Bhogal, 2004; Watson, Laughlin, & McGeough, 2009), whereas SU fertiliser products have been shown in comparison to contribute lower levels of both NH₃ and N₂O to the environment (Cowan et al, 2019).

In a Mediterranean maize – maize-wheat rotation it was reported that SU fertilisers reduced N₂O emissions from deep soil but urease inhibitors did not abate direct N₂O emissions (Mateo-Marín, Quílez, Guillén, & Isla, 2020). In New Zealand, where approximately half the country's GHG emissions were estimated to come from agriculture, it was proposed that mitigation would include the use of nitrification inhibitors to reduce N₂O emissions (Wilcock, Elliott, Hudson, Parkyn, & Quinn, 2008). Research has demonstrated that using stabilised urea fertiliser, which can be formed with urease inhibitors, nitrification inhibitors or both, instead of CAN, reduces N₂O emissions (Harty et al., 2016).

Grass and Silage Production

Successful silage production is well understood (Teagasc, 2016), as are the required grass composition parameters prior to ensiling such as in vitro dry matter digestibility (DMD), water soluble carbohydrate (WSC) and crude protein (CP) concentration, and buffering capacity (BC) (Burns, Gilliland, Grogan, Watson, & O'Kiely, 2013; Conaghan, O'Kiely, Halling, O'Mara, & Nesheim, 2012; Wilkins & Lovatt, 2011) to ensure a rapid fermentation to reach a steady acidic pH and achieve anaerobic conditions for storage. The production of high quality silage is dependent on many factors including prevailing meteorological conditions, degradation of nitrates (Spoelstra, 1985), addition of enzymes (Jacobs, Cook, & McAllan, 1991), the grass varieties and their ploidies (Conaghan, O'Kiely, Howard, O'Mara, & Halling, 2008), the time of grass ear emergence (Humphreys & O'Kiely, 2006) and the cutting date (Gilliland, Camlin, & Johnston, 1995). High nitrate concentrations in grass, especially after a third silage cut, are thought to be associated with poorer ensilability as they lead to an increased BC during silage fermentation, extending the time taken for a stable pH to be achieved and increasing the opportunities for spoilage organisms to proliferate (Patterson et al., 2021). However, this effect of higher nitrate concentrations increasing the buffering capacity of silages during fermentation can be overcome with sufficient WSC levels (2-3% on a fresh-matter basis, AHDB 2019) to still fuel an effective fermentation when nitrate concentration is lower than 800 ppm (Teagasc, 2016). The nutritional quality of silage can be assessed by analysing factors including DMD, neutral detergent fibre (NDF), acid detergent fibre (ADF), metabolisable energy (ME), ash, CP and WSC content, whereas the conditions during the fermentation process are indicated by the relative proportions and concentrations of lactic acid (LA), ethanol, the ratio between the primary volatile fatty acids (VFAs) produced during anaerobic fermentation; acetic, propionic and butyric acid, the concentration of NH₃-N and the pH and BC of the silage (McEniry, O'Kiely, Clipson, Forristal, & Doyle, 2007).

Grassland crops in Northern Ireland have a typical estimated N requirement balance (other than livestock manure) of 272 kg N ha⁻¹ yr⁻¹ for dairy cattle and 222 kg N ha⁻¹ yr⁻¹ for other livestock (DAERA, 2019) and many of the 1.6 million cattle in Northern Ireland (NI) (DAERA, 2020a) are dependent on grass silage forage when housed between October and March. Grass silage is the predominant conserved forage offered to ruminant livestock and is made annually from approximately 300,000 of NI's 816,000 ha of grassland (DAERA, 2020a).

Following the "Making Ammonia Visible" (DAERA, 2017) report's recommendation for farmers to move to using SU products in Northern Ireland there is therefore a need to ascertain the effects of SU on grass and silage production in comparison to the currently favoured N fertiliser product (CAN) in order to develop improved fertiliser recommendations for multi-harvest silage systems to support the continued production of high yields of high-quality grass silage. The yield response of unamended urea is more variable than with CAN (Frame & Laidlaw, 2014) therefore a less variable yield response for SU compared to CAN would be a desirable outcome.

In order to determine if SU can be utilised in the place of CAN fertiliser product by Northern Irish farmers to reduce the associated levels of nitrogenous emissions from grass silage production systems, this study was undertaken to compare the effects of N applied as CAN or SU to perennial ryegrass (*Lolium perenne* L.) dominant grass plots for three silage cuts per year over each of two years, relative to an unfertilised control treatment. Effects of N type were measured on herbage yield, composition and ensilability

and on N uptake by the grass plant over a seven week growing period at different stages of the growing season.

Materials and Methods

Site

The study, which was undertaken in 2018 and 2019, lasted for 21 weeks each year at Agri Food and Biosciences Institute, Hillsborough, County Down ($54^{\circ}27'N$, $6^{\circ}04'W$). The experimental plots were established on a perennial ryegrass and white clover sward which had been last re-seeded in 2013 with a seed mixture comprising of intermediate and late maturing perennial ryegrass varieties and white clover. The soil type was a slightly gleyed sandy clay-loam (48% sand, 31% silt and 21% clay) overlying Silurian shale (greywacke) till. All herbage samples were from managed plots ($5m \times 1.5m$) harvested as per a '3 cut' silage harvest system according to the harvest regime indicated in Table 1.

				20)18								
		Cut 1	l			Cut 2				Cut 3			
		kg N/ł	na ap	plied	kg N/ha applied				kg N/ha applied				
		Cont	S	CA		Cont	S	CA		Cont	S	CA	
Week	Date	rol	U	Ν	Date	rol	U	Ν	Date	rol	U	Ν	
0 - initial trim/fertiliser	11-		12	12	29-		10	10	24-		10	10	
application	Apr	0	0	0	May	0	0	0	Jul	0	0	0	
	25-				13-				07-				
2	Apr				Jun				Aug				
2	02-				20-				14-				
3	May				Jun				Aug				
1	09- May				20- Jun				22- Aug				
4	15-				03-				78-				
5	May				In1				20- Απσ				
5	22-				10-				04-				
6	Mav				Jul				Sep				
-	29-				24-				12-				
7	May				Jul				Sep				
				20)19								
		Cut 1	l			Cut 2	2			Cut 3	3		
		kg N/ł	na ap	plied	kg N/ha applied				kg N/ha applied				
		Cont	S	CA		Cont S CA				Cont	S	CA	
Week	Date	rol	U	Ν	Date	rol	U	Ν	Date	rol	U	Ν	
0 - initial trim/fertiliser	26-		12	12	21-		10	10	09-		10	10	
application	Mar	0	0	0	May	0	0	0	Jul	0	0	0	
	09-				04-				23-				
2	Apr				Jun				Jul				
2	16-				11- T				30-				
3	Apr 24				Jun				Jul				
1	24- A pr				10- Iun				00- Aug				
4	30-				25-				Aug 13-				
5	Apr				Lun				13- Διισ				
5	07-				02-				20-				
6	May				Jul				Aug				
-	14-				09-				27-				
7	May				Jul				Aug				

 Table 1: Schedule of fertiliser applications and grass plot harvesting for sample collection in 2018 and 2019.

Weather

Northern Ireland has a temperate climate which typically lends itself to the production of high yields from perennial ryegrass dominated pastures across the province. During the two grass growing seasons within the described experiment some significantly atypical weather conditions were encountered, which impacted on the 2nd cut 2018 silage harvest in particular. Table 2 shows the 30-year average monthly precipitation and air temperatures for the experimental site compared to 2018 and 2019 (Met Office, 2021).

Table 2: Summary of the maximum and minimum air temperatures and total rainfall recorded during the experimental period (March-September 2018 and 2019) compared to the 1991-2020 30-year average for the Met Office weather station located at the experimental site (Met Office, 2021).

Month	Max temperature (°C)	Min temperature (°C)	Rainfall (mm)
	1991-20	20 average	
March	9.4	2.7	66.5
April	11.7	4.2	57.6
May	14.7	6.5	60.0
June	17.1	9.3	69.7
July	18.6	11.0	81.1
August	18.3	10.9	83.2
September	16.2	9.2	72.4
	2	2018	
March	10.3	-3.8	235.8
April	18.3	-1.1	51.8
May	22.7	2.5	47.6
June	28.6	6.5	52.9
July	25.6	8.3	92.3
August	21.2	6.1	78.2
September	20.0	3.7	30.1
	2	2019	
March	14.3	0.7	125.2
April	19.2	-0.5	68.6
May	18.2	0.8	30.8
June	22.5	5.9	97.8
July	24.2	7.0	71.9
August	23.2	8.9	112.7
September	20.3	7.2	71.8

Treatments

The design was a randomised block comprising 4 replicates of 18 treatments in a 3 x 6 factorial design (4 x 3 x 6 = 72 plots per row) for each of 3 silage harvest periods in 2018 and repeated in 2019. The 18 treatments comprised of 3 fertiliser treatments (CAN, SU and a no fertiliser Control) with 6 harvesting intervals per cut (at 2, 3, 4, 5, 6 and 7 weeks post fertiliser application) and 3 cuts per year (1st, 2nd and 3rd Cuts) over 2 years (2018 and 2019). In total there were 432 plots destructively harvested over two years (4 replicates x 3 fertilisers x 6 harvests x 3 cuts x 2 years). Herbage was therefore harvested from 12 plots (4 replicates x 3 treatments) on each of six occasions, during the 1st cut

silage period. This process was repeated for the 2nd cut silage period with another 72 plots, and again for the 3rd cut silage period totalling 216 plots in 2018. The 2018 experiment was repeated in 2019 using a new set of randomised plots with a two year total of 432 plots analysed.

Fertiliser

The CAN fertiliser was SulfaCAN (26.6:0:0:12.5 NPKS) and the SU was a Koch Advanced Nitrogen (KaN) product (38:0:0:17.5 NPKS) as urea with the urease inhibitor n-butyl thiophosphoric triamide (nBTPT) plus sulphur. Following January soil sampling (6.4 pH, P index 3, K index 2+), the experimental area received no slurry or manure applications and the experimental fertiliser treatments along with indicated P₂O₅ and K₂O applications were applied in March - July as per RB209 guidelines (AHDB, 2010). Maintenance P₂O₅ and K₂O applications were made alongside the N fertiliser products as follows: phosphate was applied once only at a rate of 20kg/ha P₂O₅ prior to first cut in 2018. A Potash product was applied as a split dressing before 1st cut, 2nd cut and 3rd cut as 60; 60; 40 kg K₂O ha⁻¹ to supply a total of 160kg K₂O ha⁻¹ across all plots in 2018, with a further 30 kg K₂O ha⁻¹ applied prior to 1st cut in 2019. All plots received P and K dressings including all control plots. The P and K fertilisers were applied by hand as a top-dressing first to each plot, with the N dressing applied last to ensure an even spread of the N fertiliser granules within each plot. The full schedule of experimental fertiliser applications for the plots is shown in Table 1.

Plot management

The 216 grass plots utilised each year measured 5m x 1.5m (2018 and 2019, total 432 plots) and were trimmed off to a stubble height of 4cm using an Agria mower (scythe width 1.1m) in March each respective year to get a consistent sward at the point of plot establishment, with all cut grass removed from the site. All 216 plots (per year) were fertilised for '1st cut Silage' on 10th April, 2018 (and 26th March 2019) and 12 plots were destructively harvested in each of weeks 2-7 post '1st Cut' fertilisation (4 replicates x 3 N regimes x 6 weeks = 72 plots per 'Cut' treatment). The total herbage from each harvested plot was removed for dry matter (DM) yield assessment and analysed by Near Infra-Red Spectroscopy (NIRS) for ensilability and grass quality parameters. On week 7 after fertiliser application, the herbage remaining from the final 12 "1st Cut" plots after sub-

samples for DM%, ensilability and grass quality had been collected was ensiled in a 6 kg mini pipe silos as described below (2.6). All 144 remaining grass plots to be assessed for Cuts 2 and 3 were cut off (not recorded) to simulate normal practice and received '2nd cut Silage' fertiliser. Twelve plots were then harvested in each of the following 2-7 weeks as described for the '1st cut' series with the week 7 cut ensiled. Finally, the 72 remaining plots were cut off, given '3rd cut Silage' fertiliser applications as detailed (Table 1) and harvested in the next 2-7 weeks according to the same protocol, with the final week 7 cut again ensiled.

Fresh herbage analysis and yield determination

The total fresh weight of herbage harvested from each individual plot was recorded and three replicate sub-samples were taken, weighed, oven-dried at 85 °C for 48 hours then re-weighed for DM% determination, to facilitate the calculation of plot DM yields. A total of 12 fresh grass samples were submitted for laboratory analysis each week, from Week 2 - 7 for each of the three grass harvests, over the two years. Fresh grass samples were analysed using NIRS for Nitrate, BC, ME, ADF, CP, DM and WSC. Due to rapid wilting following the 1st cut - Week 6, 2nd cut - Weeks 2 and 3 harvests (13th and 20th June 2018) and 1st cut - Week 7 harvest (15th May 2019) no NIRS data were recorded for nitrate, BC or ME from the fresh grass plots. However silage was made from the latter harvest as per normal.

Silage preparation and analysis

The Silage Cut at 'Week No 7' was chopped and placed into 6 kg mini pipe silos and a 2kg sample was analysed after 100 days by chemical analysis for DM, volatile corrected organic dry matter (VCODM), ammonia nitrogen as a fraction of total nitrogen (NH₃-N/ Total N), pH, lactic acid (LA), acetic acid (AA), propionic acid (PA), butyric acid (BA), ethanol, propanol, and by NIRS for crude protein (CP), neutral detergent fibre (NDF) and dry matter digestibility (DMD). A separate sample of 600g was dried at 60°C for 48 hours, milled and analysed for Ash content, ADF and WSC. A further ODM content was determined on a 600g sample dried at 85°C for 48 hours. Following the drought in 2018 and resultant poor grass yields (Figure 1) no second cut silage data were produced for that year.

Statistics

The ANOVA and REML functions within Genstat (19th Edition) (VSNI, 2017) were utilised to assess the fixed effects of week, cut, fertiliser treatment and their interactions, as appropriate. To account for the significant impact of a summer drought during 2018 affecting the experimental area, the sum of rainfall recorded in the 7-days prior to each plot sampling was included as a co-variate in all analyses performed. Mean, standard error of mean (SEM) and effective standard error (ANOVA only), standard error of the difference (SED), variety F statistic and probability of significance of F statistic (F pr) (ANOVA) or the chi probability statistic (chi. pr) (REML), were calculated as required for comparisons of parameters from fresh grass and silage samples.

For grass quality, dry matter yields and yield gain in kg of DM per kg of applied Nitrogen (N) the main fixed effects of week, cut and treatment, and the 2- and 3-factor interactions, were assessed adjusting for the covariate total rain while accounting for the random variability of year and replication of the experiment within year. For these responses it was possible to fit the model using ANOVA. The residual diagnostics were visually assessed for normality and were deemed to be acceptable. The 3-factor interaction was not significant for any of the responses.

For grass ensilability and silage quality the data were unbalanced therefore analysis was performed using REML. As above, the main fixed effects of week, cut and treatment, and the 2- and 3-factor interactions, were assessed adjusting for the covariate total rain while accounting for the random variability of year and replication of the experiment within year. The residual diagnostics were visually assessed for normality. The residual diagnostics for normality were deemed to be acceptable, although nitrate show some minor departure from normal On all occasions where a significant difference was indicated, Fishers protected least-significant-difference test was employed for post-hoc analysis to indicate where the significant difference occurred between specific treatments and treatment x factor interactions.

Results

A number of significant differences between sampling weeks and between cuts were identified for the grass quality, silage quality and grass ensilability parameters measured and in the grass DM yields recorded, but there was frequently no treatment effect identified for these metrics. Therefore, those differences will be attributable to environmental conditions and or plant physiology changes during the course of the experiment and are not relevant to our hypothesis investigating differences between CAN and SU in terms of silage/grass yields and quality. There were also several significant week x cut interactions identified in the analysis, but again where no treatment effect was also identified these are not presented. The summary data for all variables across all weeks and cuts is provided in Tables S1 and S2 for grass quality and yield and grass ensilability respectively. Table 2 shows the combined May and June rainfall of 100.5 mm in 2018 notably below the 30-year average total for those months of 129.7 mm. May 2019 was also exceptionally dry, with rainfall for that month just 50% of the 30-year average (Met Office, 2021).

Grass yield

There were no significant differences in total yield between CAN and SU fertilised plots, with average DM yields from the week 7 cuts of 15,513 and 16,132 kg DM/ha respectively. However, the N fertilised plots had significantly (p<0.001) greater total yields than the control plots, with an average yield of just 8,694 kg DM/ha across both years (Figure 1, Table 3).

Table 3: Total grass yields from all week 7 harvests of each fertiliser treatment in 2018and 2019.

		` 0	/		
	2018	2019	Average	SEM* (e.s.e)	Sig F. pr
Control	7624	9764	8694a	86.6	< 0.001
CAN	13278	17748	15513b		
SU	14981	17283	16132b		

Total Annual Yields (kg-1 DMha)

* e.s.e = effective standard error

Yields from CAN and SU were similar throughout. The pattern of grass growth in Weeks 2-7 following N fertilisation for Cuts 1-3 in 2018 relative to 2019 (data not shown) and the relative yields of Cuts 1-3 (at Week 7) in both years (Figure 1) highlight the poor growth during the second cut period of 2018 owing to the extreme drought conditions experienced at the experimental site that year (AgriSearch, 2018).



Figure 1: Grass dry matter (DM) yield per cut and per treatment sampled at week 7 for ensiling in both 2018 and 2019, highlighting the reduction in cut 2 yield because of summer drought conditions.

The yield response to nitrogen fertiliser estimated as kg of DM gained over the control plot yields (which had 0 N applied) per kg of N fertiliser applied to both the SU and CAN treatment plots after 7 weeks of post-fertilisation growth across 3 silage cutting periods showed the significant gain in grass yields achievable through the application of N fertilisers, but no difference between fertiliser products was detected (Table 8).

	kg DM gain/kg N	SEM e.s.e	F. pr					
	Cut							
1	13.89a							
2	11.85a	1.498	0.004					
3	19.00b							
Treatment								
Control	0a							
SU	21.51b	1.498	<0.001					
CAN	23.24b							

Table 8: Yield response to nitrogen fertiliser as kg of DM gained over the control with 0 N applied per kg of N fertiliser applied after 7 weeks of post-fertilisation growth across 3 silage cutting periods for both CAN and SU fertiliser treatments

During this study it was found that the 3^{rd} cut silage harvest showed a significantly (F. pr = 0.004) higher yield gain response to fertiliser of 19.0 kg DM per kg of N fertiliser applied, with no difference between fertiliser products, than either the 1^{st} cut or 2^{nd} cut harvests which saw a 13.9 and 11.9 kg DM/kg N gain respectively.

Grass quality and ensilability

There were no significance differences between responses of CAN and SU fertilised plots in terms of grass quality parameters (DM, nitrogen, ADF, ash, or WSC) (Table 4) or ensilability measures (nitrate concentration, BC, fresh WSC, CP, BC and ME) (Table 5). Results of treatment differences in grass quality mainly occurred between N fertilised plots and control plots. Significant cut x treatment interactions were identified in ADF, ash and WSC in the grass quality analysis (Table 4) and in fresh WSC, BC, CP and nitrate, but none of these were significantly different between the SU and CAN treatments at each individual cut (Table 5). **Table 4:** Grass quality variables (oven dry matter (DM), nitrogen, acid detergent fibre, ash and water soluble carbohydrates) which had a significant treatment and/or treatment x cut interaction across all of the weeks sampled (weeks 2-7 post fertiliser application). Values followed by letters denote where significant differences were identified using Fishers protected least-significant-difference test.

Variable	Treatme	Mean	SEM	Sig. F.	Cut	Treatment*Cut	SEM*	Sig. F.
	nt		(e.s.e	pr		Mean	(e.s.e)	pr
Dry Matter	Control	213.8	2.709	< 0.001	1	214.5	4.735	0.7
(g/kg)		a			2	234.6		
					3	192.6	1	
	SU	173.0			1	167.0		
		b			2	196.4		
					3	155.4	1	
	CAN	172.1			1	166.3		
		b			2	193.8		
					3	156.1		
Nitrogen	Control	21.7a	0.369	< 0.001	1	20.8	0.645	0.422
(g/kg DM)					2	20.7		
					3	23.5	1	
	SU	33.8b	1		1	33.3		
					2	32.9		
					3	35.2		
	CAN	33.9b			1	32.2	_	
					2	33.9	_	
					3	35.6		
Acid	Control	233.4	1.736	< 0.001	1	219.3a	3.034	< 0.001
Detergent		a			2	230.0b	-	
(g/kg DM)			-		3	250.8e	-	
	SU	244.4			1	245.9de	-	
		D			2	234.7bc	-	
			-		3	252.6e	-	
	CAN	242.6			1	242.2cd	-	
		U			2	232.7b	-	
		02.2	0.700	0.001	3	253.0e	1.000	0.001
Ash	Control	83.2	0.709	<0.001		73.9a	1.238	<0.001
					2	82.06	-	
	CII	01.0	-		3	93./e	-	
	50	91.0				07.00 01.5do	-	
					2	91.300	-	
	CAN	91 3	-		1	88 4cd	-	
	CAN	91.5			2	00.400 01.6da	-	
						91.000		

					3	93.8e		
Water	Control	236.8	2.758	< 0.001	1	269.6e	4.821	< 0.001
Soluble		a			2	249.2d		
Carbohydrat					3	191.6c		
(g/kg DM)	SU	155.1			1	159.1b		
		b			2	166.9b		
					3	139.3a		
	CAN	156.2			1	165.5b		
		a			2	167.4b]	
					3	135.6a	1	

* e.s.e = effective standard error

Table 5: Grass ensilability variables (nitrate concentration, crude protein content, buffering capacity, fresh water soluble carbohydrate % and metabolisable energy) which had a significant treatment and/or treatment x cut interaction across all of the weeks sampled (weeks 2-7 post fertiliser application). Values followed by letters denoting where the significant differences were identified using Fishers protected least-significant-difference test.

Variable	Treat.	Mean	SEM	Sig. chi pr	Cut	Treatment* Cut Mean	SEM	Sig. chi pr
Nitrate (ppm)	Control	149a	96.3	< 0.001	1	78a	129.3	< 0.001
					2	193a		
					3	177a		
	SU	1540b	-		1	749b		
					2	1600c		
					3	2271d		
	CAN	1520b			1	731b		
					2	1538c		
					3	2290d		
Water Soluble	Control	4.089b	0.1850	< 0.001	1	4.539d	0.2100	0.002
Carbohydrates					2	4.591d		
% Fresh					3	3.136c		
	SU	2.38a			1	2.574b		
					2	2.653b		
					3	1.911a		
	CAN	2.373a			1	2.556b		
					2	2.712b		
					3	1.851a		
Crude protein	Control	11.88a	0.4270	< 0.001	1	10.63a	0.5279	< 0.001
(% DM)					2	11.35a		
					3	13.66b		
	SU	17.77b			1	17.08cd		
					2	18.69e		
					3	17.52cd		
	CAN	17.82b			1	16.84c		
					2	18.59e		
					3	18.04de		
Buffering	Control	253.6a	29.43	< 0.001	1	279.5c	29.98	< 0.001
(meg/kg DM)					2	224.4a		
(incq/kg Divi)			_		3	256.7b		
	SU	376.0b			1	378.1d		
					2	378.6d		
			_		3	371.5d		
	CAN	376.1b			1	384.6d		
					2	369.2d		
					3	374.3d		

ME	Control	11.13b	0.1934	0.009	1	11.42	0.2014	0.111
(MJ/kg DM)					2	11.25		
					3	10.87		
	SU	11a			1	11.16		
					2	11.19		
					3	10.76		
	CAN	10.99a			1	11.10		
					2	11.23		
					3	10.75		

The pattern of weekly nitrate concentration detectable in fresh grass samples in relation to grass yield over three cuts was similar for both CAN and SU but both had significantly greater overall nitrate concentrations and DM yields than the control (Figure 2).



Figure 2: Effect of fertiliser treatment on mean weekly grass dry matter (DM) yields (week 2-7) and nitrate concentrations (ppm) in fresh grass samples for each silage cut period (1-3).

The variance of nitrate concentrations between plots within each treatment was highest at the third cut where average nitrate levels in the week 6 and 7 samples (relevant to when silage harvests may be taken on commercial farms) in CAN and SU fertilised plots were in excess of 1000 ppm (Table 7), except for the week 6 SU samples in 2019 at 951 ppm. The adjusted mean calculated in the REML analysis shown in Table 7 suggests that the overall risk for excess nitrate concentrastions in the fresh grass samples analysed from weeks 6 and 7 post fertiliser application was highest in the cut 3 period. A significant week x treatment x cut interaction was identified for nitrate concentrations in grass samples, and these are detailed in Table 7 for the week 6 and 7 average results (relevant to grass ensiling in commercial practice) recorded across both experimental seasons. Significant differences were observed over all treatments for both yield and nitrate content at the cut level (F pr. <0.001) and weekly levels (F pr. <0.001) but there were no significant interactions between CAN and SU treatments and either cut or for nitrate levels or yield. In the two-year study, one control plot was also found to have a high nitrate concentration at Week 7 (1300 ppm, cut 3, 2019).

Table 7: Nitrate levels detected in fresh grass samples tested at weeks 6 and 7 post fertiliser application (relative to the timing of silage harvests in commercial farm practice) as the average nitrate levels recorded each year (2018 and 2019) and the adjusted mean values in the significant week x treatment x cut interaction from the REML statistical analysis. Values followed by letters denoting where the significant differences were identified using Fishers protected least-significant-difference test.

Cut	Treatment	Week	2018	2019	REML adjusted Mean	SEM (e.s.e)	F. pr
	Control	6		228	145ab	2.67.7	0.048
		7	41		62ab		
1	SU	6		529	446ab		
1		7	663		684abc		
	CAN	6		592	510ab		
		7	259		280ab		
	Control	6	66	125	53a		
		7	71	333	168ab		
2	\mathbf{SU}	6	293	1168	689bc		
2		7	526	1163	809bc		
	CAN	6	579	778	637ab		
		7	593	144	334ab		
	Control	6	108	70	90ab		
		7	79	454	248ab		
2	\mathbf{SU}	6	1726	951	1339 c		
3		7	1149	1600	1356 cd		
	CAN	6	1088	2173	1631 cd		
		7	2375	1323	1831 d		

* e.s.e = effective standard error

Silage quality

In terms of silage quality parameters (DM, VCODM, NH3-N/ Total N, pH, CP, NDF, LA, AA, PA, BA, VA, ethanol, propanol, ADF, ash, WSC or DMD) there were no significant differences overall between responses of CAN and SU fertilised plots (Table 6). Any differences in silage quality mainly occurred between N fertilised plots and control plots. Significant treatment x cut interactions were observed for DM, ash, NDF, VCODM, AA, ethanol, and PA, but within individual cutting periods there were again no significant differences between the CAN and SU treatments (Table 6).

Table 6: Analysis of silage quality variables for a significant treatment and/or treatment x cut interaction for the week 7 post fertiliser application samples which were ensiled at the end of each growth period (cuts 1-3). Values followed by letters denote where the significant differences were identified using Fishers protected least-significant-difference test.

Variable	Treatme	Mean	SEM*	Sig. F.	Cu	Treatment*Cut	SEM*	Sig. F.
	nt		(e.s.e)	pr	t	Mean	(e.s.e)	pr
Dry Matter	Control	250.9	4.115	< 0.001	1	264.9d	7.230	0.018
(g/kg)		a			2	290.6e		
					3	197.3ab		
	SU	214.3			1	213.2b		
		b			2	241.9c		
					3	187.8a		
	CAN	212.1			1	211.9b		
		b			2	234.4c		
					3	190.2a		
Dry Matter	Control	698.0	5.304	0.120	1	719.3	9.319	0.400
Digestability					2	684.6		
					3	690.0		
	SU	705.8			1	736.5		
					2	671.7		
					3	709.1		
	CAN	715.2			1	742.3		
					2	686.9		
					3	716.5		
Acid	Control	290.5	3.557	0.012	1	300.4	6.250	0.646
Detergent		a			2	269.1		
$ \begin{array}{c} F 1 Dre \\ (g/kg DM) \end{array} $					3	303.2		
	SU	304.0	1		1	318.8	1	
		b			2	285.3		

CAN 303.5 b I 310.0 2 284.0 3 317.6 Ash (g/kg MD) Control 93.2 1.540 0.046* * 1 82.8a 2.707 0.032 MD) SU 96.9 * 1 91.4bc 2 87.1ab 3 109.6e 1 91.4bc 2 95.3cd 3 103.9e 1 91.4bc 2 94.3cd 3 101.0de 1 91.7bc 2 94.3cd 3 101.0de 1 106.2 4.195 0.053 1 1 122.6 3 97.5 1 1 127.8 2 155.9 3 145.9 1 129.4 2 156.2 3 142.5 1 129.4 2 3.862 0.0142 0.001 1 3.93 0.0249
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Ash (g/kg MD) Control 93.2 1.540 0.046* * 1 82.8a 2.707 0.032 MD) SU 96.9 * 1 91.4bc 2 95.3cd 3 109.6e 1 91.4bc 2 95.3cd 3 103.9e 1 91.7bc 2 94.3cd 3 101.0de 1 91.7bc 2 94.3cd 3 101.0de 1 106.2 4.195 0.053 0.053 Protein (g/kg DM) Control 108.8 2.387 <0.001 1 106.2 4.195 0.053 SU 143.2 b - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - -
Ash (g/kg MD) Control 93.2 1.540 0.046* * 1 82.8a 2.707 0.032 MD) SU 96.9 * 2 87.1ab 3 109.6e SU 96.9 - - 2 95.3cd 3 103.9e CAN 95.7 - - - - - - Crude protein (g/kg DM) Control 108.8 a 2.387 <0.001 1 106.2 4.195 0.053 SU 143.2 b - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - -
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CAN 3.92b 1 3.95
2 3.91
3 3.90
Neutral Control 450.8 3.459 0.246 1 439.1bc 6.078 0.005
Detergent 2 426.5ab
$\begin{array}{c c} \textbf{Fibre} \\ \textbf{(g/kg DM)} \end{array} \qquad 3 484.3d \\ \end{array}$
(g, kg D(v)) SU 439.9 1 453.4c
2 414.5a
3 449.5c
CAN 439.0 1 442.4bc
2 422a
3 450.2c
VCODM Control 272.5 3.923 <0.001
(g/kg) a 2 288.9a
3 239.1b
SU 1 230.1b

		222.0			2	227.6bc		
		b			3	208.3c	-	
	CAN	219.4	-		1	232.0b	-	
		b			2	214.6bc	-	
					3	211.7c	-	
NH ₃ -N/ Total	Control	0.048	0.0021	< 0.001	1	0.03512	0.00389	0.364
Ν		01	00		2	0.05727	2	
					3	0.05163	-	
	SU	0.633	-		1	0.05340	-	
		8			2	0.07852	-	
					3	0.05823	-	
	CAN	0.642	-		1	0.05237	-	
		1			2	0.07977	-	
					3	0.06048	-	
Water	Control	81.81	4.082	< 0.001	1	105.33	7.173	0.057
Soluble		a			2	94.16	-	
Carbohydrat					3	45.93	-	
	SU	19.14	-		1	22.82	-	
		b			2	31.87	-	
					3	2.74	-	
	CAN	18.92	-		1	21.83	-	
		b			2	28.45	-	
					3	6.49	-	
Acetic Acid	Control	5.026	0.1833	0.255	1	2.547a	0.3396	< 0.001
(g/kg)					2	8.876e		
					3	3.654bc		
	SU	4.431			1	2.613ab		
					2	6.794d	_	
					3	3.887c		
	CAN	4.404			1	3.127abc		
					2	6.396d	_	
					3	3.690c		
Ethanol	Control	8.791	0.7416	< 0.001	1	13.480a	1.372	0.033
(g/kg)		a			2	4.331bc		
					3	8.563b		
	SU	3.913	-		1	5.101bc		
		b			2	4.205bc		
					3	2.434c		
	CAN	5.102	1		1	7.750b	1	
		b			2	4.472bc	1	
					3	3.084c	1	
Lactic Acid	Control		0.7911	0.007	1	24.74	1.468	0.282

(g/kg)		18.93			2	15.95		
		a			3	16.09		
	SU	22.01	-		1	24.9		
		b			2	21.84		
					3	19.28		
	CAN	23.02			1	26.68		
		b			2	21.08		
					3	21.3		
Propanol	Control	0.017	0.0267	0.314	1	0.0466	0.04941	0.702
(g/kg)		2	3		2	0.0000		
					3	0.0490		
	SU	0.047			1	0.1268		
		9			2	0.0000		
					3	0.0407		
	CAN	0.055			1	0.1559		
		7			2	0.0000		
					3	0.0407		
Propionic	Control	0.012	0.0150	0.270	1	0.0000a	0.02695	0.031
Acid		4	8		2	0.0000a		
(g/kg)					3	0.0566ab		
	SU	0.032			1	0.1041b		
		2			2	0.0000a		
					3	0.0102a		
	CAN	0.026			1	0.04373ab		
		1			2	0.0000a		
					3	0.0522ab		
n-Butyric	Control	0.011	0.0388	0.449	1	0.0450	0.06936	0.723
Acid (g/kg)		8	2		2	0.0439		
(g/Kg)					3	0.0000		
	SU	0.058			1	0.1561		
		3			2	0.0439		
					3	0.0000		
	CAN	0.010			1	0.0140		
		9			2	0.0439		
					3	0.0000		

* e.s.e = effective standard error ** For Ash (g/kg DM) although the initial REML analysis identified a significant treatment effect, this was not found following the Fishers LSD post-hoc analysis.

Discussion

The results of this study have indicated primarily that no significant differences in yield or grass quality parameters occur between CAN fertilised treatments and SU fertilised treatments under simulated grass silage production. Further to this the results have indicated that no significant differences occur in any quality parameters of silage produced from either CAN or SU fertilised grass. These results are reassuring and support previous research indicating SU is a suitable alternative N source for grass production (Forrestal et al., 2016) over the more widely used CAN products. Whilst CAN fertilisers are highly effective as a N supply to support grass growth, are associated with higher levels of nitrogenous emissions than SU fertilisers. The study indicates that swards managed for silage production in Northern Ireland are equally able to utilise nitrogen supplied in the form of SU as in CAN for growth and plant protein production, with no differences observed between total DM yields produced, or measures of grass or silage quality, between these two fertiliser treatments.

A drought occurred in Northern Ireland in the early summer of 2018 leading to a reduction in grass growth, particularly that for second cut silage (AgrisearchNI, 2018), as highlighted by Figure 1. To minimise the impact of these extreme conditions in our statistical analysis the sum of rainfall recorded in the 7-days prior to each plot sampling was included as a co-variate in the analyses performed, with year as a fixed effect because separate areas of the field were used for the plot study in 2018 and 2019, and the growing seasons were very different in terms of the weather conditions experienced (specifically rainfall). Despite this some anomalies arising from this period are reflected in the results, with the drought conditions likely contributing to the lower DMD of cut 2 silage samples, and higher cut 2 DM values within both the grass guality and silage figures (Tables 4 and 6). The yield-gain as kg DM/ha of grass produced after 7 weeks of growth over the average yield of the control plots per kg N/ha applied during each cutting period was significantly (F. pr 0.004) higher for the 3rd cut period regardless of CAN or SU treatment (Table 8). Given the extremely dry conditions of summer 2018 the uptake of applied fertiliser during the 2nd cut period is likely to have been restricted and any residual nitrogen remaining in the soil may have contributed to this increased 3rd cut yield gain value. If soil moisture had been low or there had been little rain at the time of silage cutting in a commercial farm setting, the following N application would have been delayed in practice and reduced pro rata until conditions improved. Due to the lag between N

assimilation and utilisation, significant differences in yield between N fertilised plots and control plots were not expressed in the first 2-3 weeks after N application (Figure 2).

High average nitrate concentrations (>1000 ppm) were found in herbage samples from both the SU and CAN plots across the sampling weeks for each cut (Table S2), however, the majority of high nitrate plots were from fertilised 3rd cut plots (Table 7). Nitrates are degraded in silage production (Spoelstra, 1985) and this process required a closer examination of the effects on nitrate concentrations from SU fertilisation in comparison with CAN. It is reported that grass will ensile correctly with up to 800ppm nitrate provided sugars are adequate i.e. >3% or, 2-3% Fresh Weight (FW) if the grass can be wilted (Teagasc, 2016; AHDB 2019). High concentrations of nitrate in forage can cause toxicity in cattle however nitrate concentrations of 0-3,000 ppm (DM) are generally considered safe for all cattle, though concentrations <2,500 ppm are preferred for dairy cattle, whereas 3,000-5,000 ppm are generally considered safe for non-pregnant beef cattle (Strickland, C., H., & Step, 2013). If higher concentrations of nitrates in herbage occurred when fertilised with SU this would reduce its effectiveness and desirability as a replacement N fertiliser for CAN due to the association between high nitrate concentrations, especially at third cut, and poor ensiling (CAFRE, 2017; Teagasc, 2016), but also its safety for use on grazing ground as many farms alternate activity on specific fields between silage production and grazing. The highest average nitrate concentrations throughout the experiment were recorded at week 2 post-fertiliser application in the 3rd cut plots, at 3758 and 3453 ppm in the SU and CAN treatment groups respectively (Table S2). These high concentrations may be linked to residual nitrogen remaining in the soil after the 2nd cut period in 2018 because of the weather conditions, as mentioned above. Nonetheless, the difference between nitrate concentrations on SU or CAN fertilised plots was not significant at comparable timepoints (Table 5, Table 7), indicating there is no additional risk of excess nitrate concerntations inhibiting ensilability of harvested grass or the safety of fresh grass for grazing when using SU products compared to CAN. Lower levels of N fertilisation lead to lower nitrate content and improved ensilability by increasing WSC content of grass (Huhtanen, Jaakkola, & Nousiainen, 2012). It is therefore desirable to reduce high concentrations of nitrates in fresh grass cut for silage as they are converted to NH₃ during silage fermentation which in turn increases BC by counteracting the necessary drop in pH required for effective silage fermentation. However, the herbage plots with nitrate concentrations >1000 ppm did not coincide with poor silage quality

parameters in this study. The nitrate concentrations in CAN and SU fertilised herbage were not significantly different from each other overall but both were higher than in Control yield herbage at the week, cut and year levels.

The significant treatment x cut interaction for nitrate shows no difference between SU and CAN but highlights 3rd cut silages had higher average nitrate concentrations than 1st and second cut. Looking specifically at the samples analysed for ensilability in weeks 6 and 7 post-fertiliser application, as these represent the stages of plant growth and maturity that in practice would be targeted by farmers aiming to conserve high yields of high quality silage, and when average nitrate concentrations had dropped below 1000 ppm (Table 7) which is the industry recommendation to ensure silage fermentation and rapid production of an acidic pH and anaerobic conditions for preservation of nutritional quality are achieved (AHDB, 2019), 3rd cut silage had the highest nitrate ppm average in both week 6 and 7, above the recommended 1000 ppm threshold. This is an important finding as it may hold relevance for appropriate fertiliser application recommendations in future. This study followed the established UK recommendations for grass silage production defined in the fertiliser management guide RB209 (AHDB, 2010), yet these results suggest nitrate concentrations remained undesirably high for silage preservation at both week 6 and 7 post fertiliser application. With these results on a grass ensilability test the recommended action would have been to delay harvest until nitrate levels have fallen, potentially leading to reduced DMD (AHDB, 2019), or other action taken to remedy the inhibition of fermentation because of high nitrate concentrations, eg: addition of molasses to raise sugar levels (Keady, 1996; Muck, O'Kiely, & Wilson, 1991), incurring additional production costs.

Within this experiment the nitrate concentrations (Table 7) and nitrogen yield response for 3rd cut harvests at week 7 (Table 8) do not tally however, with the highest nitrate concentration recorded at 3rd cut indicative of the applied nitrogen not being converted to plant proteins during grass growth, yet grass yields at the 3rd cut showed the highest yield gain figure for conversion of applied N fertiliser to grass DM. Therefore, it would be prudent to repeat this measurement across more typical silage production seasons to determine if there is any indication for fertiliser recommendations for late-season silage harvests (3rd or subsequent cuts) to be revised in order to reduce nitrate concentration at harvest and improve the efficiency of nitrogen use or establish if this pattern was solely linked to the weather conditions experienced during this trial. The broadly similar effects of CAN and SU on silage quality indicate that the method of N fertilisation, including at the third cut, does not differentially effect silage quality.

Conclusion

SU was shown to be a suitable substitute for CAN in that it performs as well as CAN in terms of yield response as previously reported (M.A. Harty et al., 2017) and leads to no significant difference in terms of grass quality or silage quality. Ensilability measures of grass quality such as BC, WSC and nitrate concentrations were unaffected by fertiliser type. The rate of uptake and utilisation of nitrate, whether from SU or CAN is variable across weeks and cuts and although the expected trends were demonstrated, plot effects were also seen. Therefore, this supports the view that there should be no detrimental effects on either silage yields or quality where farmers transitioning to using SU fertilisers in place of CAN. This transition would allow farmers to reduce the nitrogenous emissions associated with silage production in Northern Ireland as the agriculture industry moves to address the challenges of climate change.

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ni.gov.uk/sites/default/files/publications/daera/20.21.177%20Nutrients%20Action%20Pr ogramme%202019-2022%20Guidance%20Booklet%20Final.PDF

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Appendices:

Table S1: Table of means per week/cut for	grass quality	(wet chemistry	analysis)	and yield
averaged over 2 seasons (2018 and 2019)				

Wee Contr ol SU CAN Contr ol SU CAN Contr ol SU CAN 2 195.0 206.7 205.8 244.4 230.2 227.0 273.1 253.3 255.4 3 198.3 210.7 201.9 223.8 223.1 222.6 261.1 240.8 244.9 4 214.7 247.6 240.3 208.2 211.8 212.8 84.5 244.5 246.1 248.2 A 216.5 251.1 215.2 223.3 243.9 268.5 256.4 6 8 9 6 2 4 7 6 6 1 258.5 7 256.2 289.9 291.5 248.6 275.8 267.7 243.0 253.4 254.1 210.5 8 2 9 1 102.7 100.5 8 9 1 101.8 101.1 101.1 101.1 101.1 101.1 101.1 101.1		Cut 1			Cut 2			Cut 3			
$ \begin{array}{c ccccccccccccccccccccccccccccccccccc$		Wee k	Contr ol	SU	CAN	Contr ol	SU	CAN	Contr ol	SU	CAN
$ { ADF (g/kg DM) } \left({ \begin{array}{*{20}c} 2 & 9 & 8 & 3 & 5 & 6 & 3 & 3 & 6 & 1 \\ 198.3 & 210.7 & 201.9 & 223.8 & 223.1 & 222.6 & 261.1 & 240.8 & 244.9 \\ 2 & 7 & 4 & 1 & 9 & 4 & 212.8 & 264.1 & 248.2 \\ 8 & 1 & 9 & 4 & 212.8 & 213.3 & 243.9 & 268.7 \\ 7 & 25 & 220.7 & 25 & 5 & 251.1 & 215.2 & 223.8 & 223.3 & 243.9 & 268.7 \\ 7 & 256.5 & 251.1 & 215.2 & 223.8 & 243.3 & 242.4 & 239.1 & 251.0 & 258.5 \\ 8 & 9 & 6 & 2 & 4 & 7 & 6 & 6 & 1 \\ 7 & 256.2 & 284.9 & 291.5 & 248.6 & 275.8 & 267.7 & 243.0 & 253.4 & 254.1 \\ 7 & 256.2 & 284.9 & 291.5 & 248.6 & 275.8 & 267.7 & 243.0 & 253.4 & 254.1 \\ 7 & 256.2 & 284.9 & 291.5 & 248.6 & 275.8 & 267.7 & 243.0 & 253.4 & 254.1 \\ 7 & 256.2 & 284.9 & 3 & 3 & 5 & 8 & 2 & 1 & 8 \\ 2 & 84.31 & 93.75 & 97.81 & 95.99 & 101.8 & 106.9 & 101.3 & 101.1 & 100.1 \\ 3 & 76.93 & 91.15 & 93.66 & 89.87 & 101.8 & 106.9 & 101.3 & 101.1 & 100.1 \\ 3 & 76.93 & 91.15 & 93.66 & 89.87 & 101.8 & 106.9 & 101.3 & 101.1 & 100.1 \\ 4 & 72.12 & 90.19 & 87.53 & 76.47 & 91.6 & 90.69 & 95.26 & 93.71 & 94.64 \\ 5 & 74.03 & 89.8 & 87.73 & 76.86 & 89.7 & 90.46 & 93.69 & 96.44 & 93.74 \\ 6 & 69.73 & 84.06 & 84.46 & 80.92 & 85.95 & 85.73 & 90.3 & 84.26 & 85.56 \\ 7 & 66.31 & 73.31 & 79.08 & 71.84 & 75.81 & 79.23 & 86.87 & 87.43 & 88.19 \\ \\ Nitrogen (g/kg DM) & 5 & 19.59 & 33.32 & 29.56 & 16.31 & 29.62 & 31.4 & 22.67 & 31.58 & 32.28 \\ 6 & 17.55 & 27.35 & 26.22 & 16.27 & 27.46 & 28.03 & 20.24 & 28.69 & 29.3 \\ 7 & 15.01 & 21.35 & 22.9 & 16.02 & 23.43 & 23.76 & 20.1 & 25.65 & 27.94 \\ 2 & 217.3 & 180.4 & 178.1 & 198.5 & 7 & 6 & 7 & 1 \\ 3 & 193.9 & 156.5 & 154.8 & 216.0 & 174.0 & 175.9 & 170.6 & 134.1 & 134.3 \\ 3 & 5 & 9 & 3 & 9 & 3 & 3 & 2 & 5 \\ 7 & 281.1 & 221.1 & 215.0 & 252.3 & 197.3 & 199.5 & 194.3 & 175.1 & 171.1 \\ 8 & 6 & 8 & 2 & 2 & 3 & 7 & 5 & 4 \\ 4 & 9 & 8 & 5 & 6 & 5 & 3 & 1 & 1 \\ 5 & 201.7 & 153.9 & 146.7 & 256.6 & 25.0 & 209.5 & 181.5 & 157.3 \\ 1 & 52 & 2 & 7 & 1 & 8 & 4 & 3 & 3 \\ 1 & 1 & 7 & 4 & 6 & 8 & 4 & 1 \\ \end{array} \right)$		2	195.0	206.7	205.8	244.4	230.2	227.0	273.1	255.3	255.4
ADF (g/kg DM) 3 198.3 210.7 201.9 223.8 223.1 222.6 261.1 240.8 244.5 4 214.7 8 1 9 4 212.8 243.5 244.5 246.1 248.2 5 220.7 265.5 251.1 215.2 223.8 223.3 243.9 268.5 256.4 6 230.6 259.4 262.2 239.8 243.3 242.4 239.1 251.0 258.4 7 5 284.9 291.5 248.6 275.8 267.7 243.0 253.4 254.1 7 5 284.9 291.5 248.6 104.1 96.41 94.78 9 1 3 76.93 91.15 93.66 89.87 101.8 106.9 91.3 101.1 100.7 100.5 3 76.93 91.15 93.66 89.87 106.9 96.9 49.74 49 2 9 4 20.7 <td></td> <td>2</td> <td>9</td> <td>8</td> <td>3</td> <td>5</td> <td>6</td> <td>3</td> <td>3</td> <td>6</td> <td>1</td>		2	9	8	3	5	6	3	3	6	1
ADF (g/kg DM) 2 7 4 247.6 240.3 208.2 211.8 212.8 244.5 246.1 248.2 ADF (g/kg DM) 5 20.7 265.5 251.1 215.2 223.8 223.3 243.9 268.5 256.4 6 8 9 6 2 4 7 6 6 1 7 256.2 284.9 215.5 248.6 275.8 267.7 243.0 25.4 254.4 7 256.2 284.9 291.5 248.6 275.8 267.7 243.0 25.4 254.1 7 256.2 284.9 29.15 248.6 275.8 26.77 24.0 251.0 258.5 3 76.93 91.15 93.66 89.87 101.8 106.9 101.3 101.7 100.5 8 2 9 1 74.03 89.8 87.73 76.68 89.7 90.46 93.69 94.4 93.74		3	198.3	210.7	201.9	223.8	223.1	222.6	261.1	240.8	244.9
ADF (g/kg DM) 4 214.7 247.6 240.3 208.2 211.8 212.8 243.5 246.1 248.2 ADF (g/kg DM) 5 220.7 2 7 2 3 9 9 1 256.4 6 230.6 259.4 262.2 239.8 243.3 242.4 239.1 251.0 258.5 7 256.2 284.9 291.5 248.6 275.8 267.7 243.0 253.4 254.1 7 5 284.9 3 5 8 2 1 8 2 84.31 93.75 97.81 95.99 104.1 96.41 94.78 102.7 100.5 3 76.93 91.15 93.66 89.87 101.8 106.9 101.3 101.1 100.1 4 72.12 90.19 87.53 76.47 91.6 90.69 95.26 93.71 94.64 5 74.03 89.8 87.73		5	2	7	4	223.0	6	3	5	4	4
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$ \begin{array}{c ccccccccccccccccccccccccccccccccccc$		5	220.7	203.3	231.1 7	213.2	225.8	225.5 Q	243.9 Q	208.5	256.4
$ \begin{array}{c c c c c c c c c c c c c c c c c c c $			230.6	2594	262.2	239.8	243 3	242.4	239.1	251.0	258 5
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$ \begin{array}{c c c c c c c c c c c c c c c c c c c $		7	256.2	294.0	291.5	248.6	275.8	267.7	243.0	253.4	254.1
$ \begin{array}{c ccccccccccccccccccccccccccccccccccc$		/	5	284.9	3	3	5	8	2	1	8
$ \begin{array}{c ccccccccccccccccccccccccccccccccccc$		2	84.31	93.75	97.81	95.99	104.1 8	96.41	94.78	102.7 9	100.5 1
$ \begin{array}{c ccccccccccccccccccccccccccccccccccc$		3	76.93	91.15	93.66	89.87	$\begin{array}{c}101.8\\4\end{array}$	106.9 9	101.3 8	101.1	100.1 9
$ \begin{array}{c c c c c c c c c c c c c c c c c c c $	Ash (g/kg DM)	4	72.12	90.19	87.53	76.47	91.6	90.69	95.26	93.71	94.64
$ \begin{array}{c c c c c c c c c c c c c c c c c c c $		5	74.03	89.8	87.73	76.86	89.7	90.46	93.69	96.44	93 74
$ \begin{array}{c ccccccccccccccccccccccccccccccccccc$		6	60.73	84.06	8/ /6	80.02	85.05	85 73	00.3	84.26	85.56
$ \begin{array}{c c c c c c c c c c c c c c c c c c c $		07	66 21	72 21	70.09	71.94	75 91	70.22	90.5	04.20 97.42	85.50 99.10
$ \begin{array}{c ccccccccccccccccccccccccccccccccccc$		/	26.5	15.51	19.00	71.04	20.62	19.25	00.07	07.45	47.05
$ \begin{array}{c ccccccccccccccccccccccccccccccccccc$		2	26.5	45.05	44.68	28.02	38.63	40.36	27.3	47.23	47.25
Nitrogen (g/kg 4 20.99 34.08 30.64 20.99 38.31 38.45 24.5 35.03 34.68 DM) 5 19.59 33.32 29.56 16.31 29.62 31.4 22.67 31.58 32.28 6 17.55 27.35 26.22 16.27 27.46 28.03 20.24 28.69 29.3 7 15.01 21.35 22.9 16.02 23.43 23.76 20.1 25.65 27.94 2 217.3 180.4 178.1 198.5 7 193.6 6 5 1 3 3 5 9 3 9 3 3 2 5 4 193.9 156.5 154.8 216.0 174.0 175.9 170.6 134.1 134.3 3 3 5 9 3 9 3 3 2 5 4 197.4 145.7 149.4 240.3 193.7 189.8 187.5 142.1 146.2 5 195.3		3	25.38	38.75	38.97	26.36	39.99	41.42	26.45	42.8	42.4
$ \begin{array}{c ccccccccccccccccccccccccccccccccccc$	Nitrogen (g/kg	4	20.99	34.08	30.64	20.99	38.31	38.45	24.5	35.03	34.68
$ \begin{array}{c ccccccccccccccccccccccccccccccccccc$	DM)	5	19.59	33.32	29.56	16.31	29.62	31.4	22.67	31.58	32.28
$ \frac{7}{2} \frac{15.01}{2} \frac{21.35}{22.9} \frac{22.9}{16.02} \frac{23.43}{23.76} \frac{20.1}{20.1} \frac{25.65}{25.65} \frac{27.94}{27.94} \\ \frac{2}{1} \frac{217.3}{5} \frac{180.4}{5} \frac{178.1}{5} \frac{198.5}{7} \frac{194.1}{7} \frac{193.6}{6} \frac{216.7}{6} \frac{167.0}{6} \frac{166.5}{5} \frac{1}{1} \\ \frac{3}{3} \frac{193.9}{5} \frac{156.5}{9} \frac{154.8}{3} \frac{216.0}{9} \frac{174.0}{3} \frac{175.9}{3} \frac{170.6}{3} \frac{134.1}{134.3} \frac{134.3}{3} \\ \frac{3}{5} \frac{5}{9} \frac{3}{3} \frac{9}{3} \frac{3}{3} \frac{2}{2} \frac{5}{5} \\ \frac{4}{197.4} \frac{197.4}{145.7} \frac{149.4}{149.4} \frac{240.3}{240.3} \frac{193.7}{193.7} \frac{189.8}{189.8} \frac{187.5}{142.1} \frac{146.2}{146.2} \\ \frac{4}{4} \frac{8}{3} \frac{6}{5} \frac{5}{7} \frac{6}{6} \frac{7}{8} \\ \frac{195.3}{144.3} \frac{153.6}{153.6} \frac{243.9}{243.9} \frac{194.1}{194.2} \frac{194.2}{2} \frac{202.6}{2} \frac{156.5}{161.6} \\ \frac{3}{3} \frac{1}{1} \frac{1}{5} \frac{5}{2} \frac{209.5}{2} \frac{181.5}{2} \frac{157.3}{12} \frac{156.5}{165.5} \frac{161.6}{16.6} \\ \frac{4}{9} \frac{9}{8} \frac{5}{5} \frac{6}{5} \frac{5}{3} \frac{3}{1} \frac{1}{156.5} \\ \frac{7}{281.1} \frac{221.1}{221.1} \frac{215.0}{252.3} \frac{252.3}{197.3} \frac{199.5}{194.3} \frac{175.1}{175.1} \frac{171.1}{171.1} \\ \frac{8}{8} \frac{6}{8} \frac{8}{2} \frac{2}{2} \frac{3}{3} \frac{7}{5} \frac{5}{4} \\ \frac{2}{2} \frac{7}{1} \frac{8}{8} \frac{4}{3} \frac{3}{3} \frac{66.74}{63.51} \frac{63.51}{3} \\ \frac{272.4}{3} \frac{158.3}{160.4} \frac{160.4}{211.5} \frac{211.5}{129.7} \frac{120.5}{120.5} \frac{150.5}{102.8} \frac{104.1}{104.1} \\ \frac{3}{3} \frac{1}{1} \frac{1}{7} \frac{7}{4} \frac{6}{6} \frac{8}{8} \frac{4}{1} \\ \frac{1}{1} \end{array}$		6	17.55	27.35	26.22	16.27	27.46	28.03	20.24	28.69	29.3
$ \begin{array}{c ccccccccccccccccccccccccccccccccccc$		7	15.01	21.35	22.9	16.02	23.43	23.76	20.1	25.65	27.94
$ \begin{array}{cccccccccccccccccccccccccccccccccccc$		2	217.3 1	180.4 5	178.1 3	198.5	194.1 7	193.6	216.7 6	167.0 5	166.5 1
$ \begin{array}{cccccccccccccccccccccccccccccccccccc$		3	193.9 3	156.5 5	154.8 9	216.0	174.0 9	175.9 3	170.6	134.1 2	134.3
$\frac{4}{2} = \frac{1000}{4} + \frac{1000}{4} + \frac{1000}{2} + 1000$			197.4	145 7	149.4	240.3	1937	189.8	187 5	142.1	146.2
$\frac{\text{DM (g/kg)}}{\text{S}} = \frac{195.3}{3} \begin{array}{ccccccccccccccccccccccccccccccccccc$		4	4	8	3	6	5	7	6	7	8
$\frac{5}{6} = \frac{3}{201.7} + \frac{1}{153.9} + \frac{1}{146.7} + \frac{5}{256.6} + \frac{2}{225.0} + \frac{194.2}{209.5} + \frac{2}{181.5} + \frac{157.3}{157.3} + \frac{1}{156.5} + \frac{2}{7} + \frac{281.1}{8} + \frac{221.1}{215.0} + \frac{252.3}{252.3} + \frac{197.3}{199.5} + \frac{194.3}{194.3} + \frac{175.1}{175.1} + \frac{171.1}{171.1} + \frac{8}{8} + \frac{6}{8} + \frac{2}{2} + \frac{2}{3} + \frac{7}{5} + \frac{5}{4} + \frac{2}{3} + \frac{2}{3} + \frac{2}{7} + \frac{1}{188.3} + \frac{8}{4} + \frac{3}{3} + \frac{3}{3} + \frac{66.74}{3} + \frac{63.51}{3} + \frac{272.4}{3} + \frac{158.3}{160.4} + \frac{211.5}{211.5} + \frac{129.7}{129.7} + \frac{120.5}{150.5} + \frac{102.8}{102.8} + \frac{104.1}{104.1} + \frac{1}{160.4} + $	DM (g/kg)	F	195.3	144.3	153.6	243.9	194.1	104.2	202.6	156.5	161.6
$\frac{6}{4} \begin{array}{cccccccccccccccccccccccccccccccccccc$		5	3	1	1	5	2	194.2	2	5	2
$\frac{4 9 8 5 6 5 3 1 150.5}{7 281.1 221.1 215.0 252.3 197.3 199.5 194.3 175.1 171.1 \\ \hline 8 6 8 2 2 3 7 5 4} \\ \frac{2 245.9 126.2 129.4 149.9 108.6 118.3 132.1 \\ 2 2 7 1 8 4 3 3 66.74 63.51 \\ \hline 3 272.4 158.3 160.4 211.5 129.7 120.5 150.5 102.8 104.1 \\ \hline 3 1 1 7 4 6 8 4 1 \\ \hline \end{array}$		6	201.7	153.9	146.7	256.6	225.0	209.5	181.5	157.3	156 5
$\frac{7}{281.1} \begin{array}{c} 221.1 \\ 221.1 \\ 8 \\ 6 \\ 8 \\ 2 \\ 2 \\ 2 \\ 7 \\ 3 \\ 272.4 \\ 3 \\ 1 \\ 1 \\ 1 \\ 7 \\ 4 \\ 6 \\ 8 \\ 4 \\ 1 \\ 7 \\ 4 \\ 6 \\ 8 \\ 4 \\ 1 \\ 7 \\ 4 \\ 6 \\ 8 \\ 4 \\ 1 \\ 199.5 \\ 199.5 \\ 199.5 \\ 199.5 \\ 199.5 \\ 199.3 \\ 199.5 \\ 199.3 \\ 199.5 \\ 199.3 \\ 199.5 \\ 199.5 \\ 199.3 \\ 199.5 \\ 199.3 \\ 199.5 \\ 199.3 \\ 199.5 \\ 199.3 \\ 199.5 \\ 199.3 \\ 199.5 \\ 199.5 \\ 199.3 \\ 132.1 \\ 66.74 \\ 63.51 \\ 63.51 \\ 102.8 \\ 104.1 \\ 3 \\ 1 \\ 1 \\ 7 \\ 4 \\ 6 \\ 8 \\ 4 \\ 1 \\ 1 \\ 1 \\ 1 \\ 7 \\ 4 \\ 6 \\ 8 \\ 4 \\ 1 \\ 1 \\ 1 \\ 1 \\ 1 \\ 1 \\ 1 \\ 1 \\ 1$		0	4	9	8	5	6	5	3	1	150.5
$\frac{8}{2} = \frac{6}{2} + \frac{8}{2} + \frac{2}{2} + \frac{3}{3} + \frac{7}{3} + \frac{5}{4} + \frac{4}{3} + \frac{149}{3} + \frac{149}{3$		7	281.1	221.1	215.0	252.3	197.3	199.5	194.3	175.1	171.1
$ \frac{2}{3} = \frac{243.9}{2} + \frac{120.2}{129.4} + \frac{149.9}{149.9} + \frac{108.0}{108.0} + \frac{118.5}{132.1} + \frac{152.1}{66.74} + \frac{63.51}{63.51} + \frac{108.0}{3} + \frac{118.5}{3} + \frac{132.1}{3} + \frac{149.9}{6} + \frac{108.0}{3} + \frac{118.5}{3} + \frac{132.1}{3} + \frac{149.9}{3} + \frac{149.9}{3} + \frac{108.0}{3} + \frac{118.5}{3} + \frac{132.1}{3} + \frac{149.9}{3} + \frac{149.9}{3} + \frac{118.5}{3} + \frac{132.1}{3} + \frac{149.9}{3} + \frac{149.9}{3} + \frac{118.5}{3} + \frac{132.1}{3} + \frac{149.9}{3} + \frac{149.9}{3} + \frac{149.9}{3} + \frac{118.5}{3} + \frac{132.1}{3} + \frac{149.9}{3} + 149.9$			<u>8</u> 245.0	0	<u>8</u> 120.4	<u> </u>	<u> </u>	<u> </u>	/ 122.1	3	4
$ \begin{array}{c} \text{WSC (g/kg DM)} \\ \underline{3 272.4 158.3 160.4 211.5 129.7 120.5 150.5 102.8 104.1 \\ 3 1 1 7 4 6 8 4 1 \end{array} $		2	243.9 7	120.2 7	129.4 1	149.9 8	108.0 A	110.5	3	66.74	63.51
$\qquad \qquad $	WSC (g/kg DM)		272.4	158.3	160.4	211.5	129.7	120.5	150.5	102.8	104.1
		3	3	1	1	7	4	6	8	4	1

	4	254.1	148.1	176.2	280.7	167.1	171.0	185.8	143.9	138.7
	4	8	3	7	1	2	9	5	4	6
	5	284.4	138.6	170.6	314.2	205.5	199.4	205.9	143.7	157.3
5	4	2	4	3	2	3	3	3	8	
	6	281.4	181.3	182.1	277.6	202.0	200.7	239.2	185.5	172.1
	0	5	3	8	2	7	200.7	1	8	8
	7	279.3	201.7	173.9	260.8	188.2	194.3	235.8	192.9	177 8
	,	4	8	1	8	3	1	233.8	2	177.0
	2	1590	1750	1830	430	490	450	380	840	830
	3	1820	2570	2380	690	1050	990	550	1500	1680
Yield (kg-1 DM ha)	4	2470	3700	3660	1200	1650	1710	1020	2520	2530
	5	3270	4710	4990	1540	2440	2480	930	3320	3400
	6	4460	6010	6240	2130	2880	3150	1480	4180	4200
	7	4870	7660	7070	2360	3990	4290	1470	4480	4150

Table S2: Table of means per week/cut for fresh grass ensilability over 2 seasons (2018 and2019)

	Cut 1 Cut 2			Cut 3						
	Wee k	Contro 1	SU	CAN	Contro 1	SU	CAN	Contro 1	SU	CAN
	2	4.44	2.84	2.68	2.4	1.43	1.65	2.98	1.29	1.31
	3	4.4	2.79	2.71	3.64	1.86	2.04	2.2	1.19	1.26
Water Soluble	4	4.66	2.5	2.75	4.91	2.93	2.81	2.95	1.63	1.78
Carbonydrates % Fresh	5	4.38	2.03	2.23	5.83	3.09	3.2	3.64	2.24	2.24
70 T Tesh	6	4.3	2.66	2.49	5.76	3.88	3.51	3.73	2.5	2.38
	7	5.03	2.6	2.45	4.91	2.65	2.96	3.48	2.78	2.29
	2	62	973	1407	668	3111	2596	147	3758	3453
	3	75	927	853	82	2559	2662	122	2968	2843
Nituata (mmm)	4	77	466	632	92	1554	1770	309	2466	1906
Mitrate (ppm)	5	75	1023	731	84	868	1215	75	1668	2009
	6	228	529	592	96	731	678	89	1338	1630
	7	41	663	259	202	844	369	266	1374	1849
	2	307	366	374	270	409	395	227	374	371
	3	296	388	386	220	402	393	310	438	403
Buffering	4	284	407	394	221	400	391	252	386	366
Capacity (med kg-1 DM)	5	316	469	492	199	397	373	233	334	347
	6	280	343	377	221	339	351	246	364	379
	7	222	326	314	250	359	346	253	313	361
	2	15.88	22.8 3	22.7 9	15.05	22.1 3	22.0 5	15.91	22.4 9	22.4 5
	3	13.35	19.9 5	20.2 1	13.39	21.0 4	21.0 5	15.63	21.1 5	20.4 1
Crude Protein	4	11.35	18.9	17.4 8	9.75	19.1	18.9	15.23	19.1 6	19.1
(% DM)	5	9.59	16.3 4	15.9	8.23	16.4 9	16.3 9	12.68	15.5 8	16.4 1
	б	9.28	13.5 5	13.4	9.6	15.9 1	16.3 4	9.21	12.2 6	13.2 5
	7	7.03	13.6 3	13.9 5	10.95	16.3	15.6 6	11.6	12.8 3	14.9 3
	2	12	12.0 5	11.9 8	10.88	11.2	11.3 8	10.6	10.9 9	10.9 3
	3	11.99	11.8 1	11.8 4	11.36	11.3 4	11.4	10.81	10.8 9	10.8 4
	4	11.86	11.4	11.4 3	11.46	11.6	11.4 5	11	10.8	10.8 1
ME (MJ/Kg DM)	5	11.26	10.9 8	10.9 4	11.56	11.4 5	11.3 9	11.18	10.7 6	10.8 3
	6	10.66	10.3 1	10.1 3	11.14	11.3	11.2	10.75	10.5 5	10.5 4
	7	10.03	9.65	9.5	10.89	10.2 5	10.6 1	10.86	10.5 9	10.5 6

Chapter 4

Delayed autumn closing and its effects on Silage DM production and sward quality

Introduction

There is a need to produce more milk from forage in Northern Ireland, from grazed grass and grass silage (McConnell, 2017). Northern Ireland has a significant silage feeding requirement because the grass growing season is limited by low solar radiation and soil temperatures during the late autumn and winter months (Humphreys & O'Kiely, 2006). Increased rainfall during this period often creates saturated field conditions, which limit grass growth and soil trafficability. Between October and March many of the 1.6 million cattle in Northern Ireland are housed and fed grass silage, made annually from approximately 300,000 ha of Northern Ireland's 816,000 ha of grassland (DAERA, 2020). Climate projections (Nolan, 2015) predict the average growing season in Ireland will increase by >35 days per year, but in Northern Ireland increasingly frequent warmer, wetter winters are expected (Fig.1a and b).

The utilisation of high quality grass silage in dairy cow diets is not fully exploited and there is significant dependence on imported concentrates to support dairy production in Northern Ireland.. This reliance on imported 'high protein' feeds such as soya-bean meal leaves the livestock sector vulnerable to price volatility and instability of supply (AgrisearchNI, 2020b). Improved home grown grass quality can result in a significant increase in the metabolisable energy (ME) and digestibity value (D value) of silage to help reduce this reliance on concentrates. Variation in silage quality over a twenty year period in Northern Ireland between first, second and third cuts was reported (Patterson, Sahle, Gordon, Archer, Yan, Grant & Ferris, 2021) although no significant improvement in silage quality over time was noted.



Fig. 1a Mean temperature (°C) for the period November – February in 20 year time periods from 1903-2022 in Northern Ireland (Met Office, 2022) and number of years in each time period where mean temperature (November – February) was greater than 6°C.



Fig. 1b Mean total rainfall (mm) for the period November – February in 20 year time periods from 1903-2022 in Northern Ireland (Met_Office, 2022) and number of years in each time period where total rainfall (November – February) was greater than 500mm.

First cut silage, generally made in mid-May, is typically the highest yielding and highest quality grass cut, although both yield and quality may be influenced by winter carry over (Looney, Hennessy, Wingler, Claffey, & Egan, 2021) and meteroligical conditions (Carozzi, Martin, Klumpp & Massad, 2022). Routine practice in the region is to take a second cut in mid-summer and a third and final annual silage cut around mid-September. Herbage which grows following third harvest may remain un-grazed over the winter to be ensiled with first cut herbage the following year, potentially reducing grass silage quality. How the timing of the last defoliation following each growing season affects the quantity and quality of first cut silage in the next season is not fully understood. Effects of closing date on autumn and winter growth were reported regarding the following spring's herbage yield and quality for grazing (Hennessy, O'Donovan, French, & Laidlaw, 2006). Here, closing off perennial ryegrass dominated swards by mid-September decreased herbage growth in spring due to reduced tiller density, and grazing in winter and early spring reduced herbage mass between April and June. The effect of closing date on the following season's silage quality and subsequent cow performance does not appear to have been examined previously.

This study aimed to identify the effects of delayed closing in either mid-November or mid-January compared to a typical mid-September close on first cut grass silage DM yield, composition and fermentation characteristics in the following growing season to determine whether delayed closing could improve first cut silage quality from perennial ryegrass based swards in Northern Ireland.

Materials and Methods

Site

The study was undertaken between September 2018 and May 2020 at AFBI Hillsborough (54°27′N, 6°04′W) on two different sets of plots. Swards were re-seeded in 2016 with a mixture of intermediate heading diploid (65%) and tetraploid (28%) perennial ryegrass varieties (heading dates 18th – 25th May) and a white clover blend (7%).

Fertiliser

Following January soil sampling, stabilised urea fertiliser (KAN Agrotain 38% N, Koch Agronomic Services) was applied in mid-March 2019 and 2020 as per RB209 guidelines (AHDB, 2017) at a rate of 120kg N ha⁻¹. Across the full season N fertiliser application rate for first cut was 120 kg N ha⁻¹ each year, followed by 100 and 80 kg N ha⁻¹ for second and third cuts.

Based on soil analysis at the beginning of the study (6.4 pH, P index 3, K index 2+) Phosphate was applied as 20 kg P_2O_5 ha⁻¹; and Potash was applied as a split dressing after each cut to supply a total of 160kg K₂O ha⁻¹.

Plot management

All 24 grass plots measuring 6m x 3m were trimmed off to approximately 4 cm on the 25th September 2018 using an Agria mower (Agria, Germany) with a scythe width of 1.1m. Following the September defoliation, 8 of these plots were defoliated again in mid-November 2018 another 8 plots were defoliated in mid-January 2019. Dates of last defoliation, March fertilisation and 1st cut in May are given (Table 1).

Year		Last Defolia	ation	Fertilisation	1 st Cut		
			Season	1 (2018/ 2019)			
2018 2019	25 th Sep	16 th Nov	15 th Jan	27 th Mar	14 th May		
Season 2 (2019/ 2020)							
2019 2020	17 th Sep	28 th Nov	21 st Jan	23 rd Mar	12 th May		

Table 1	Dates of last	defoliation,	fertilisation	and first	cut in	2018/	2019	and 2	2019/	2020
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All 24 plots were cut off in May 2019 to simulate normal silage making practice and grass samples were removed for silage preparation. A 3m strip was mown first and this material provided the herbage which was removed for dry matter (DM) yield assessment. Further samples of 1kg of fresh herbage were assessed by manual separation for presence of dead grass material (in 2019 only) quantified by DM weight. The experiment was replicated with new plots in the period September 2019- May 2020.

Silage preparation and analysis

Fresh grass was wilted, chopped and a bacterial inoculant additive, (Silo-King® GS: product code 874. Agri-King Inc., Fulton, Illinois, USA) was applied by spraying the solution onto the grass using a hand sprayer and mixing by gloved hand. Exactly 6 kg of the mixed herbage with additive were placed into 6 kg mini pipe silos and a 2kg sample was analysed after 100 days by chemical analysis for organic dry matter (ODM), volatile corrected organic dry matter (VCODM), ammonia nitrogen as a proportion of the total nitrogen (NH₃-N/ Total N), pH, crude protein (CP), lactic acid (LA), acetic acid (AA), propionic acid (PA), butyric acid (BA), valeric acid (VA), ethanol, propanol, acid-detergent fibre (ADF), ash, and water soluble carbohydrates (WSC) and by Near Infrared Reflection Spectroscopy (NIRS) for dry matter digestibility (DMD) as described by Park, Agnew, Gordon & Steen (1998).

Meteorological data

Met Office databases (Met Office, 2022) provided monthly data for Northern Ireland for bright sunshine hours, total rainfall (mm) and mean daily air temperature (°C) (Tables 2 and 3, Fig. 1a and b). Soil moisture deficit (SMD) (kPa) data for AFBI Hillsborough (April and May 2020) was obtained through GrassCheckNI (AgrisearchNI, 2020a) (Fig. 2). Additional proxy daily meteorological data were accessed from the Plant Testing Station, AFBI-Crossnacreevy, (54°33' N. 5°32' W) located 23km from the test site (mean temperature (°C), total solar radiation (W m⁻²) (Fig. 3) and total rainfall (mm)) in the period 15th April – 15th May 2019 and 15th April – 15th May 2020 (Table 3). The fraction of total solar radiation considered to be photosynthetically active radiation (PAR) is in the region of 0.45 - 0.50 (Talling, 1961; Tsubo & Walker, 2005) therefore total solar radiation ratios served as a proxy for PAR ratios.



Fig. 2 Soil moisture tension (kPa) in 2020 for the period with weeks beginning 13th April -11th May in 2020 from Hillsborough GrassCheck site (AgrisearchNI, 2020). Above dotted line at 60 kPa moisture deficit may restrict grass growth. Below solid line at 10 kPa is very wet – saturated soil.

A soil temperature of 5.6 °C at 10cm depth is generally accepted as the minimum for grass growth (bi, 1968) therefore an air temperature of 6 °C was taken as a baseline in this report to calculate growing degree days (GDD) (degree days > 6 °C) from Crossnacreevy mean temperature data (Table 3).

	Ŧ		14		14	d .
	Jan	Feb	Mar	Apr	May	Spring
			Mean Tem	perature (°C	2)	
2019	4.8	6.7	6.7	8.5	9.8	8.3
2020	5.3	4.6	5.6	9.0	11.1	8.5
30 Year mean	4.5	4.7	6.0	7.9	10.5	8.1
			Total Rain	(mm)		
2019	55	79	161	74	60	294
2020	70	232	70	24	31	125
30 Year mean	115	92	87	74	74	235
			Bright Sun	shine (hours	5)	
2019	36	79	86	118	152	356
2020	42	74	129	207	223	559
30 Year mean	43	67	101	148	183	433

Table 2 Meteorological data for Northern Ireland (January – May in 2019 and 2020)

Statistics

Analysis of Variance in Genstat (19th Edition) (VSNI, 2017) was applied as the appropriate General Linear Model (GLM) to assess the fixed effects of defoliation time, year of harvest and any interactions, blocking on year and the replicated experiment within year. Means, standard error of the difference (SED), least significant difference (LSD), variety F statistic and probability of significance of F statistic (F pr) were calculated. Predictions from the GLM were calculated for unbalanced comparisons where required using the Genstat regression model. Pearson's correlation coefficient, r, was calculated with probability values in Genstat. The strength of association for absolute values of r (both positive and negative) was described as follows: strong (0.60 - 0.79) or very strong (0.80 - 1) (Swinscow, 1997).

Results

Meteorological Conditions

Mean temperatures between January and May in 2019 and 2020 were broadly similar. Meteorological spring (March – May) indicated average temperatures for 2019 and 2020 with above average rain in 2019 and below average rain in 2020 based on 30 year means (Table 2). In 2019 bright sunshine hours in spring were below average (356) but spring 2020 had the highest number of bright sunshine hours (559) since records began in 1919. In the three weeks prior to mid-May in 2020 a significant SMD was recorded (Fig. 2) and accumulated PAR was greater, as indicated by accumulated total solar radiation (Fig. 3 and Table 3). Growing degree days were reduced in 2020 compared with in 2019 (Table 3).

			Results Ratio				
	2019	2020	2020: 2019				
Defoliation Month	<u>Yield (k</u>	<u>g DM ha⁻¹)</u>					
September	8180	10430	1.3				
November	7779	9587	1.2				
January	6513	9098	1.4				
Mean	7491	9705	1.3				
	<u>SED</u>	<u>F pr.</u>					
Time	240.8	<.001					
Year	196.6	<.001					
Time x Year	340.6	0.282					
		L					
	Local data 15 ^t	ⁿ April-15 [™] May					
	<u>(Crossn</u>	snacreevy)					
Growing Degree Days (degree days > 6°C)	290	274	0.9				
Mean Temperature (°C)	9.4	9.1	0.97				
Total Solar Radiation (W m ⁻²)	451	623	1.4				
Total rainfall (mm)	48	23	0.5				
	NI data April and May						
Mean Temperature (°C)	9.2	10 1	1 1				
Total Bright Sunshine Hours	135	215	1.1				
Total rainfall (mm)	122	213	1.0				
	133	55	0.4				

 Table 3 Year effect between 2019 and 2020 and relationship between yield and meteorological data.



Fig. 3 Comparison of daily cumulative total solar radiation (W m⁻²) at the Plant Testing Station for the period 15th April -15th May in 2019 and 2020.

Grass yield

Grass yield was significantly greater overall in 2020 and for each defoliation timing treatment compared with 2019 and significantly greater following last defoliation in September compared with January in both seasons (Table 3). The timing of last defoliation in 2019/2020 resulted in significantly lower first cut yields following each treatment whereas in the 2018/2019 season there was no significant drop in first cut yield between the September and November last defoliations (Table 3). The ratio of mean yield in 2020 compared to 2019 was 1.3 whereas the ratio of PAR and Bright sunshine hours were 1.4 and 1.6 respectively (Table 3).

The ratio of live: dead material harvested for first cut silage in 2019 was not significantly different between treatments (Table 4).

Table 4 Proportions of living tissue from grass samples harvested in May 2019 from three
different months of last defoliation cuts

	Range (%)	Mean Live tissue (%)	SEM
September	87-97	92	1.1
November	93-99	95	0.9
January	91-97	95	0.6
Mean	87-99	94	0.6

Grass yield and silage ME

Silage ME values in 2020 were significantly lower than in 2019 however there were no significant differences in ME value between any treatments in 2020 (Fig.4, Table 5). The ME values for first cut silage in 2019 from both the November and January defoliation treatments were significantly higher than for the September defoliation, and higher than all 2020 silage harvest values regardless of treatment.



Fig. 4 Grass yields and corresponding silage ME values for 1^{st} cut in 2019 and 2020 following each defoliation treatment. Error bars are LSD (yield LSD = 489 kg DM ha⁻¹, ME LSD = 0.190 MJ kg⁻¹ DM).

The year effect on grass yield and silage ME resulted in greater yield and lower ME overall in 2020 compared with 2019. The ME values were differentiated between treatments in the first season but not the second (Fig. 4).

	VCO g k	DM* kg ⁻¹	Amm g kg ⁻¹	ionia N Total N	р	Н	Crude g kg	e protein g ⁻¹ DM
Defoliation	2019	2020	2019	2020	2019	2020	2019	2020
September	218	269	76	66	4.01	4.15	162	125
November	207	249	77	53	4.00	4.09	157	117
January	215	255	62	54	4.03	4.10	157	118
	SED	F pr.	SED	F pr.	SED	F pr.	SED	F pr.
Time	3.7	<.001	2.7	<.001	0.015	0.055	3.0	0.087
Year	3.0	<.001	2.2	<.001	0.013	<.001	2.4	<.001
ТхҮ	5.2	0.317	3.8	0.014	0.022	0.098	4.2	0.928
	Laction g kg ⁻	c Acid ¹ DM	Acet g kg	Acetic Acid		nic Acid ¹ DM	n-Buty g kg	yric Acid ⁻¹ DM
Defoliation	2019	2020	2019	2020	2019	2020	2019	, 2020
September	85.6	55.5	25.1	19.6	0.000	0.000	0.000	0.000
November	87.6	56.4	28.2	18.5	0.000	0.032	0.000	0.000
January	86.3	51.6	20.5	19.1	0.000	0.042	0.000	0.000
	SED	F pr.	SED	F pr.	SED	F pr.	SED	F pr.
Time	4.25	0.774	1.70	0.113	0.022	0.609	0	n/a
Year	3.47	<.001	1.39	<.001	0.018	0.175	0	n/a
ТхҮ	6.01	0.851	2.41	0.068	0.031	0.609	0	n/a
	i-Valer	ric Acid	Etl	nanol -1 DM	Prop	anol	A	DF* -1 DM
Defoliation	2019	2020	g مع 2019	2020	2019	2020	2019	2020
September	0.000	0.000	44.8	45.8	0.181	0.000	275	265
November	0.000	0.000	39.6	55.7	0.291	0.000	287	263 267
January	0.000	0.000	44.1	57.2	0.325	0.000	274	264
	SED	F pr.	SED	F pr.	SED	F pr.	SED	F pr.
Time	0	n/a	3.67	0.360	0.161	0.896	3.2	0.036
Year	0	n/a	2.99	0.002	0.131	0.050	2.6	<.001
ТхY	0	n/a	5.19	0.108	0.227	0.896	4.5	0.173
	А	sh	W	SC*	DN	ID*	N	ÍE *
	g kg	¹ DM	g kg	⁻¹ DM	g kg	¹ DM	M	J kg ⁻¹
Defoliation	2019	2020	2019	2020	2019	2020	2019	2020
September	93	75	8.2	41.0	749	749	11.7	11.5
November	92	73	7.3	50.3	772	750	12.0	11.6
January	94	73	7.9	41.5	778	745	12.2	11.6
	SED	F pr.	SED	F pr.	SED	F pr.	SED	F pr.
Time	15	0.571	7 92	0.831	6.6	0 143	0.07	$< 0.00^{\circ}$

 Table 5
 Silage quality data for all defoliation treatments for first cut silage in May 2019 and 2020.

Year	1.2	<.001	6.49	< 0.001	5.4	0.001	0.05	< 0.001
ТхҮ	2.1	0.643	12.91	0.678	9.5	0.043	0.09	0.006

* VCOMD = volatile corrected organic dry matter, ADF = acid detergent fibre, WSC = water soluble carbohydrates, DMD = dry matter digestability, ME= metabolizable energy

Silage quality

Year effects were noted in 13 out of 16 recorded parameters (Table 5) with treatment effects in 4 parameters (VCODM, NH₃N/Total N, ADF and ME) and treatment x year interactions in 3 parameters (NH₃N/ Total N, DMD and ME). Statistically, the 2019 silage had significantly higher ME for both November and January treatments (Table 5). Overall the second season levels of NH₃-N/Total N in the 1st cut silage were significantly lower than the first season levels. Significant year effects on levels of NH₃N/Total N, CP and LA per plot throughout the three defoliation treatments and two seasons are illustrated (Fig. 5).



Fig. 5 Levels of NH3, CP and LA per plot and defoliation time in 2019 and 2020.

Statistically significant (p <0.001), strong (r >0.6) correlations between a number of silage quality parameters are shown (Table 6) including the inverse relationship between CP and WSC levels. Ammonia levels per plot (n = 48) correlated strongly and significantly with CP, LA, and AA (Table 6).

	Ammonia N g kg Total N	Ash	Crude protein	Dry Matter Digestability	Lactic Acid
Acetic Acid	0.69				
Ash	0.65				
Crude protein	0.63	0.91			
Lactic Acid	0.64	0.77	0.74	0.68	
DMD		0.62			
ME MJ kg		0.66		0.90	0.68
WSC			-0.65		

Table 6 Silage quality parameters with strong (r = 0.60 - 0.69) or very strong (r > 0.70) and significant (p < 0.001) correlations from all defoliations in both seasons.

All values have a significance of p<0.001. n = 48

Discussion

Year effects on first cut yield and silage quality were greater and more numerous than treatment effects of timing of last defoliation, emphasising that whilst management has important impacts on the quality and quantity of silage production on a farm, the impacts of annual weather conditions during the growing season and at ensiling will always have a significant impact on silage production. Future studies would benefit from recording additional information on carry-over yields and any effects on sward morphology also. The main meteorological differences between the two seasons were increased light levels (with a positive effect on yield and silage quality) in 2020 and reduced soil moisture (with a negative effect on ME). Levels of CP showed no significant differences between treatments, however, overall levels of CP in 2020 were significantly lower than in 2019. Contrary to previous findings (Binnie, Mayne, & Laidlaw, 2001; Lawrence, O'Donovan, Boland, & Kennedy, 2017) there were no significant differences between treatments in the proportions of live and dead material (Table 4), however this may have been due to obtaining only a single year's data as 2020 samples could not be assessed due to UK lockdown restrictions in place because of the Covid-19 pandemic.

The experimental treatment simulating delayed closing with defoliation events (as simulated grazing cuts) in November and January compared to defoliation in September was not found to have very large effects on the quality of first-cut silage harvested the following May. Both the November and January defoliations from the second season resulted in lower NH₃ N/Total N than that for September whereas in the first season only

January defoliation resulted in lower NH₃ N/Total N levels (Tables 5 and 6). The year effect of reduced silage CP levels in 2020 (Table 5) to an optimal level (Teagasc., 2016) may have been due to physiological stress as a result of reduced soil moisture prior to first cut leading to reduced protein levels in the sward (Teagasc, 2014) and consequently leading to reduced silage NH₃-N levels.

The effects of defoliation timings on NH3-N/ Total N within each season may be indirectly due to effects of intercepted PAR through dissimilar canopy structures. The rate of tiller production depends on light availability at the plant base and is strongly influenced by defoliation management (Ryan, Hennessy, Murphy, & Boland, 2010) (Akmal & Janssens, 2004; Frame & Laidlaw, 2014) and would therefore be greater following March fertilisation of swards which had been defoliated in January compared with swards with heavier canopies that were last defoliated in September. New tillers will photosynthesise more efficiently than older leaves leading to higher ME levels, more utilised protein and lower levels of NH₃-N/ Total N (Table 5).

Conclusion

Late defoliation in November or January may result in better quality silage in terms of higher levels of ME and reduced levels of ammonia albeit with a lower first cut DM yield. This outcome may be dependent on a combination of winter and spring meteorological conditions facilitating growth.

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Chapter 5

Performance of dairy cows offered grass silage produced within either a three- or four-harvest system, when supplemented with concentrates on a feed-to-yield basis

Introduction

Grass silage is the predominant forage offered to housed dairy cows during the 'winter' months in many northern European countries, including western parts of the United Kingdom (UK) and Ireland. As herd sizes increase and dairy farming becomes more intensive, cows are being housed for longer periods, or housed full-time, which increases reliance on grass silage in these regions. While the feed value of grass silage is determined primarily by digestibility (Keady et al., 2013), the digestibility of grass silages produced within Northern Ireland (NI) has shown little improvement during the last 20 years (Patterson et al., 2020).

Harvest date is the most important factor affecting silage digestibility (Keady et al., 2013), due to the increased degree of lignification of fibre with increasing plant maturity (Randby et al., 2010). For example, silage digestible organic matter in the dry matter (D-value) declines by an average of 3.3 % for each week delay in harvest date (Keady et al., 2013). Similarly, Keady et al. (2013) demonstrated that for each 10 g/kg increase in silage DM digestibility, dry matter intake (DMI) and milk yield increased by 0.22 kg/day and 0.33 kg/day, respectively, while in northern Europe a 10 g/kg increase in D-value increased silage DMI and energy corrected milk (ECM) yield by 0.27 and 0.45 kg/day, respectively (Huhtanen et al., 2013). Increasing silage digestibility can also lead to 'concentrate sparing', with Huhtanen (2018) calculating a concentrate sparing effect of 0.81 kg concentrate DM per 10 g/kg DM increase in silage D-value.

While the impact of silage feed value on cow performance has been examined in many studies, these have often involved silages made from primary growth herbage. However, on commercial farms silages made from primary, secondary and indeed tertiary regrowth herbage is often offered. While regrowth herbage is leafier than primary growth herbage (Beever et al., 2000; Kuoppala et al., 2003), it frequently contains a higher proportion of indigestible NDF than the latter (Huhtanen et al., 2006). This highlights the need to examine the impact of the whole season harvesting system on cow performance, including the impact of harvesting earlier and more frequently.

The impact of harvesting frequency was examined by Ferris et al. (2003) almost two decades ago, who found that concentrate inputs were reduced by an average of 55% without loss in milk production when cows were offered silages produced within a four-

compared to a two-harvest system. However, since this work was undertaken, the majority (65%) of farmers in NI have moved to a three-harvest system (Ferris et al., 2019), while milk yields have also increased, and concentrate allocation systems have largely moved from 'flat-rate' to 'feed-to-yield' (FTY) systems. Consequently, the current study was designed to examine the impact of offering silages made within either a three- or a four-harvest system on intakes and performance of higher yielding dairy cows offered concentrates on a FTY basis. While systems involving four or more harvests are already used in some parts of Europe where summer climate is influenced by continental air masses, this is less common in UK and Ireland, which have a maritime climate. Furthermore, offering concentrates on a FTY basis necessitates adopting a 'systems' feeding approach, as concentrate levels cannot be fixed with each silage type, but are determined by actual cow performance within each silage type, relative to average energy intakes from the basal diet. In addition, as is normal practice on progressive dairy farms, bespoke concentrates were designed specifically for each individual silage. Preliminary results from this study have been published previously in the form of a conference abstract (Craig et al., 2020).

Materials and methods

This study was conducted at the Agri-Food and Biosciences Institute (AFBI), Hillsborough, NI (54°27'N; 06°04'W).

Experimental animals

This study involved 80 Holstein dairy cows, 24 primiparous and 56 multiparous (mean lactation number 2.8 (SD 1.77)), with a mean calving date of 1 November 2018 (range, 5 October to 12 December 2018). Cows had a mean predicted transmitting ability (PTA₂₀₁₈) for milk yield, fat yield and protein yield of 251 (SD 157.9) kg, 14.9 (SD 5.4) kg and 14.0 (SD 4.7) kg, respectively. During the three week period pre-partum cows were given *ad-libitum* access to grass silage, supplemented with a pre-calving mineral/vitamin mix and with calcined magnesite, to achieve target intakes of 100 and 50 g per cow/day, respectively.

Treatments

Cows were moved to a free-stall house within 24 hours post-calving. Two treatments, namely silage produced within either a three-harvest system (3H) or a four-harvest

system (4H), were examined. Cows were allocated at random to each of the two treatments at calving (28 multiparous and 12 primiparous cows per treatment), while ensuring that treatment groups remained balanced for calving date, lactation number, PTA for milk yield, milk fat yield and milk protein yield, BW and body condition score (BCS) at calving, and, in the case of multiparous cows, previous 305-day milk yield. The silages offered were produced from perennial ryegrass (*Lolium perenne*) based swards, with target intervals between harvests of approximately 50 days with the 3H system and 35 days with the 4H system. Actual cutting dates for the 3H system were 29 May, 24 July, and 11 of September (harvests one to three, respectively), while actual cutting dates for the 4H system were 17 May, 25 June, 8 August and 11 September, 2018 (harvests one to four, respectively).

Each cow was offered each of its treatment silages consecutively (i.e. harvest one, followed by harvest two, etc.,) for a target number of days, with the change from one silage to another taking place on a single day each week, while the target number of feeding days for each silage was in proportion to the herbage DM yield for each harvest. Target feeding days for harvests one to three within the 3H treatment were 70, 55, and 57 days respectively, while target feeding days for harvests one to four within the 4H treatment were 52, 44, 41 and 45 days, respectively, although shortages (due to higher than anticipated intakes) of the final silage offered within 3H and 4H treatments meant that study was reduced in length to 175 days (25 weeks).

All rations were formulated using NutriOpt (Nutreco, Amersfoort, Netherlands). The grass silage component of the diet was mixed with a concentrate to form a partial mixed ration (basal ration). The concentrate fraction was included in the mix at a rate of 8.6 kg per cow/day, to achieve a target concentrate intake of 8.0 kg per cow/day (rations offered *ad libitum* at 107.5 % of the previous day's intake). A separate concentrate was formulated for each silage type (ingredient list and chemical composition of each concentrate are presented in Table 1). The rations were prepared daily using a mixer wagon (Vari-Cut 12, Redrock, Armagh, UK) and offered between 09.00 and 10.00 hours, while uneaten food was removed the following day at approximately 08.00 hours. The appropriate silage was placed in the wagon, and mixed for approximately five minutes, after which the appropriate concentrate was added to the wagon and the silage and concentrate mixed for a further five minutes. The ration was transferred from the mixer wagon to a series of

individual feed boxes mounted on weigh scales (Controlling and Recording Feed Intake, Bio-Control, Rakkestad, Norway). Cows were given access to these boxes via an electronic identification system, and individual cow intakes were recorded daily.

Cows were offered additional concentrates (ingredient list and chemical composition in Table 1) on a FTY basis, with 2.0 kg/day of this concentrate offered via an in-parlour feeding system (fixed throughout the duration of the study; 1.0 kg at each milking) and the remainder offered via an out-of-parlour feeding system (OPF). Concentrates offered via the OPF increased during the first 21 days post-calving by 0.25 kg/day (from 0 to 5.25 kg/day) for multiparous cows and by 0.20 kg/day (from 0 to 4.2 kg/day) for primiparous cows. These concentrate feed levels remained unchanged until 28 days post-calving, after which these concentrates were offered on a FTY basis. Concentrate levels were reviewed weekly (on the same day that cows moved from one silage to another), and adjusted on the basis of milk yields during the previous two weeks. The first step in the process involved determining average daily silage and concentrate intakes from the basal ration (on a group basis, recorded using the feed intake system) over the previous 14 day period. Total metabolisable energy (ME) intake from the basal ration was then determined based on the predicted ME concentration of the silage offered (based on weekly analysis), and the estimated ME concentration of the concentrate (based on formulated values, NutriOpt). This intake was assumed to support the cow's maintenance energy requirement (based on equations in the current UK dairy cow rationing system (Feed into milk; Thomas, 2004)), plus the production of a certain amount of milk (based on an assumed ME requirement of 5.2 MJ/kg milk). The milk produced by each cow not supported by the basal ration was determined as the difference between the actual milk yield over the previous two weeks, and the milk yield that the basal ration was calculated to support. Concentrates were offered at a rate of 0.45 kg, for each kg of milk not supported by the basal ration. The basal ration in the 3H treatment (excluding the buildup period) was calculated to provide sufficient ME to meet the cows maintenance energy requirements plus 23.8 (19.1), 24.1 (19.4), and 23.6 (18.8) kg milk/day for cows (heifers) offered silage from harvests one to three, respectively. Similarly, excluding the build-up period, the basal ration in the 4H treatment was calculated to provide sufficient energy to meet the cows maintenance energy requirements plus 26.2 (21.2), 26.1 (20.9), 25.9 (20.9) and 26.0 (20.8) kg milk/day for cows (heifers) offered silage from harvests one to four, respectively. If individual cow milk yields fell below the yield which the basal ration

as able to support, concentrate levels offered through the OPF were held at 1.0 kg per cow/day for three weeks, and thereafter concentrate feeding via the OPF ceased.

Cow measurements

Throughout the experimental period, cows were milked twice daily (between 06.00 and 08.00 hours and between 15.00 and 17.00 hours) using a 50-point rotary milking parlour (Boumatic, Madison, USA). Individual cow milk yields were automatically recorded at each milking, and a daily milk yield for each 24 hour period calculated. Each week a milk sample from each cow was taken during two consecutive milkings, analysed for fat, protein and lactose concentrations using an infrared milk analyser (Milkoscan Combifoss[™]7; Foss Electric, Hillerød, Denmark), and a weighted concentration of each constituent calculated for the 24 hour sampling period.

Individual cow BW was recorded using an automated weigh bridge twice daily (immediately after each milking), and a mean weekly BW for each cow was determined. The BCS of individual cows was estimated on a five-point (including quarter points) scale by the same trained technician each fortnight, according to Edmonson et al. (1989). The daily EB (MJ of ME/d) for each cow was calculated using equations contained within 'Feed into Milk' the current UK dairy cow rationing system, as the difference between the cow's total ME requirements (maintenance, milk production, and activity) and total ME intake (Agnew and Yan, 2004). Blood samples were collected from the tail of each cow prior to feeding at 4, 8, 12, 16 and 20 week of lactation, and centrifuged (3000 rpm for 15 minutes) to isolate either the serum (tubes with a clot activator) or the plasma (fluoride oxalate tubes). Serum β HB, non-esterified fatty acids (NEFA) and urea concentrations, and plasma glucose concentrations were determined as described by Little et al. (2016).

Feed analysis

Each day a sample of the grass silage was taken from throughout the pile of mixed silage and dried at 85°C for 18 hours to determine oven DM content. At two time points in each week a sample of grass silage was dried at 60°C, this was bulked for each fortnight, milled through a sieve with 0.85 mm aperture and subsequently analysed for NDF, ADF and ash. A fresh silage sample was analysed on a weekly basis for ME concentration using NIRS according to Park et al. (1998). Furthermore, a fresh silage sample was also analysed on a weekly basis for gross energy, N, pH, ammonia-N and volatile components. A weekly sample of each concentrate offered was collected. A sub-sample was dried at 85°C for 24 hours to determine oven DM content, while a second sub-sample was dried at 60°C for 48 hours, bulked for each fortnight, milled through a 0.85 mm sieve, and subsequently analysed for N, NDF, ADF, ash and starch. All chemical analysis of the feedstuffs offered where undertaken as described by Purcell et al. (2016).

Statistical analysis

Weekly data for DMI, milk yield, milk composition, BW, and energy balance (EB), fortnightly data for BCS, and periodic blood metabolite data (4, 8, 12, 16 and 20 weeks), were analysed using REML repeated measures analysis with week post-calving included as the repeated measure. The model included the following terms as fixed effects: lactation number + week + treatment + (week × treatment). Cow within week was also included as a random effect. The correlation between weeks was modelled using an autoregressive model of order. In addition to lactation number, PTA for milk yield, fat yield, protein yield, fat plus protein yield and milk composition were used as covariates in the analysis the corresponding variables. For variables where significant treatment effects were identified (P < 0.05), differences between the 3H and 4H treatments were tested using Fisher's protected-adjusted multiple comparisons. All data were analysed using GenStat (19.1; VSN International Limited, Oxford, UK).

Results

Herbage DM yields at harvests one, two and three in the 3H system were 5.2, 4.1 and 4.1 (total, 13.4) t DM/ha, while the corresponding values at harvests one, two, three and four within the 4H system were 3.4, 3.6, 2.6 and 2.8 (total, 12.3) t DM/ha. The oven DM content of herbage at ensiling, and the chemical composition of the silages produced, are presented in Table 2. Dry matter of herbage ensiled, and of the resultant silages, varied between harvests within systems, reflecting the variability of weather conditions encountered during the season. Silage protein levels tended to increase from first through to the last harvest within each system. Silages produced within the 3H system tended to have higher fibre, and a correspondingly lower ME concentration, than silages produced within the 4H system.

When averaged over the entire experimental period, cows on the 4H treatment had a higher silage DMI than those on the 3H treatment (P < 0.001), while concentrate and total DMI were unaffected by treatment (Table 3). Intakes of all parameters varied over time

(Figure 1, P < 0.001), and there was a significant interaction (P < 0.001) between treatment and week for silage DMI (Table 3).

Cows on 4H treatment had a higher milk yield (P = 0.009), fat yield (P = 0.002), protein yield (P < 0.001) and fat + protein yield (P < 0.001), and produced milk with a higher protein content (P = 0.004) than those on 3H, while cows on 3H produced milk with a higher fat content (P = 0.002; Table 4). Treatment had no effect on milk lactose content. Milk yield, milk protein content, milk fat content and milk fat plus protein yield varied (P >0.001) over the experimental period (Figure 2a – 2d, respectively). There were no interactions between treatment and week of lactation for any milk production parameter (P < 0.05).

Treatment had no effect on BW (average 637 kg) or BCS (average 2.5) over the experimental period, or on nadir BW or days to reach nadir BW (62 days). Cow BW and BCS varied over time (Table 5; P < 0.001), but there was no significant interaction between treatment and week of lactation. Mean EB over the experimental period was 5.6 and 0.6 MJ/d for cows on 4H and 3H, respectively (P < 0.030; Table 5). While EB was affected by week of lactation (P < 0.001; Figure 3), there was no significant interaction between treatment and lactation week.

Treatment had no effect on plasma glucose levels (mean 3.49 mM/L). However, cows on 4H tended to have lower serum β HB and higher serum NEFA concentrations (*P* = 0.085 and *P* = 0.070, respectively; Table 5) compared to cows on 3H. Serum urea was significantly higher in cows on 4H compared to cows on 3H (*P* = 0.032). All blood metabolites were affected by week of lactation (*P* < 0.001) and there was an interaction between treatment and week for serum β HB and serum urea (*P* = 0.034, *P* < 0.001, respectively; Table 5).

Discussion

Impact on silage composition and feed value

Silage DM concentrations were variable (225 – 491 g/kg DM), with concentrations above target (300 g/kg DM at ensiling) arising due to excellent weather conditions and unexpected machinery related delays, while concentrations below target highlight the limitations of a 24 hour period of field wilting within a temperate maritime climate. Silages

were generally well fermented, with lactic acid concentrations and pH largely a function of DM content. Silage CP concentration tended to increase, and ME to decrease, with later harvests, agreeing with the trends identified within a 20 year dataset of NI silages (Patterson et al., 2020). In general, ammonia N concentration, an indicator of the extent of deamination of plant protein by both plant and microbial enzymes, was lower in drier silages, and increased with increasing CP content in later harvests. When silage composition within each harvest is 'weighted' using herbage DM yield within that harvest, mean CP, NDF and ME concentrations over all harvests were 143 g/kg DM, 519 g/kg DM and 10.7 MJ/kg DM respectively for silage within the 3H treatment, and 164 g/kg DM, 472 g/kg DM and 11.3 MJ/kg DM for silage within the 4H treatment. The higher nutritive value of the 4H silage reflects the earlier cutting and shorter re-growth intervals adopted.

Impact on cow intakes and performance

Few studies have compared the impact of harvesting frequency over an entire season on subsequent cow performance. Comparisons between each harvest within the current study were not meaningful, due to the confounding effects of differences in lactation stages when each harvest was offered, and the absence of a fourth harvest within the 3H system. Thus performance over the entire study period was compared, with individual harvest data presented within Figures 1 - 3 aiding interpretation of the outcomes.

The weighted ME composition of the silages produced within the 4H system was 0.55 MJ/kg DM higher than for those produced within the 3H system, equivalent to an increase in D-value of 34 g/kg. This increase would be expected to increase silage DMI by 0.92 kg/day (Huhtanen et al., 2013), with this similar to the increase observed, demonstrating the benefits of more frequent harvesting. However, differences in intakes were inconsistent within the 3H and 4H treatments, with Figure 1 highlighting that intakes of the second harvest within the 3H treatment were similar to intakes of the 4H silages offered at this time, while intakes of the first and third harvest silages were lower. Silage composition data provides no obvious explanation for these inconsistencies.

The adoption of a FTY approach makes interpretation of the concentrate intake data more challenging, with concentrate intakes and concentrate types differing between harvests within each system, and at the same stage of lactation between systems. Nevertheless, offering concentrates FTY is common on most NI farms, with the approach taken allowing

harvesting systems to be compared under conditions similar to those used in practice on farms. Despite the variation in concentrate intakes between silages, overall concentrate intakes were unaffected by treatment, although numerically lower for 4H. Nevertheless, substitution effects are likely to have been greater with the 4H silages due to their higher nutritive value (Keady et al., 2004; Huhtanen et al., 2008). Total DMI did not differ between treatments, with the two intake curves for total DMI following largely identical patterns until approximately week 18 of lactation, when intakes with the 3H treatment declined more rapidly than those of the 4H treatment, coinciding with the change to the third harvest of 3H which was the wettest silage within the study.

Difference in milk yield between treatments became apparent shortly after calving, milk yields on 3H remaining lower than for 4H throughout the study (mean difference over the experiment of 2.4 kg/day). This difference was larger than the response of 1.5 kg/day that might have been expected (0.45 kg milk per 10 g/kg increase in D-value according to Huhtanen et al. (2013)). Although milk yields peaked at the same time with both treatments (week 8; Figure 2a), the decline in yield tended to be slower in 4H, although, there was no interaction between treatment and time.

Both milk fat and milk protein content exhibited the normal early lactation decline, with concentrations beginning to increase from approximately week 6 - 7 of lactation onwards. The increased milk protein content with 4H (0.7 g/kg greater) was similar to the 0.5 g/kg increase expected based on Rinne et al. (1999), namely a 0.14 g/kg increase in milk for each 10 g/kg increase in silage D-value. While a greater milk protein content normally suggests cows with an improved energy status, cows on 4H actually had a poorer overall EB than cows on 3H. Differences in CP likely reflect an improved balance of energy and available protein in the 4H silages (Rinne et al., 1999; Kim et al., 2000), resulting in an increased supply of microbial protein. The lower milk fat content with 4H (1.0 g/kg lower) appeared to arise between week 7 – 19 of lactation, when silages from harvests two and three were offered. At this time cows on both treatments were generally in positive EB, and consequently differences are likely to have been driven more by the lower NDF content of the diets containing 4H silages, rather than differences in tissue mobilisation.

The cumulative effect of the differences in milk yield and milk composition was that milk fat plus protein yield was 0.2 kg/day higher in 4H cows compared to 3H cows. Part of

this response can be attributed to the higher ME intake with the 4H treatment, a consequence of both the higher DMI and higher ME content of the diets offered (on average 0.55 MJ per cow/day higher). However, over the entire experiment the average EB of cows on the 4H treatment was lower than for cows on 3H (5.6 v. 0.6 MJ/day ME). While cows on both treatments returned to positive EB at approximately week five of lactation, the energy status of cows on 4H appeared to be particularly affected during the weeks after they transitioned between harvests. However, this was not reflected in any significant differences between treatments in either BW or BCS. While treatment effects on βHB and NEFA concentrations tended towards significance, concentrations of the former were higher with 3H, while concentrations of the latter were higher with 4H. This is difficult to explain as both of these metabolites are considered to be indicative of adipose tissue break down (Macrae et al., 2012). Nevertheless, differences between treatments were small, and likely to be of little biological significance, as concentrations of both NEFA and βHB remained below 0.7 mM/L, and 1.0 mM/L, respectively, the maximum value suggested as normal by Whitaker (1997). Plasma glucose concentrations increased during the weeks post-calving, but did not differ between treatments, and remained above the optimal value of 3.0 mM/L (Whitaker et al., 1983). Therefore, it appears that cows on 4H may have partitioned a greater proportion of energy consumed to milk solids, than cows on 3H.

Blood serum urea was significantly higher in 4H compared to 3H cows (4.44 v. 4.15 mM/L), except at week 12 when 3H cows had elevated serum urea. The higher levels of urea, combined with the higher levels of NEFA, could indicate an excess of effective rumen degradable protein relative to fermentable ME with 4H silages (Whitaker, 1997). Indeed, total diet CP levels, weighted across all diets offered, was higher in 4H compared to 3H diets, namely 179 and 171 g/kg DM, respectively. However, N utilisation efficiency was similar with the 3H and 4H treatments, namely 0.32 and 0.33, respectively.

Whole systems comparison and practical considerations

A whole system comparison was conducted to examine the impact of differences in herbage yield and DMI between treatments, with this analysis examining inputs and outputs associated with feeding a 100 cow dairy herd over a 180 day winter housed period. The 1.1 t DM/ha reduction in yield with the 4H compared to the 3H system (12.3 v. 13.4 t DM/ha, respectively) is in agreement with the outcome of earlier AFBI studies

indicating reduced yield with more frequent harvesting (Binnie and Chestnutt, 1991; Ferris et al., 2003). Assuming in-silo and feed-out losses of 15% within both systems, the utilisable silage yields available with the three- and four-harvest systems are 11.4 and 10.5 t DM/ha, respectively . Based on measured silage intakes within the current study, the total silage requirement over the 180 day housed period is 171 t and 187 t DM for the three- and four-harvest system, respectively (daily intake x herd size x housed period), a 9.5% higher requirement with the latter. Dividing the total silage requirement associated with each system (t DM) by the utilisable silage yield available within each system (t DM/ha), indicates a land requirement of 15 and 18 ha to supply the winter feed requirements of the three- and four-harvest system respectively, a 19.3% higher requirement with the latter. The greater land requirement associated with a four-harvest system may be an issue in situations where land is a limiting resource. However, based on concentrate intake and milk output values in the current study, the four-harvest system requires 2.2% less concentrates (241 v. 236 t DM for three- and four-harvest system, respectively), and is associated with the production of a 6.9% higher yield of fat plus protein (49.5 v. 52.9 t for three- and four-harvest systems, respectively).

While a four-harvest system offers opportunities to reduce concentrate inputs and to increase milk solid output, the system is not without its challenges. To produce high digestibility silages herbage must be harvested within a relatively short time-window which can be challenging given the high reliance on contractor usage in many countries (> 60% in NI: Ferris et al., 2019). Secondly, in order to achieve satisfactory fermentation, which can be challenging given the higher N content and possibly high nitrate levels in herbage, adequate wilting is necessary. However, unlike many parts of mainland Europe, where the summer climate is influenced by continental air masses and tend to be relatively stable, western areas of the UK and the Ireland have a temperate maritime climate, with a significant proportion of annual rainfall between the months of April to September. Therefore, the short-time window for harvesting to ensure high digestibility must also be associated with weather conditions when wilting can be achieved. However, herbage harvested within a four-harvest system due to the lower herbage yield at each harvest.

Conclusion

Increasing the harvesting frequency of grass for silage production from three- to fourharvests per season has potential to improve silage feed value and cow performance. Silage produced under a four-harvest system had a higher nutritional value which increased silage DMI, milk yield and milk fat plus protein yield, compared to silage produced under a three-harvest system. The lower DM yield (t/ha) with the four-harvest system would increase land area required by 19.3% compared to a three-harvest system; however, this was accompanied by a 2.2% reduction in concentrate use and a 5.9% increase in milk solids output. Therefore, producing grass silage within a four-harvest system is an effective way to improve silage feed value and increase milk production from forage.

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Table 1 Ingredient list (g/100 g fresh) and chemical composition (SD in parenthesis) of the concentrate offered to all cows through the out-of-parlour feeding system (OPF), and of individual concentrates used to supplement each silage type within the three- (3H) and four-harvest (4H) silage production systems.

	Concentrate	3 harvest system (3H)			4 harvest system (4H)			
	via OPF	Harvest 1	Harvest 2	Harvest 3	Harvest 1	Harvest 2	Harvest 3	Harvest 4
Ingredients								
Maize meal	12.6	25.8	44.1	44.6	30.7	45	44.6	44.6
Wheat		22.2	18.4	17		20	10	10.5
Barley		7.1	10	10				
Rapeseed meal	12.5				18.5	5	11.5	5
Soyabean meal (high protein)	6.4	16.6	5	5	14	5	5	5
Maize gluten	15	11.3	7.6	8.5				
Distillers dried grains with solubles	10	11	5	5				
Soya hulls (toasted)	15.75				26.6	15.1	19	25
Wheat feed meal	11							
Palm kernel meal	9.5							
Molaferm ¹	4							
Protected protein (Sopralin) ²			3.75	3.75	4	3.75	3.75	3.75
Protected fat (Maxfat) ²		2.5	2.5	2.5	2.5	2.5	2.5	2.5
Limestone (CaCO ₃)	1.3	1.25	1.25	1.25	1.25	1.25	1.25	1.25
Rumen buffer (Acid buff) ³	0.6	0.89	1	1	1.05	1	1	1
Sodium chloride	0.65	0.65	0.65	0.65	0.65	0.65	0.65	0.65
Mineral and vitamin pre-mix ²	0.4	0.4	0.4	0.4	0.4	0.4	0.4	0.4
Calcined magnesite	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3
Yeast (Actisaf) ⁴		0.04	0.05	0.05	0.06	0.05	0.05	0.05
Chemical Composition								
Oven dry matter (g/kg)	890 (16.1)	905 (4.1)	898 (6.4)	897 (6.2)	906 (9.5)	892 (11.8)	898 (5.2)	899 (5.8)
Starch (g/kg DM)	151 (27.8)	384 (28.0)	437 (31.6)	457 (18.2)	220 (20.1)	401 (23.3)	363 (8.8)	359 (32.7)
Crude protein (g/kg DM)	211 (5.7)	205 (4.0)	156 (8.2)	164 (4.8)	230 (7.8)	163 (15.6)	169 (5.2)	150 (6.6)
NDF (g/kg DM)	421 (30.5)	219 (5.8)	191 (16.2)	196 (17.0)	361 (17.3)	260 (17.9)	281 (29.3)	321 (38.0)
Ash (g/kg DM)	88 (2.4)	75 (4.3)	65 (2.5)	67 (2.7)	83 (5.2)	77 (8.1)	75 (2.1)	73 (4.3)
Metabolisable energy [‡] (MJ/kg DM)	12.0	13.3	13.5	13.5	12.6	13.2	12.9	13.0

	3 harvest system (3H)			4 harvest system (4H)			
	Harvest 1	Harvest 2	Harvest 3	Harvest 1	Harvest 2	Harvest 3	Harvest 4
Herbage pre-ensiling							
Oven dry matter (g/kg)	403 (37.4)	289 (36.1)	234 (29.4)	322 (53.1)	513 (62.2)	263 (45.4)	266 (15.7)
Silage							
Oven dry matter (g/kg)	387 (29.4)	282 (29.6)	225 (20.6)	279 (16.8)	491 (33.1)	261 (31.8)	252 (14.9)
VCODM (g/kg)	400 (40.5)	292 (18.2)	239 (18.7)	296 (12.7)	505 (14.1)	275(12.7)	269 (10.5)
Crude protein (g/kg DM)	106 (9.4)	161 (19)	176 (19)	124 (9.8)	164 (14.2)	180 (9.5)	203 (8.7)
Ash (g/kg DM)	70 (1.9)	92 (5.5)	125 (5.1)	74 (1.7)	90 (4.0)	110 (3.0)	125 (6.1)
Acid detergent fibre (g/kg DM)	326 (7.5)	289 (14.9)	286 (10.0)	264 (4.6)	287 (6.5)	265 (11.2)	249 (14.1)
Neutral detergent fibre (g/kg DM)	562 (7.9)	504 (28.1)	476 (15.2)	469 (13.2)	505 (18.6)	474 (28.5)	432 (21.0)
Gross energy (MJ/kg DM)	18.7 (1.18)	19.1 (0.64)	18.5 (0.99)	19.1 (1.17)	19.2 (0.38)	18.4 (0.67)	18.5 (0.55)
Metabolisable energy (MJ/kg DM)	10.9 (0.36)	10.6 (0.52)	10.6 (0.38)	12.1 (0.39)	11.2 (0.50)	10.7 (0.39)	10.8 (0.47)
pH	4.34 (0.19)	4.07 (0.16)	4.14 (0.23)	3.73 (0.21)	4.85 (0.16)	4.04 (0.11)	4.21 (0.22)
Lactic acid (g/kg DM)	46 (12.1)	66 (34.2)	89 (53.4)	127 (32.0)	20 (7.5)	101 (26.9)	106 (42.4)
Acetic acid (g/kg DM)	10.3 (3.34)	28.7 (11.25)	39.2 (18.62)	15.4 (3.41)	7.3 (1.74)	13.3 (3.07)	22.0 (4.14)
Ethanol (g/kg DM)	5.7 (3.04)	5.5 (1.68)	6.2 (3.27)	18.1 (10.12)	5.7 (2.75)	10.1 (4.60)	7.3 (3.83)
Ammonia (g/kg total N)	66 (1.2)	71 (0.5)	86 (1.9)	58 (1.2)	49 (0.6)	66 (0.5)	80 (2.2)

Table 2 Dry matter of herbage at ensiling, and the chemical composition (SD in parenthesis) of the resultant silage as producedwithin a three- (3H) or four-harvest (4H) system.

VCODM., volatile corrected oven dry matter

	5	Trea	itment		P Value			
		3 harvest system (3H)	4 harvest system (4H)	SED	Treatment	Week	Treatment × Week	
Harvest 1	Silage DMI (kg/day)	8.5 (1.00)	9.3 (1.45)					
	Concentrate DMI (kg/day)	14.2 (2.10)	12.5 (1.81)					
	Total DMI (kg/day)	22.7 (2.90)	21.8 (3.10)					
Harvest 2	Silage DMI (kg/day)	10.9 (1.04)	10.3 (1.39)					
	Concentrate DMI (kg/day)	13.5 (2.97)	14.0 (3.28)					
	Total DMI (kg/day)	24.4 (3.39)	24.3 (4.04)					
Harvest 3	Silage DMI (kg/day)	9.7 (0.84)	11.2 (1.41)					
	Concentrate DMI (kg/day)	12.8 (2.62)	13.3 (2.88)					
	Total DMI (kg/day)	22.5 (3.02)	24.5 (3.55)					
Harvest 4	Silage DMI (kg/day)		10.8 (1.22)					
	Concentrate DMI (kg/day)		12.6 (2.75)					
	Total DMI (kg/day)		23.4 (3.44)					
All harvests	Silage DMI (kg/day)	9.5	10.4	0.30	< 0.001	< 0.001	< 0.001	
	Concentrate DMI (kg/day)	13.4	13.1	0.43	0.165	< 0.001	0.123	
	Total DMI (kg/day)	23.0	23.4	0.59	0.131	< 0.001	0.172	

Table 3 Effect of offering silage made within either a three- (3H) or four-harvest (4H) system on average daily dry matter intake (DMI) of each silage within each harvest (SD in parenthesis) and on the average DMI across all harvests.

		Treatment				P Value	
	-	3 harvest	4 harvest system	SED	Treatment	Week	Treatment ×
		system (3H)	(4H)				Week
Harvest 1	Milk yield (kg/day)	40.3 (8.85)	40.7 (9.36)				
	Fat (g/kg)	41.3 (4.56)	40.8 (3.85)				
	Protein (g/kg)	32.6 (1.76)	34.1 (2.10)				
	Fat plus protein yield (kg/day)	2.95 (0.546)	3.04 (0.685)				
Harvest 2	Milk yield (kg/d)	38.3 (8.77)	42.0 (9.61)				
	Fat (g/kg)	42.4 (5.29)	38.9 (5.41)				
	Protein (g/kg)	32.6 (1.93)	33.0 (1.95)				
	Fat plus protein yield (kg/day)	2.84 (0.537)	2.99 (0.566)				
Harvest 3	Milk yield (kg/day)	33.8 (7.67)	39.2 (8.45)				
	Fat (g/kg)	43.0 (5.54)	42.2 (5.24)				
	Protein (g/kg)	33.3 (2.01)	33.7 (2.19)				
	Fat plus protein yield (kg/day)	2.56 (0.492)	2.94 (0.526)				
Harvest 4	Milk yield (kg/day)		36.1 (8.24)				
	Fat (g/kg)		42.5 (5.62)				
	Protein (g/kg)		33.7 (2.12)				
	Fat plus protein yield (kg/day)		2.72 (0.529)				
All harvests	Milk yield (kg/day)	37.3	39.7	1.08	0.009	< 0.001	0.501
	Fat (g/kg)	42.1	41.1	1.28	0.022	< 0.001	0.587
	Protein (g/kg)	32.9	33.6	0.45	0.004	< 0.001	0.140
	Lactose (g/kg)	48.3	48.2	0.11	0.178	< 0.001	0.737
	Fat yield (kg/day)	1.54	1.61	0.023	0.002	< 0.001	0.416
	Protein yield (kg/day)	1.22	1.32	0.032	< 0.001	< 0.001	0.524
	Fat plus protein yield (kg/day)	2.75	2.94	0.087	< 0.001	< 0.001	0.516

Table 4 Effect of offering silage made within either a three- (3H) or four- harvest (4H) system on average daily milk production and milk composition for each silage within each harvest (SD in parenthesis), and on average daily performance across all harvests.

	Treatment			P Values		
	3 harvest system (3H)	4 harvest system (4H)	SED	Treatment	Week	Treatment × Week
Bodyweight (kg) ¹	637	636	9.6	0.788	< 0.001	0.349
Nadir bodyweight (kg)	613	609	11.5	0.721		
Days to nadir body weight	63	61	12.1	0.871		
Body condition score ¹	2.5	2.4	0.05	0.792	< 0.001	0.362
End of study body condition score	2.5	2.5	0.08	0.916		
Energy balance (MJ/day) ¹	5.6	0.6	0.23	0.030	< 0.001	0.148
Blood metabolites ²						
βHB (mM/L)	0.43	0.39	9.614	0.085	< 0.001	0.034
NEFA (mM/L)	0.16	0.18	0.020	0.070	< 0.001	0.150
Glucose (mM/L)	3.52	3.46	0.327	0.679	< 0.001	0.550
Urea (mM/L)	4.15	4.44	0.184	0.032	< 0.001	< 0.001

Table 5 Effect of offering silage made within either a three- (3H) or four-harvest (4H) system on cow body weight, body condition score and mean energy balance over the experimental period, and on mean blood metabolites.

¹ Mean across entire experimental period ² Mean analysis of samples taken at 4, 8, 12, 16 and 20 weeks post-calving. β HB, beta-hydroxybutyrate; NEFA, non-esterified fatty acid



Figure 1 Mean weekly silage DMI kg/day (dry matter intake; dotted lines), concentrate DMI kg/day (dashed lines) and total DMI kg/day (solid lines) of cows offered silage produced within either a three- (3H; ◆) or four-harvest (4H; •) system¹.

¹Arrows indicate when cows changed to harvests 2,3 and 4 (4H; black arrow) and harvests 2 and 3 (3H; grey arrow)



Figure 2 Mean weekly milk yield (kg/day) (a), milk fat composition (g/kg) (b), milk protein composition g/kg (c), and fat plus protein yield (kg/day) (d) of cows offered silages produced within either a three- (3H; \bullet) or four-harvest (4H; \bullet) system¹. ¹Arrows indicate when cows changed to harvests 2,3 and 4 (4H; black arrow) and harvests 2 and 3 (3H; grey arrows)

Chapter 6

Comparison of the performance of dairy cows offered grass silage produced within either a three- or five-harvest system

INTRODUCTION

The digestibility of grass silage produced within Northern Ireland (**NI**) has shown little change during the last twenty years (Patterson et al., 2021) despite clear evidence of the animal performance benefits associated with improving silage digestibility. For example, in a review of 10 studies, Keady et al. (2013) observed that for each 10 g/kg increase in silage digestibility (digestible organic matter in the dry matter: **D-value**), DMI and milk yield increased by an average of 0.22 kg/d and 0.33 kg/d, respectively. Similarly, an analysis of four datasets from northern Europe indicated that silage DMI and ECM yield increased by an average of 0.27 and 0.45 kg/d per 10 g/kg increase in D-value (Huhtanen et al., 2013). As a result, increasing the D-value of grass silage can result in a concentrate sparing effect saving approximately 2.35 kg/d of concentrates per 5 percentage units increase in silage D-Value (Keady et al., 2013). Thus, improving silage digestibility can result in substantial improvements in cow performance.

Plant maturity at harvest is one of the primary factors influencing silage digestibility due to the negative relationship between the degree of lignification of fibre and digestibly (Randby et al., 2010; Keady et al., 2013), with an average decline in D-value of 3.3% for each one-week delay in harvest of primary growth herbage (Keady et al., 2013). Similarly, in northern Europe D-value has been observed to decline by approximately 5 g/kg DM per day with primary growth herbage (Kuoppala et al., 2008; Sairanen et al., 2022). Thus, harvesting grass at a less mature growth stage is likely to play a key role in improving silage nutritive value, which over the course of a season is likely to involve more frequent harvesting.

The majority of studies investigating the impact of silage digestibility on cow performance have focused on silages produced from primary growth herbage. Silages produced from regrowth herbage contain greater proportions of NDF, which reduces digestibility (Huhtanen et al., 2006) and in turn has a negative effect on intake and subsequent milk production. Even when differences in digestibility are accounted for, the intake potential of silages produced from regrowth herbage are generally lower than that of silages produced from primary growth herbage (Huhtanen et al., 2007). Furthermore, timing of the primary harvest remains key to improving silage quality over the course of the growing season. For example, in systems with two or three-harvests,

an early primary harvest improved regrowth quality, feed intake, cow performance and energy efficiency compared to regrowth following a later first harvest (Pang et al., 2021). This demonstrates the importance of examining the effects of harvesting silage made from regrowth herbage.

While the use of two- or three-harvest systems on dairy farms have been the norm within the United Kingdom (UK) and Ireland for many years, there has recently been a growing interest in the use of 'multi-cut' systems, with the term generally taken to mean more than three harvests. For example, while a recent survey of NI dairy farmers indicated that 65% harvested grass for silage on three occasions during the year, a small number (12%) harvested either four or five times (Ferris et al., 2022). However, relatively few studies have examined the impact of harvesting frequency on silage nutritive value and subsequent cow performance. In one early study, Ferris et al. (2003) demonstrated a 55% reduction in concentrate input without loss of milk production when cows were offered silages produced within a four-harvest system compared to a two-harvest system. More recently, Craig et al. (2023) found that cows offered silage produced within a four-harvest system had a greater DMI, milk yield and milk fat and protein yield compared to cows offered silage produced within a threeharvest system. However, in this study concentrates were offered on a feed-to-yield basis, meaning that concentrate intakes differed with each silage type. While it is recognised that increasing the number of silage harvests per year increases the cost per tonne silage DM, an economic modelling exercise in Norway indicated that, assuming land is not a limiting factor, a three-harvest system could be more profitable in terms of gross margin than a traditional two-harvest system (Flaten et al., 2015). The current study was designed to build on the results of previous research (Craig et al., 2023) by examining the effects of a three- vs. a five-harvest system on silage nutritive value, cow performance and overall efficiency.

MATERIALS AND METHODS

This study was conducted at the Agri-Food and Biosciences Institute (**AFBI**), Hillsborough, NI (54°27'N; 06°04'W). All experimental procedures were conducted under an experimental licence granted by the Department of Health, Social Services & Public Safety for Northern Ireland in accordance with the Animals (Scientific Procedures) Act 1986.

Experimental animals and housing

This study involved 34 mid-lactation Holstein dairy cows (average of 147 DIM: range 136 to 163 d) of which 30 were multiparous and 4 were primiparous (mean lactation number 2.7 (s.d. 0.98)). From calving until the start of the experiment cows were housed and offered a partial mixed ration comprising grass silage, fermented whole crop silage and concentrates (in a 45 : 20 : 35 DM ratio), while an additional 8.0 kg of concentrate was offered daily, 4.0 kg at each milking.

Throughout the experimental period cows were housed in a free-stall house with concrete flooring and had access to individual cubicles fitted with rubber mats and bedded with sawdust. The cubical-to-cow ratio was > 1:1 at all times, meeting the recommendations of FAWC (1997). The floor area was scraped every 3 h using an automated system. Two treatments, comprising silage produced within either a three-harvest system (**3H**) or a five-harvest system (**5H**), were examined. Treatment groups (15 multiparous and 2 primiparous cows per treatment) were balanced for lactation number, and pre-experimental fat plus protein yield, BW and BCS.

Silage production

The silages offered were produced from a perennial ryegrass (*Lolium perenne*) based sward located within four adjoining fields (total area of 12.5 ha). Each field was divided into two equal blocks, with the first block (selected at random) managed according to a three-harvest system (target of 52 d between harvests) while the second block was managed according to a five-harvest system (target of 32 d between harvests).

Total target nitrogen (**N**) application rates over the season were 250 kg/ha for 3H (110 kg/ha pre harvest 1, and 70 kg/ha after each of harvest 1 and 2, respectively) and 270 kg/ha for 5H (80 kg/ha pre harvest 1, and 60, 60, 40 and 30 kg/ha after harvests 1, 2, 3 and 4, respectively). Rates were based on RB209 (2021), with the greater target application rate with 5H reflecting the planned longer growing season with this treatment. With 3H, inorganic N applications comprised 65, 44 and 51 kg N/ha in the form of protected urea (proportionally 0.28 or 0.40 N), pre harvest 1, and after harvests 1 and 2, respectively, while with 5H this comprised 35, 46, 49 and 21 kg inorganic N/ha of inorganic N in the form of protected urea prior to harvest 1, and after harvests

1, 2, 3 and 4, respectively. The remaining N was applied in the form of dairy cow slurry prior to each of harvests 1, 2 and 3 to achieve applications of 45, 26, 20 kg organic N/ha (3H system), and prior to each of harvests 1, 2, 3 and 4 to achieve applications of 45, 14, 11 and 20 kg N/ha, respectively (5H system) (assumed DM and N content of slurry of 6% and 2.6 kg/m³, respectively, and assumed availability of N of 30% (RB209, 2021)).

Cutting dates for the 3H system where 17 May, 28 June, and 23 August 2021 (Harvests 1 – 3, respectively), while cutting dates for the 5H system were 30 April, 1 June, 29 June, 10 August and 7 September 2021 (Harvests 1 – 5, respectively). With both systems grass was mown using a Class 3200 mower (Harsewinkel, Ostwestfalen-Lippe, Germany), tedded to facilitate wilting, placed into rows using a Class 3100 grass rake (Harsewinkel, Ostwestfalen-Lippe, Germany), and harvested using a John Deere 7450 precision-chop forage harvester (Moline, IL, USA). Average field wilting period for the 5H system was 12 h (range 5 to 27 h), while average field wilting for the 3H system was 25 hrs (range 22 to 28 h), with a target DM at harvesting of approximately 300 g/kg. Grass was treated at harvest with a bacterial inoculant (Silo-King GS, Agri-King, Canada) containing 5 × 10¹⁰ cfu/kg fresh herbage of *Lactobacillus plantarum, Pediococcus pentosaceus*, and *Enterococcus faecium*, at a target rate of 1 litre per ton of fresh herbage. Herbage from each harvest was ensiled in separate bunker silos (100 – 200 t capacity), covered in polythene sheeting, and sheets weighed down with rubber mats.

The fresh weight of herbage harvested at each of the eight harvests during the study was determined by weighing all trailer loads of grass on a commercial weighbridge. A herbage sample was taken from throughout each load of herbage after it had been emptied from the trailer and the sample dried at 60°C for 48 h to determine oven dry matter (**ODM**) content. The yield of herbage DM harvested at each harvest within each of 3H and 5H was subsequently determined.

Experimental diets

Within 3H and 5H the experimental silages were offered consecutively over a 21 wk period, the period of time over which each silage was offered being in proportion to the yield of herbage DM at each harvest (9, 7 and 5 wks for harvests 1, 2 and 3 within

3H, and 5, 5, 4, 4 and 3 wks for harvests 1, 2, 3, 4 and 5 within 4H, respectively). Cows were offered fresh silage daily between 10.00 and 11.00 h, with silage mixed for approximately 5 min in a diet feeder (Vari-Cut 12, Redrock, Armagh, UK) before being placed into a series of feed boxes mounted on weight scales (Controlling and Recording Feed Intake, Bio-Control, Rakkestad, Norway). Cows accessed these boxes via an electronic identification system, enabling individual cow intakes to be recorded daily. Silages were offered ad libitum, at 107% of the previous day's intake, while uneaten food was removed the following day at approximately 09.00 h.

Cows on both treatments were offered a common commercial concentrate in the form of a pellet (CP: 190 g/kg DM, ME:13.0 MJ/kg). This was offered through an out-of-parlour concentrate feeding system at a rate of 11.0 kg per cow/d over the first 15 wks of the study, being reduced to 7.0 kg per cow/d during wks 16 – 19, and 5.0 kg per cow/day during wks 20 – 21. In addition, all cows were offered an additional 1.0 kg/d of this concentrate through an in-parlour feeding system (0.5 kg at each milking).

Cow measurements

All cows were milked twice daily (between 05.30 and 08.00 h and between 16.00 and 18.30 h) throughout the experiment using a 50-point rotary milking parlour (Boumatic, Madison, USA). Milk yields were recorded automatically at each milking and a total daily milk yield for each cow for each 24 h period calculated. Milk samples were taken during two consecutive milkings each week, treated with a preservative tablet (Broad Spectrum MicroTabs II, Advanced Instruments, Massachusetts, USA) and stored at 4°C until analysed (normally within 48 h). Milk samples were analysed for fat, protein, lactose, casein and MUN concentrations using an infrared milk analyser (Milkoscan Combifoss[™]7; Foss Electric, Hillerød, Denmark), and a weighted concentration of each constituent determined for the 24 h sampling period. Milk gross energy (**GE**) was calculated using the following equation (Tyrell and Reid, 1965):

Milk GE = (fat $\times 0.0384$) + (protein $\times 0.0223$) + (lactose $\times 0.0199$) – 0.108. Energy corrected milk yield (kg/d) was calculated assuming the GE content of 1 kg 'standard milk' to be 3.1 MJ/kg (i.e., for milk containing 40 g/kg fat, 32 g/kg protein, and 48 g/kg lactose, as described by Muñoz et al. (2015)) as follows:

ECM $(kg/d) = (milk yield (kg/d) \times GE (MJ/kg))/3.1$

A further milk sample was taken from each cow, in proportion to milk yield, during two successive milkings at the end of the final week during which each silage was being offered, and samples frozen. These samples (5 per cow on the 5H treatment and 3 per cow on the 3H treatment) were subsequently pooled as follows: samples where defrosted and homogenised using an Ultra Turrax (IKA England, Oxford), and a subsample (in proportion to individual milk yield) from each harvest combined and rehomogenised to provide a single pooled sample per cow. The final sample was then analysed for milk fatty acids (FA), as follows: milk fat was extracted from 1.0 ml of homogenised milk using a chloroform methanol extraction method (Bligh and Dyer 1959), and FA determined as methyl esters (FAME). The FA composition was determined using gas-liquid chromatography, with an aliquot (1.0 ul) of the FAME extract injected onto a CP Sil88 capillary column (100 meters x 0.25 mm id x 0.2 µm film thickness) in a Agilent 7890 gas chromatograph (both Agilent Technologies, Santa Clara, USA), equipped with a temperature programmable injector operated in the split mode and a flame ionisation detector. The oven was initially held at 50°C for 4 min then ramped at 8°C/min to 110°C, then 5°C/min to 170°C (hold time 10 min) and finally ramped at 2°C/min to 225 °C (hold time 30 min). Fatty acids were identified by their retention time with reference to commercially available FA standards (37 Supelco FAME mix) and individual standards for those not in the mix (SigmaAldrich Co. Ltd., Gillingham, UK), and were quantified using C13 FAME as an internal standard.

Rumen fluid samples were obtained from each cow during the final week when silage from each harvest was being offered. Rumen fluid was collected via an oro-ruminal probe (Ruminator; Profs-products, Wittibreut, Germany), with a hand pump. The oro-ruminal probe was inserted into the oesophagus, and the cow allowed to swallow the probe. After insertion into the rumen, a pump was attached and an initial rumen fluid sample of approximately 100 ml collected into a collecting jar, the pump disconnected, and the initial sample discarded. The collection jar was then washed under running water, the pump reattached to the sample tube and a second sample (approximately 300 ml) collected, the pump detached, and the oro-ruminal probe slowly removed from the cow. The rumen fluid sample was decanted into a 250 ml sterile container, set on ice and transferred to the lab within 30 minutes. Rumen fluid was diluted with demineralised water (1:5 dilution) and filtered through Whatmans No. 6 paper. Two drops of saturated mercuric chloride solution were added, and samples stored at 4°C

prior to analysis. A one ml sample from above, one ml of internal standard solution (0.504 mg/ml 3-methyl –n-valeric acid prepared in 0.15M oxalic acid solution) and three ml of distilled water were mixed and filtered through Whatman 0.45 micron polyethersulphone membrane filter. The filtrate was used to determine VFA concentrations using gas chromatography (GC 456, SCION Instruments UK Ltd, Scotland). Rumen fluid pH and ammonia (NH₃) concentrations were determined using a 815 Robotic Sample Processor XL (Metrohm, UK). Following analysis, results where weighted according to the length of time that cows where offered each silage.

Body weight was recorded twice daily (immediately after each milking) using an automated weighbridge, and a mean weekly BW for each cow was determined. The BCS of each cow was estimated by a trained technician once every 2 wks, according to Edmonson et al. (1989) using a 5 point (including quarter points) scale.

Feed analysis

The grass silage offered was sampled daily throughout the experiment and dried at 60°C for 48 h to determine ODM content. Samples of dried silage were collected thrice weekly and pooled for each 14-d period, with pooled samples milled through a sieve with 0.85 mm aperture and analysed for NDF, ADF and ash concentrations. In addition, a fresh silage sample was collected weekly and analysed for GE, N, pH, ammonia-N and volatile components, while the ME concentration of fresh silage samples was predicted using near infrared reflectance spectroscopy (NIRS) according to Park et al. (1998). The concentrate offered was sampled weekly and dried at 60°C for 48 h to determine ODM content. Samples of dried concentrate were pooled for each 14-d period, milled through a 0.80 mm sieve, and analysed for N, NDF, ADF, ash, GE and starch concentrations. Chemical analysis of all feedstuffs offered where undertaken as described by Purcell et al. (2016).

Statistical analysis

Weekly data for DMI, milk yield, milk composition, BW, and fortnightly data for BCS were analysed using REML analysis, with week included as the repeated measure The model included the following terms as fixed effects: lactation number + week + treatment + (week × treatment). Cow within week was also included as a random effect. The correlation between weeks was modelled using an autoregressive model

of order one. Weighted data for rumen fluid analysis (VFA, NH3 and pH) and milk FA were analysed using ANOVA. All data were analysed using GenStat (21; VSN International Limited, Oxford, UK).

RESULTS

Herbage DM yields at harvests 1, 2 and 3 in the 3H treatment were 5.3, 4.2 and 3.1 (total, 12.6) t DM/ha, while the corresponding values at harvests 1 - 5 within the 5H system were 2.8, 2.9, 2.0, 2.0 and 1.5 (total, 11.2) t DM/ha. The ODM content of herbage at ensiling, and the chemical composition of the silages produced, are presented in Table 1. The DM content of herbage ensiled, and of the resultant silages, varied between harvests. Silages were reasonably well fermented as indicated by the generally high concentrations of lactic acid. The one exception was harvest 5 of the 5H treatment which had a lactic acid and acetic acid concentration of 12 and 15 g/kg DM, respectively. The ammonia content of all silages was below 80 g/kg total N. Silage composition varied between harvests within each treatment, with CP content ranging from 111 to 156 g/kg DM in 3H and 140 to 189 g/kg DM in the 5H treatment. Metabolisable energy concentrations tended to decrease with later harvests, being 11.5, 10.8 and 9.8 MJ/kg DM (harvests 1 - 3) within the 3H treatment, and 11.9, 12.1, 11.3, 10.7 and 10.8 MJ/kg DM (harvests 1 - 5) within the 5H treatment. When silage composition from each harvest was 'weighted' using herbage DM yield within that harvest, mean CP, ME and NDF concentrations over all harvests were 131 g/kg DM, 10.9 MJ/kg DM and 413 g/kg DM respectively for silage within the 3H treatment, and 152 g/kg DM, 11.5 MJ/kg DM and 341 g/kg DM silage within the 5H treatment.

Mean silage DMI were 12.1, 11.6 and 10.9 kg/d (harvests 1 - 3, 3H treatment) and 14.4, 13.2, 15.6, 12.2 and 15 kg/d (harvests 1 - 5, 5H treatment), while the respective values for total DMI were 22.4, 22.0 and 17.3 kg/d, and 24.1, 23.8, 26.4, 21.2 and 20.8 kg/d (Table 2). Mean silage DMI and total DMI across all harvests was greater for cows on the 5H treatment than for those on the 3H treatment (+ 2.4 kg; P < 0.001). All intake parameters varied over time (P < 0.001), and there was a significant treatment × week interaction for both silage DMI (Figure 1a; P < 0.001) and total DMI (P < 0.001).

For harvests 1 – 3 within 3H, mean values were 37.2, 32.5 and 21.9 kg/d for milk yield, 46.6, 45.5 and 46.8 g/kg for milk fat content, 34.4, 35.4 and 35.4 g/kg for milk protein

content, 113, 119 and 158 mg/kg for MUN, 3.02, 2.62 and 1.80 kg/d for milk fat plus protein yield and 40.8, 35.3 and 24.1 kg/d for ECM yield. Similarly, within harvest 1 – 5 within the 5H system, mean values were 37.9, 37.0, 34.7, 28.8 and 24.4 kg/d for milk yield, 46.7, 47.2, 46.1, 46.8 and 47.6 g/kg for milk fat content, 35.1, 35.2, 37.0, 35.9 and 36.4 g/kg for milk protein content, 105, 108, 122, 137 and 154 mg/kg for MUN, 3.12, 3.02, 2.87, 2.37 and 2.03 kg/d for milk fat plus protein yield and 42.2, 40.7, 38.4, 31.9 and 27.2 kg/d for ECM yield.

Across the 21 wk period cows on 5H had a higher milk yield (+ 1.5 kg; P = 0.009; Figure 1 b), protein yield (+ 0.08 kg/d; P = 0.008), fat yield (+ 0.08 kg/d; P = 0.010), fat plus protein yield (+ 0.16 kg/d; P = 0.004; Figure 1e) and ECM yield (+ 2.07kg/d; P = 0.004; Figure 1f) compared to those on 3H. Fat, protein, MUN and casein content of milk was unaffected by treatment, while milk lactose was greater in 5H (P = 0.003). All milk production parameters in Table 2 varied over the time, while there was significant Treatment × Week interaction all parameters except milk fat content. Treatment had no effect on BW (average 656 kg) or BCS (average 2.7) over the experimental period, while both increased with time (P < 0.001 and P = 0.031, respectively).

Total concentrations of C4:0 – C16:0 FA in milk were unaffected by treatment (P > 0.05; Table 3). Concentrations of CLA C18:2cis-9, trans-11 (P = 0.019), total n-3 (P < 0.001) and total n-7 (P = 0.044) unsaturated fatty acids (UFA) were greater in 5H cows. There was no treatment effect on concentrations of total monounsaturated fatty acids (MUFA) or total polyunsaturated fatty acids (PUFA). Ratio of n-6 to n-3 FA was higher in cows offered 3H.

Rumen fluid pH was unaffected by treatment, while ammonia concentration in rumen fluid was greater (7.9 vs. 6.2 mg/dL; P = 0.009) within the 3H treatment (Table 3). Concentrations of VFA in rumen fluid differed between the two treatments with cows on 3H having higher acetate (66 vs. 61 mMol/L; P = 0.040) and total butyrate concentrations (17.1 vs 13.7 mMol/L; P < 0.001) compared to 5H. Acetate to propionate and acetate plus butyrate to propionate ratios where not affected by treatment. Concentrations of VFAs in rumen fluid were similar between harvests within each system (Figure 2), excepting higher acetate production in harvest 5 in the 5H system.

DISCUSSION

A key factor influencing physical and economic performance of dairy cows within grass-based systems is the nutritional value of grass silage offered. This study investigated the effect of managing grass silage crops within either a three- or a five-harvest system and the subsequent effect on cow performance at feed out.

Effect on silage yield and composition

While the 'grey' literature indicates that a multi-cut silage system (five or six harvests per year) can increase the DM yield/ha, this has not been the case under research conditions. For example, moving from a two-harvest to a four-harvest system reduced yields by 0.5t DM/ha (Ferris et al., 2003) while moving between a three-harvest to a four-harvest system reduced DM yields by 1.1 t/ha (Craig et al., 2023). Similarly in the current study, the 5H system reduced DM yields by 1.4 t/ha. This is a substantial decrease in DM yields and will have an impact on land requirements and cost of production if producers seek to adopt a multi-cut system.

The silages were well preserved as evidenced by low pH (mean 3.95), high lactic acid concentration (> 60 g/kg DM, with the exception of Harvest 5 in 5H), and low levels of ammonia-N (mean 66 g/kg DM). Despite aiming for a target herbage DM at ensiling of 280 to 300 g/kg, actual silage DM contents within this study were variable. In particular, the DM content of the 5H silages were extremely variable reflecting the difficulty in balancing the narrow-time frame for maintaining silage digestibility with favourable weather conditions for wilting. Excepting the lower CP content in Harvest 2 of both systems, there was a trend for CP content to increase as harvests progressed. This is reflective of previous work carried out at the institute (Patterson et al., 2021; Craig et al., 2023). Due to the substantial increase of CP within the 5H silages, the average total diet CP was 167 and 197 g/kg DM for the 3H and 5H systems respectively. As the growing season progressed there was a general decrease in silage D-value (D-value x 0.16 = ME content in MJ/kg DM) in the current study which also aligns with previous work (Patterson et al., Craig et al.). As expected, the decline of D-value was less marked within the 5H system, reflecting the change in NDF.

Impact on cow intakes and performance

Within the current study comparisons of intake and milk production data between individual harvests within the 3H and 5H systems are not useful, due to the

confounding effects of differences in lactation stages and the number of weeks offered each harvest. Consequently, cow performance over the entire study period was compared, although individual harvest data presented within Figures 1 a-f aid interpretation of the outcomes. The weighted ME composition of the silages produced within the 5H system was 0.65 MJ/kg DM higher than for those produced within the 3H system, which equates to an increase in D-value of 40 g/kg. According to Huhtanen et al. (2013), this increase would be expected to result in 1.1 kg/d increase in silage DMI; however, within this study silage DMI was increased by 2.4 kg/d. The difference in intake was observed early on in the study when cows were offered the primary harvest within each system; however, the larger than expected difference in intake was likely driven by the extremely dry (401 g/kg) harvest 3 in 5H. Despite the subsequent decrease in DMI when 5H cows where then offered the wettest silage (218 g/kg), intakes returned to their previous level demonstrating the benefits of increasing forage digestibility through early and more frequent harvesting.

Despite the substantial increase in silage DMI, milk yields were not affected to the same extent. While the mean difference in milk yield between 3H and 5H was 0.3 kg/d greater than the response expected (0.33 kg milk per 10 g/kg increase in D-value; Keady et al., 2013), the 1.8 kg/d increase in ECM found in the 5H treatment was as expected (0.27 kg ECM per 10g/kg increase in D-Value). Within the 5H treatment, milk yield and ECM yield did not decline to the same extent while cows were offered harvest 4 and harvest 5 compared to 3H cows offered harvest 3. This likely reflects the greater ME of the final harvests within the multi-cut system compared to the traditional 3H system.

Rinne et al. (1999) observed that for each 10 g/kg increase in silage D-value, milk protein concentration increased by 0.14 g/kg milk; however, there was no significant effect of treatment on either fat or protein content of milk in this study, which was likely due to the similar concentrations of acetate + butyrate to propionate ratio within the rumen (Sutton et al., 1988). The increase in fat yield, protein yield and fat plus protein yield was a reflection of the increase in milk yield. The increase in lactose content within the 5H treatment was likely a function of the increased energy available to the 5H cows (Osorio et al. 2016), but while this was significant the difference was small (0.6 g/kg). Total energy intake was 249 and 284 MJ/d within the 3H and 5H treatments

respectively. The increase of 1.8 kg/d of ECM would amount to an extra energy requirement of 9.4 MJ/d; however, the remaining 25.6 MJ remain unaccounted for as there was no difference in BW or BCS between the treatments. Therefore, the increased energy intake within the 5H treatment may not have been used efficiently.

Cows offered the 5H treatment had an improved milk FA profile in terms of increased concentrations of CLA (+ 0.23 of a percentage unit) and n-3 FA (+ 0.09 of a percentage unit). Both CLA and n-3 FA are of interest due to possible human health benefits (Griinari and Bauman, 1999; Ellis et al., 2006). The increase in the concentrations of n-3 FA in the 5H treatment may have been due to the increased proportion of forage in the diet (Ellis et al., 2006), as due to the increase in silage DMI, forage proportion was 60% for the 5H as opposed to 55% for the 3H treatment. The increase in n-3 FA within the 5H treatment reduced n-6 to n-3 ratio. Despite the changes in individual FA within the profile, there was no significant difference between treatments in total saturated or unsaturated FA.

Despite the higher dietary CP level of the cows offered the 5H, ammonia concentration in the rumen tended to be greater in cows offered 3H silage. The concentrate offered to both treatments was a low starch, high fibre compound; therefore, the increased fermentable energy available from the silages within the 5H treatment likely supported more rumen energy while the 3H treatment might have been short of rapidly fermentable carbohydrates. The concentration of ammonia in the rumen is determined by the production of ammonia by micro-organisms, recycling of urea and rate of passage and absorption (Keady and Mayne, 2001). Therefore, the increased concentration of rumen ammonia in the 3H treatment is possibly due to an imbalance between availability of N and energy for microbial protein synthesis.

The increased rumen acetate concentrations are likely the result of the increased fibre content of the forage fraction of the diet; however, the total NDF of the both diets was 29%. Individual harvest within system had very little effect on rumen VFA. The exception to this was harvest 5 within the 5H system, which produced greater acetate concentrations in the rumen, likely a result of the low DM and the acetic acid dominated fermentation (Keady and Mayne, 2001).

Practical implications

This study provides an opportunity to examine the overall 'system impact' of a threev. a five-harvest silage production system on a dairy enterprise. The impact of the lower herbage yield, together with the higher intakes associated with increasing the frequency of harvest should be considered before adopting a five-harvest system. The economic (margin-over-feed costs) impact of moving to a five-harvest system will be largely dictated by silage cost, concentrate cost and the value of milk produced, all of which vary considerably between countries, and from year to year. However, silage production costs (per t DM) will be higher as the number of harvests increases, as many field operations (fertiliser application, mowing, tedding, rowing, harvesting) and yard operations (filling, rolling, sealing of silos) will have to be repeated. A recent survey by Ferris et al. (2022) indicated that the vast majority of farmers in NI not only rely on contractors for silage making, but also are charged per ha irrespective of yield, thus, for many farmers there are few savings associated with the lower herbage yield at each harvest. Therefore, the increase of 1.8kg of ECM is unlikely to have improved margin-over-feed costs due to the additional cost of the five-harvest system. However, there is growing interest in multi-cut systems as they provide opportunities for improved physical performance (Craig et al., 2022) and reduction in concentrate input (Ferris et al. 2003).

Conclusion

Silage produced under a five-harvest system had higher nutritional value (ME and CP), but lower DM yields than silage produced under a three-harvest system. Cows offered silages within the five-harvest treatment has higher silage DMI and ECM yield compared to the three-harvest treatment. Cows offered silages made within a threeharvest system had greater rumen concentrations of ammonia which may indicate poor balance of fermentable energy and protein. The economic benefits of increasing harvesting frequency will be dependent on a range of factors, particularly DM yield/ha.

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	3 ha	arvest system (2	3H)	5 harvest system (5H)				
	Harvest 1	Harvest 2	Harvest 3	Harvest 1	Harvest 2	Harvest 3	Harvest 4	Harvest 5
Herbage pre-ensiling								
Oven dry matter (g/kg)	283 (24.5)	348 (30.4)	311 (28.8)	265 (13.2)	283 (13.5)	438 (25.7)	227 (12.0)	326 (16.1)
Silage								
Oven dry matter (g/kg)	243 (7.0)	340 (15.8)	293 (22.8)	241 (6.6)	251 (4.9)	401 (16.2)	218 (3.7)	308 (12.9)
VCODM (g/kg)	264 (9.2)	350 (15.0)	300 (23.0)	267 (5.8)	267 (5.3)	414 (16.1)	226 (3.5)	317 (14.5)
Crude protein (g/kg DM)	132 (4.3)	111 (11.7)	156 (4.6)	144 (4.1)	140 (5.5)	146 (11.4)	158 (1.1)	189 (10.9)
Gross energy (MJ/kg DM)	18.6 (0.55)	17.6 (1.97)	17.9 (0.66)	18.3 (0.51)	18.5 (0.59)	18.0 (1.12)	19.1 (1.27)	18.3 (0.62)
Metabolisable energy (MJ/kg DM)	11.5 (0.13)	10.8 (0.19)	9.8 (0.10)	11.9 (0.25)	12.1 (0.21)	11.3 (0.15)	10.7 (0.21)	10.8 (0.29)
pH	3.8 (0.04)	4.0 (0.04)	4.1 (0.05)	3.9 (0.04)	3.7 (0.04)	4.2 (0.03)	3.8 (0.04)	4.1 (0.05)
Lactic acid (g/kg DM)	142 (16.8)	78 (7.6)	94 (12.4)	108 (15.7)	158 (17.7)	60 (6.0)	127 (10.5)	121 (2.6)
Acetic acid (g/kg DM)	14.6 (2.7)	38 (9.1)	14.9 (4.6)	11 (1.5)	17 (1.2)	13 (2.1)	14 (1.6)	15 (3.7)
Ethanol (g/kg DM)	54.4 (14.4)	4.1 (1.0)	2.7 (0.2)	79.8 (10.8)	35.2 (10.6)	17.8 (3.5)	10.9 (1.0)	6.2 (3.5)
Ammonia (g/kg total N)	65 (7.1)	63 (3.3)	78 (10.0)	69 (6.9)	57 (4.5)	49 (1.0)	77 (0.7)	68 (3.1)

Table 1: Dry matter of herbage at ensiling, and the chemical composition (standard deviation in parenthesis) of silages -produced

within a three- (3H) or five-harvest (5H) system, as offered

VCODM, volatile corrected oven dry matter

	Trea					
-	3 Harvest System (3H)	5 Harvest System (5H)	SED	Treatment	Week	Treatment × Week
Silage DMI (kg/day)	11.7	14.1	0.40	< 0.001	< 0.001	< 0.001
Total DMI (kg/day)	21.1	23.4	0.44	< 0.001	< 0.001	< 0.001
Milk yield (kg/day)	31.9	33.5	1.02	0.037	< 0.001	< 0.001
Fat (g/kg)	46.3	46.7	0.88	0.393	< 0.001	0.588
Protein (g/kg)	35.0	35.8	0.78	0.273	< 0.001	< 0.001
Lactose (g/kg)	47.4	48.0	0.29	0.003	< 0.001	< 0.001
Urea (mg/kg)	126	122	3.5	0.123	< 0.001	< 0.001
Casein (g/kg)	27.6	28.2	0.52	0.150	< 0.001	< 0.001
Fat yield (kg/day)	1.47	1.56	0.040	0.010	< 0.001	0.029
Protein yield (kg/day)	1.11	1.19	0.034	0.008	< 0.001	< 0.001
Fat plus protein yield (kg/day)	2.59	2.74	0.068	0.004	< 0.001	< 0.001
Milk gross energy (MJ/kg)	3.39	3.44	0.042	0.111	< 0.001	0.327
Energy Corrected Milk (kg/day)	35.6	37.4	0.94	0.007	< 0.001	< 0.001
Bodyweight (kg)	655	657	19.9	0.991	< 0.001	0.351
Body condition score	2.7	2.7	0.10	0.747	0.031	0.974

Table 2: Effect of offering silage made within either a three- (3H) or five- harvest (5H) system on average daily intakes, milk production and composition and body tissue reserves.

DMI, dry matter intake

	3 Harvest System (3H)	5 Harvest System (5H)	SEM	P Value
Total C4:0 to C16:0	63.5	63.1	0.97	0.793
C18:0	10.3	10.1	0.32	0.734
C18:1cis-9	0.7	0.8	0.04	0.642
CLA, 18:2cis-9, trans-11	0.5	0.6	0.03	0.019
C18:2cis-9,12	1.7	1.6	0.05	0.539
C20:0	0.1	0.1	0.01	0.108
Total Saturated	73.5	73.0	0.68	0.639
Total MUFA	21.9	21.9	0.55	0.946
Total PUFA	2.6	2.6	0.07	0.551
Total n-3 UFA	0.6	0.7	0.02	< 0.001
Total n-6 UFA	2.0	1.9	0.06	0.709
Total n-7 UFA	1.9	2.1	0.06	0.044
Total n-9 UFA	18.8	18.6	0.55	0.881
Ratio n-6:n-3	3.3	2.8	0.04	< 0.001
Total Saturated:Unsaturated	3.1	3.0	0.12	0.598

Table 3: Effect of offering silage made within either a three- (3H) or five- harvest (5H) system on milk fatty acid concentration (% total FA identified; weighted mean across all harvests).

FA, fatty acids; MUFA, monounsaturated fatty acids; PUFA, poly-unsaturated fatty acids; UFA, unsaturated fatty acids

	3 Harvest System (3H)	5 Harvest System (5H)	SED	P Value
pH	7.0	7.0	0.16	0.607
NH ₃ -N (mg/dL)	7.9	6.2	0.62	0.009
Individual VFA (mMol/L)				
Acetate	65.6	61.0	2.19	0.040
Propionate	21.8	19.8	1.03	0.071
i-butyrate	0.9	1.0	0.03	0.737
n-butyrate	16.2	12.7	0.66	< 0.001
Total butyrate	17.1	13.7	0.68	< 0.001
i-valerate	1.7	2.1	0.149	0.004
n-valerate	1.8	1.7	0.10	0.308
Total valerate	3.5	3.9	0.18	0.057
Acetate:Propionate	3.1	3.2	0.12	0.278
Acetat+Butyrate:Propionate	3.9	3.9	0.13	0.783

Table 4: Effect of offering silage made within either a three- (3H) or five- harvest (5H) system on rumen fluid pH, ammonia nitrogen concentrations, and volatile fatty acid concentrations (VFA) (weighted mean across all harvests).





Figure 1. Mean weekly silage matter intake (kg/d) (a), milk yield (kg/d) (b), milk fat content (g/kg) (c), milk protein content g/kg (d), milk urea content (mg/kg) (e) and fat plus protein yield (kg/d) (f) of cows offered silages produced within either a three- (solid line) or five-harvest (dashed line) system¹

¹Arrows indicate when cows changed to harvests 2 and 3 (3H; black arrow) and harvests 2, 3, 4 and 5 (5H; grey arrows).



Figure 2: Impact of individual silage harvest on rumen fermentation within a three-harvest (a) or five-harvest system (b)

Chapter 7

Supplementation strategies for lactating dairy cows offered very high quality grass silages: starch-based or fibre-based concentrates offered with or without straw
Introduction

Achieving high nutrient intakes is a key objective in the management of high yielding dairy cows. For housed cows managed within grassland based production systems, this can be achieved by improving the quality of the grass silage component of the diet, and/or increasing concentrate feed levels (Ferris et al., 1997; 2001). The benefits of increasing silage quality are well known, with a review by Keady et al. (2013) indicating that for each 10 g/kg increase in silage dry matter (DM) digestibility, DM intake (DMI) and milk yield are increased by 0.22 kg/day and 0.33 kg/day, respectively. In addition, the concentrate sparing effects of higher quality silages have been clearly demonstrated (Ferris et al., 2003).

A recent survey of silage making practices in Northern Ireland (NI) demonstrated that while 22.4% and 64.9% of farmers still adopt either a two or three harvest silage production system, a significant number (12.7 %) now adopt a 'multi-harvest' system (four or more harvests) in an attempt to improve silage feed value (Ferris et al., 2019). While anecdotal evidence indicates that the adoption of multi-harvest systems is increasing, concerns are often raised that highly digestible silages are not utilised efficiently by dairy cows. Earlier or more frequent harvesting reduces the fibre concentration of silages (Kuoppala et al., 2008; Randby et al., 2012), and the reduction in fibre could have a negative impact on rumen function and digestive efficiency (Mertens, 1997). This situation may be exacerbated if cows offered very high quality silages are supplemented with high levels of starch-based concentrates which may depress rumen pH, leading to acidosis, a reduction in fibre digestibility and decreased intakes (Martin et al., 1994: Keady et al., 1999). The compromised rumen function associated with high starch concentrates has been shown to reduce milk fat concentrations on both grass silage based diets (Keady et al., 1998; 1999) and grazed grass based diets (Sayers et al., 2003). Similarly, Boerman et al. (2015) offered a high quality maize silage based diet to high yielding cows (46 kg milk/cow/day), and found milk fat content and fat corrected milk yield to be reduced by 3.7 g/kg and 1.5 kg/day, respectively, when a starch-based concentrate was offered compared to a fibre-based concentrate. As a consequence, supplementing very high quality silages with more fibrous concentrates is often advocated. However, there are benefits of offering starch-based concentrates, including: increased milk protein concentrations (Keady et al., 1998), milk yields and DMIs (Boerman et al., 2015).

The addition of chopped straw to the diet of high yielding cows offered high quality silage is often advocated in the UK and Ireland to combat the negative effects of the lower fibre content of the silage. Straw inclusion in the diet is associated with increased retention time of digesta in the rumen (Nandra et al., 1993), which may allow other feed components to be more efficiently digested and absorbed. Neutral detergent fibre (NDF) is associated with chewing activity, increased cudding, and increased saliva production which in turn helps stabilise rumen pH (Welch and Smith, 1970). On the other hand, straw inclusion can reduce total DMI due to the high concentration of slowly fermentable carbohydrate (Van Soest, 1975). Indeed, there is little evidence that improvements in animal performance can be achieved by incorporating straw into the diets of dairy cows (Brown et al., 1990; Ferris et al., 2000), while high levels of straw inclusion (>1 kg/head/day) have been found to reduce animal performance due to dilution of the ME concentration of the diet (Ferris et al., 2000).

To date, no studies appear to have examined the interaction between concentrate type and straw inclusion in high quality grass silage based diets. In addition, given that modern dairy cow rationing programmes can account for fermentable energy and protein, the effectiveness of the fibre content of the diet, and predict the acid load in the rumen, it may be possible to design starch-based concentrates that can be offered as a supplement to a very high quality grass silage, without negative effects on rumen function, while still delivering the benefits of starch-based concentrates. Consequently, the current study was designed to examine the effect of concentrate type (starchbased or fibre-based), and straw inclusion (straw or no straw), on cow performance and nutrient utilisation, when offered alongside a very high quality grass silage.

Materials and methods

This study was conducted at the Agri-Food and Biosciences Institute (AFBI), Hillsborough, Northern Ireland. All experimental procedures were conducted under an experimental licence granted by the Department of Health, Social Services & Public Safety for Northern Ireland in accordance with the Animals (Scientific Procedures) Act 1986.

Animals and housing

Twenty-four mid-lactation (mean of 149 (s.d. 52) days calved) multiparous (mean lactation number 3.8 (s.d. 1.2)) Holstein-Friesian dairy cows were used in a threeperiod, each of four weeks duration, partially balanced change-over design experiment involving four treatments. Each four-week period consisted of a 21 day feed adaption period, and a seven day measurement phase. Cows were blocked according to preexperimental milk fat + protein yield into six blocks, each of four cows, and cows within each block randomly allocated to one of the four treatments. Cows had a mean preexperimental milk yield and body weight (BW) of 37.3 (s.d. 5.4) kg per day, and 633 (s.d. 53.0) kg, respectively.

For the two week period prior to the study commencing, cows were offered a nonexperimental grass silage supplemented with approximately 10 kg concentrate per day. Approximately half of the concentrate was offered mixed with the silage using a diet feeder, and half offered via an out-of-parlour feeding system (OPF). Three days prior to the start of the study, concentrates were removed from the OPF, with the full concentrate allocation mixed with the silage in the form of a total mixed ration (TMR) comprising 43% concentrate and 57% forage on a DM basis.

Throughout the 12 week experimental period cows were housed in a free-stall house with concrete flooring, and had access to individual cubicles that were fitted with rubber mats and bedded with sawdust. The cubicle-to-cow ratio was \geq 1:1 at all times, thus meeting the recommendations of FAWC (1997). The floor area was cleaned every 3 hours using an automated scraper system.

Treatments

The four treatments were organised in a 2 × 2 factorial arrangement, comprising two concentrate types (High-starch or High-fibre) and two levels of straw inclusion (Straw or No-straw). A high quality grass silage was offered throughout the study (volatile corrected oven DM, 418 g/kg; CP, 170 g/kg DM (CP = N × 6.25); ME, 12.1 MJ/kg DM). The silage was produced from a perennial ryegrass (*Lolium perenne*) based sward. Grass was harvested using a precision-chop harvester on 3rd May 2017, following a 24 hour period of field wilting. Grass was treated at harvest with a bacterial inoculant

(ULV50, Biotal, Malvern, UK) at approximately 20 ml per tonne of fresh herbage, before being ensiled in a bunker silo.

With the No-straw treatments, grass silage and concentrates were offered in the form of a total mixed ration (TMR) comprising 57% silage and 43% concentrate, on a DM basis. Concentrates were formulated and total rations balanced using NutriOpt (Nutreco, Amersfoort, Netherlands) dairy cow rationing software. While the two concentrates differed in NDF and starch content, they had a similar ME and CP content. The total rations were designed to promote rumen function and nutrient utilisation, and took account of a number of parameters, including acid load, structural fibre content and fermentable energy and protein balance. This approach was taken to reduce the common confounding factors encountered when comparing fibre and starch diets. The ingredient composition of the two concentrates is presented in Table 1.

With the Straw treatments, chopped barley straw was included in the diet at 4% of total DM, replacing part of the grass silage component of the diet. Straw was chopped with a Kverneland 850 bale chopper (Klepp, Norway) to a nominal chop length of approximately 5 cm (hand separation of a 10 g sub sample indicated that 5.6, 35.4, 20.9, 12.7, 9.5 and 6.4% of straw by weight had chop lengths of < 2 cm, 2 - 3 cm, 3 - 5 cm, 5 - 7 cm, 7 - 9 cm, 9 - 15 cm and > 15 cm, respectively).

The rations were prepared daily at approximately 09.00 hours, and offered *ad libitum* at 107% of the previous day's intake. Uneaten ration was removed the following day at approximately 08.00 hours. Rations were prepared using a mixer wagon (Vari-Cut 12, Redrock, Armagh, NI). The total quantity of silage required for all four treatments was initially mixed for approximately five minutes and then deposited on a clean silo floor. The quantity of silage required for each individual treatment was then removed from this 'pile' in turn, placed back in the mixer wagon, and the appropriate quantities of the concentrate and straw added to the mix, and mixing continued for another five minutes. The rations were then transferred from the mixer wagon to a series of feed boxes mounted on weigh scales, with cows accessing food in these boxes via an electronic identification system, thus enabling individual cow intakes to be recorded

daily (Bio-Control Feeding System, Bio-Control, Rakkestad, Norway). Cows had free access to fresh water at all times.

Cow measurements

Feed intakes were measured as described above. All cows were milked twice daily (between 06.00 and 08.00 hours and between 15.00 and 17.00 hours) throughout the experiment using a 50-point rotary milking parlour (Boumatic, Madison, WI, USA). Milk yields were automatically recorded at each milking, and a total daily milk yield for each cow for each 24 hour period calculated. Milk samples were taken during four consecutive milkings at the end of the fourth week of each period, treated with a preservative tablet (lactab Mark III, Thompson and Cooper Ltd., Runcorn, UK), and stored at 4°C until analysed (normally within 48 hours). Milk samples were analysed for fat, protein and lactose concentrations using an infrared milk analyser (Milkoscan Combifoss[™]7; Foss Electric, Hillerød, Denmark), and a weighted concentration of each constituent determined for each 24 hour sampling period. A mean composition over the two day sampling period was subsequently calculated for each cow.

A further milk sample was taken, in proportion to milk yield, during two successive milkings at the end of the final week of each experimental period. Samples were analysed for milk fatty acids (FA), as follows: milk fat was extracted from 1.0 ml of homogenised milk using a chloroform methanol extraction method (Bligh and Dyer 1959), and FA determined as methyl esters (FAME). The FA composition was determined using gas-liquid chromatography, with an aliquot (1.0 ul) of the FAME extract injected onto a CP Sil88 capillary column (100 meters x 0.25 mm id x 0.2 µm film thickness) in a Agilent 7890 gas chromatograph (both Agilent Technologies, Santa Clara, USA), equipped with a temperature programmable injector operated in the split mode and a flame ionisation detector. The oven was initially held at 50°C for 4 minutes then ramped at 8°C/min to 110°C, then 5°C/min to 170°C (hold time 10 min) and finally ramped at 2°C/min to 225 °C (hold time 30 min). Fatty acids were identified by their retention time with reference to commercially available FA standards (37 Supelco FAME mix) and individual standards for those not in the mix (SigmaAldrich Co. Ltd., Gillingham, UK), and were quantified using C13 FAME as an internal standard.

Body weight was recorded twice daily during the final week of each experimental period (immediately after each milking) using an automated weighbridge, and a mean BW for each cow determined. The body condition score (BCS) of each cow was estimated by a trained technician at the end of the fourth week of each period, according to Edmonson et al. (1989) on a 5 point (including quarter points) scale. Blood samples were collected from the coccygeal vein of each cow prior to feeding at the end of the fourth week of each period, and centrifuged (3000 rpm for 15 minutes) to isolate either the serum (tubes with a clot activator) or the plasma (fluoride oxalate tubes). Serum beta-hydroxybutyrate (β HB) concentrations were determined according to McMurray et al. (1984), and plasma glucose concentrations were determined using the hexokinase method (Roche Diagnostics Ltd.). Serum non-esterified fatty acid (NEFA) concentrations were determined using WaKo (Wakop Chemicals GmbH, Neuss, Germany) kits. Serum urea concentrations were analysed using the Kinetic UV method (Roche Diagnostics Ltd., Burgess Hill, UK).

Faecal scores were assessed weekly during the experiment. Scoring was undertaken at a consistent time (prior to morning feeding) when cows were lying, and then compelled to rise. Scoring was on a scale of 1 - 5 as follows: 1) very watery 2) thin; when the faeces lands the 'splatter' goes a long way 3) ideal; forming a cowpat to a height of 2-3 cm 4) thick; well-formed and stacked in rings or 5) firm; stiff balls of faeces (Hulsen et al., 2006).

Nutrient utilisation

On completion of the 12 week feeding study, four cows from each treatment (n = 16) were selected for use in a nutrient utilisation study. Cows were selected from each treatment group, with selected cows balanced for daily milk yield and BW. Cows were tied by the neck in individual stalls, with stalls fitted with a rubber mat. Cows continued to access their experimental rations from feed boxes at the front of each stall. Experimental rations were offered *ad libitum* daily at 09:00 hours (+10% of previous day's intake). Uneaten food was removed the following day at 08:00 hours. Cows had access to fresh water at all times via a drinker located within each stall.

Measurement of nutrient utilisation commenced 24 hours after cows were placed in this experimental byre, with a six-day total faeces and urine collection period. Faeces were collected in a plastic collection tray (96 cm ×108 cm × 36 cm) placed behind each cow. Urine was collected into a 25 litre plastic container via a flexible plastic tube which was attached to a urine separation system. This was held in position over the vulva by attaching it using Velcro fasteners to a 'patch' which was glued (Bostik, Paris, France) either side of the cow's tail head. Approximately 300 ml of 50% sulphuric acid was added to each urine collection container daily to reduce ammonia losses. The total weight of faeces and urine produced during each 24 hour collection period was recorded, and a sample of each (5% by weight) retained for subsequent analysis. Faeces and urine samples were stored in a fridge (< 4°C) and bulked on day 3 (day 1 - 3) and day 6 (day 4 - 6). During the nutrient utilisation study, cows were milked twice daily (06.30 and 16:30 hours) within the experimental cow byre. During this time milk samples were taken at each milking, bulked in proportion to yield for days 1 - 6, and subsequently analysed for gross energy (GE), and nitrogen (N) concentrations. Each bulked urine and milk sample was analysed for N concentrations, while a further sample of each was freeze-dried (Heto Lyolab 3000, Fisher Scientific, Loughborough, Leicestershire, UK) and analysed for GE concentrations using a bomb calorimeter (Parr 6400 Bomb Calorimeter, Moline, IL, USA). Similarly, a sample of the bulked faeces sample for each cow was analysed for N concentrations (fresh basis), while a subsample was dried at 85°C for 72 hours, and the dry sample analysed for acid detergent fibre (ADF), ash and GE concentrations.

Feed analysis

A sample of the grass silage offered was taken daily throughout the experiment and dried at 85°C for 18 hours to determine oven DM content. Twice a week a sample of grass silage was dried at 60°C and dried samples bulked for each 14 day period, with the bulked sample milled through a sieve with 0.8 mm aperture, and analysed for NDF, ADF and ash concentrations. Each week a fresh silage sample was analysed using near infrared reflectance spectroscopy (NIRS) for ME concentration according to Park et al. (1998). A further fresh silage sample was taken weekly and analysed for GE, N, pH, ammonia-N and volatile components. A sample of straw and each concentrate was taken weekly, and one sub-sample dried at 85°C for 24 hours to determine oven DM content. An additional sub-sample was dried at 60°C for 48 hours, bulked for each 14 day period, milled through a 0.8 mm sieve, and subsequently analysed for N, NDF, ADF, ash and GE. The concentrates were also analysed for starch concentrations.

During the nutrient digestibility study, feed stuffs where analysed for the same chemical components as during the main study. Silages were sampled daily and analysed for oven DM (85°C), with fresh samples analysed for GE, N, pH, ammonia-N and volatiles. Dried samples were bulked for each 3-day period (day 1-3 and day 4-6), and subsequently analysed for ADF, NDF and ash concentrations. A sample of straw and each concentrate type offered during each nutrient digestibility study were sampled and analysed for oven DM (85°C). A further sample was dried at 60°C and subsequently analysed for GE, NDF, ADF, N, and ash concentrations. The concentrates were also analysed for starch concentrations. A sample of ration refused by each cow was taken daily and analysed for oven DM content. All chemical analysis of the feed stuffs offered where undertaken as described by Purcell et al. (2016).

Statistical analysis

Two cows did not complete period three due to health reasons (mastitis and oedema of the udder) and where subsequently treated as missing values during period three in the statistical analysis. Animal data recorded during the final week of each experimental period (DMI, milk yield, milk composition, BW, BCS, blood metabolites and faecal scores) were analysed using linear mixed model methodology according to the three-period change over experimental design, with constant + treatments as the fixed model, and block + block × cow + block × period as the random model. In all cases the method of residual maximum likelihood (REML) was used as the estimation method. One cow was removed from the nutrient utilisation study due to mastitis. Data from the nutrient utilisation method. Period was fitted as a random effect and a factorial arrangement of Concentrate and Straw were fitted as fixed effects. If any of the fixed effects were significant (P<0.05) then Fisher's LSD Test was used to compare individual levels of the effects. All data were analysed using GenStat (18.1; VSN International Limited, Oxford, UK).

Results

The term 'high quality silage' in this paper encompasses both the intake potential of the silage and its nutritive value. The silage offered had a DM of 418 g/kg, a CP of 170 g/kg DM, and a predicted ME content of 12.1 MJ/kg DM. The High-starch and High-

fibre concentrates had a similar CP and gross energy content, but differed in NDF (258 v. 339 g/kg DM) and starch (373 vs 237 g/kg DM) contents, as planned (Table 2).

Cow Performance

There were no interactions between concentrate type and straw inclusion for any of the parameters in Table 3, and as such only the main effects of treatment are presented. Both silage DMI and total DMI were reduced with the High-fibre concentrate (P = 0.001 and P = 0.006, respectively) and with straw inclusion in the diet (P < 0.001 and P = 0.014, respectively).

Neither concentrate type, nor straw inclusion had an effect on milk yield or milk fat content (P > 0.05) which averaged 33.1 kg/d and 45.0 g/kg respectively (Table 3). Cows offered the High-starch concentrate had a higher milk protein content than those offered the High-fibre concentrate (P < 0.001), while straw inclusion resulted in a reduction of milk protein content (P = 0.036). However, neither concentrate type nor straw inclusion had a significant effect on fat yield, protein yield, or fat + protein yield (P > 0.05).

The FA profile of the milk produced was unaffected by concentrate type, with the exception of total concentrations of C4:0 - C15:0 (greater in the High-starch treatment, P = 0.004), C16:0 concentrations (greater in the High-fibre treatment, P = 0.037) and conjugated linoleic acid (CLA; greater in the High-fibre treatment, P < 0.001). Concentrations of total saturated fatty acids (SFA), monounsaturated fatty acids (MUFA) and polyunsaturated fatty acids (PUFA) were unaffected by concentrate type (Table 3). Straw inclusion decreased total C4:0 - C15:0 concentrations (P < 0.001) and C16:0 concentrations (P = 0.002), but increased concentrations of C18:0 (P < 0.001), C20:0 (P < 0.001) and total n-9 PUFA (P < 0.001); however, there was no effect of straw inclusion on CLA concentrations. Straw inclusion reduced the concentration of SFA in milk and increased total MUFA concentrations (P < 0.001) compared to the No-straw treatments, with a consequent reduction in the Saturated:Unsaturated FA ratio (P < 0.001) in milk.

Treatment had no effect on either cow BW or BCS (Table 3; P > 0.05). Serum concentrations of β HB and NEFA, and plasma concentrations of glucose, did not differ

significantly between treatments (average 0.43 mM, 0.12 meq/L and 3.61 mM, respectively: Table 4). Cows offered the High-fibre concentrate had an increased serum urea content compared to those offered the High-starch concentrate (P < 0.001). Straw inclusion tended to decrease serum urea concentrations (P = 0.053). There was an interaction between concentrate type and straw inclusion for serum urea, with mean values for High-starch/No-straw, High-starch/Straw, High-fibre/No-straw and High-fibre/Straw being 3.13, 2.53, 3.16, 3.37 mM, respectively (SED = 0.138; P < 0.001). Serum urea was higher when straw was offered with the High-fibre concentrate (P < 0.001). Diet had no effect on mean faecal scores (average 2.6; s.d., 0.31).

Nutrient Utilisation

There were no significant interactions (P > 0.05) between concentrate type and straw inclusion in the diet for any of the nutrient utilisation parameters presented in Tables 5, 6 or 7, and consequently only the main effects of treatment are presented. Neither total DMI nor milk yields differed between treatments within the sub-group of cows used in the nutrient utilisation study (P > 0.05). Similarly, none of the digestibility coefficients examined were affected by treatment (Table 5).

Neither total N intake, nor N output in faeces, urine, manure or milk, were affected by concentrate type (P > 0.05; Table 6). When straw was included in the diet, cows had a lower N intake (P = 0.009) and a lower faecal N output (P = 0.028) compared to cows on the No-Straw treatment. None of the N use coefficients were affected by either concentrate type or straw inclusion in the diet (P > 0.05).

Neither GE intake, nor energy output in faeces, urine or milk were affected by treatment (Table 7). However, there was a trend (P = 0.050) for urinary energy output to be reduced when straw was included in the diet. None of the energy use coefficients were affected by either concentrate type or straw inclusion in the diet (P > 0.05).

Discussion

Grass silage is a major forage source for dairy cows in the more western parts of the UK and Ireland. In NI the average DM, CP and ME contents of commercial farm silages analysed by AFBI between 1996 - 2015 (n > 90,000 silages) were 280 g/kg, 123 g/kg

DM and 10.7 MJ/kg DM, respectively (Yan et al., 2017). Thus the silage used in the current study (DM, 418 g/kg; CP, 170 g/kg DM; ME, 12.1 MJ/kg DM, Table 2) was of a much higher quality than the NI average, reflecting the early cutting date and rapid field wilting. The silage was also well fermented, as indicated by its low ammonia-N content and lactate dominated fermentation.

Within NI there has been an increasing move to multi-cut systems (>3 cuts/year) in an attempt to improve the quality of silages produced (Ferris et al., 2019), and consequently high quality silages, such as the one used in the current study, are likely to become more common on NI farms. This study was designed to examine supplementation strategies for high quality silages, to ensure optimum performance and high levels of nutrient use efficiency. On many farms current practice is to supplement very high quality silages with a fibre-based concentrate, or to add straw to the diet to help 'maintain rumen function', and thus reduce the likelihood of digestive upset or metabolic diseases. Within the current study there was no interaction between concentrate type and straw inclusion for any of the parameters examined (except for plasma urea), and as such concentrate type and straw inclusion are discussed separately.

Silage intakes in the current study were higher than those recorded in many previous studies (Rinne et al., 1999; Dewhurst et al., 2003; McNamee et al., 2015), although comparable intakes to those observed in the current study have been recorded by Randby et al. (2012) and Kuoppala et al. (2008) with highly digestible silages. Both digestibility (Huhtanen et al., 2007; Steen et al., 1998) and DM content (Steen et al., 1998) are key determinants of silage DMI. Steen et al. (1998) also found a positive correlation between silage protein concentration and silage DMI. Therefore, the very high intakes observed in this study are likely attributable to the high DM, CP and digestibility of the silage offered.

Effect of concentrate type

The impact of concentrate type on DMI has not been consistent. For example, Aston et al. (1994) and Huhtanen et al. (2008) found DMI to increase as the fibre concentrations of the concentrate increased, while Keady and Mayne (2001) found no effect of either a starch- or fibre-based concentrate on DMI. The reduction in DMI

when high starch diets are offered is frequently associated with a depression in rumen pH, which may reflect subacute acidosis, a consequence of high levels of rapidly fermentable carbohydrates with some starch-based diets (Martin et al., 1994). However, in the current study DMI was 0.8 kg DM/day lower when the High-fibre concentrate was offered (Table 3).

The higher DMI with the starch-based diet is likely to reflect, in part, the fact that the High-starch concentrate offered was formulated using NutriOpt (Nutreco, Amersfoort, Netherlands) to optimise rumen health by taking parameters such as 'acid load' and 'structural fibre index' into consideration. The 'acid load' parameter within the NutriOpt rationing programme is calculated based on total fermentation products, which includes both volatile fatty acid (VFA) production in the rumen and silage fermentation products (e.g. lactic acid) consumed from the diet. The 'structural fibre index' takes into account the effectiveness of dietary fibre to promote rumination. An 'acid load' of less than 50 units and a 'structural fibre index' of greater than 100 units is considered ideal for rumen health when both parameters are considered together. The High-fibre and High-starch diets had a predicted acid load of 47 and 50, respectively, and a 'structural fibre index' of 108 and 104, respectively. Rations were also formulated taking account of 'rumen unsaturated fatty acid load (RUFAL)'. Rumen fermentation is influenced by RUFAL, which is determined as the sum C18:1, C18:2 and C18:3 FA. In a review, Walker et al. (2004) indicated that these FA are associated with disruption to rumen fermentation and with milk fat depression. Based on NutriOpt, the Highstarch and High-fibre diets were predicted to contain 21 and 20 g/kg DM RUFAL, respectively, with these values below the maximum recommended level of 25 g/kg DM (NutriOpt). The absence of effects of concentrate type on faecal scores, and on any of the digestibility and nutrient utilisation efficiency coefficients suggest both concentrate types were associated with good rumen health. The reduction in DMI with the High-fibre concentrate in the current study may have been due to increased rumen fill causing greater satiety (Allen, 1995).

While concentrate type had no effect on milk yield, milk protein content was reduced by 0.8 g/kg when the High-fibre concentrate was offered (Table 3). A similar reduction in milk protein content with fibre-based concentrates has been observed previously with grass silage based diets (Ferris et al., 2000) and grazed grass based diets (Sayers et al., 2003; Gordon et al., 1995). The reduction in milk protein concentration with the High-fibre concentrate treatments is likely related to the lower DMI with this treatment, combined with increased rumen propionate production (Rook, 1979), and increased microbial protein synthesis (Sayers et al., 2003) in the High-starch treatment.

While starch-based concentrates are often associated with a reduction in milk fat concentrations (Keady et al., 1998; 1999), no such effect was observed in the current study. While this may appear to be surprising given the difference in concentrate fibre and starch levels, it likely reflects the fact that both diets were formulated to have similar 'structural fibre indexes'. Although milk fat content was unaffected by treatment, the milk FA profile differed (Table 3). De novo synthesis of FA (C4:0 - C15:0) was greater (0.8 g/100 g total FA) in the High-starch treatments compared to the High-fibre treatments, with these FA largely synthesised by chain elongation using acetate, which is driven by fibre in the diet (Grummer, 1991). Therefore, it might be expected that the High-fibre diet would have increased the synthesis of C4:0 - C15:0 FAs, as found previously (Boerman et al., 2015). While the increase in total C4:0 - C15:0 FA in the High-starch concentrate treatments is unexplained, the actual differences between treatments were relatively small. However, C16:0, which is partly synthesised *de novo* in the mammary glands was greater with the High-fibre diet (0.5 g/100 g total FA). Concentrations of CLA were greater (0.03 g/100 g total FA) when the High-fibre diet was offered. Conjugated linoleic acid is of interest due to possible human health benefits and is formed by the biohyrogenation of dietary linoleic acid (Griinari and Bauman, 1999). Despite the changes in individual FA within the profile, there was no significant difference in total saturated or unsaturated FA when cows where offered either a High-starch or a High-fibre concentrate.

That concentrate type had no effect on cow BW, BCS (Table 3), and blood metabolites (β HB, Glucose and NEFA, Table 4)) recorded during each measurement period, suggests cows had a similar energy status. Cows gained 94 kg BW (s.d. 24.7 kg) and 0.1 (s.d. 0.11) units of BCS over the 12 week experimental period. While part of the former will can be attributed to 'gut-fill' associated with the very high silage DMI, cows were undoubtedly in positive energy balance throughout the study, a reflection of the high DMI observed. The higher serum urea concentrations observed in cows offered

the High-fibre concentrate, compared to the High-starch concentrate, occurred despite the two concentrates having similar CP concentrations, and may reflect the High-fibre diet providing less readily fermentable energy to support microbial growth to utilise rumen ammonia. Nevertheless, the nutrient utilisation study provided no evidence that concentrate treatments impacted on N utilisation efficiency, or indeed on energy utilisation efficiency (Table 6 and 7).

Again, literature evidence on the impact of concentrate type on nutrient utilisation is mixed. For example, some studies indicate increased apparent diet digestibility when high starch concentrates are offered (Aston et al., 1994). Keady et al. (1999) reported that fibre digestibility was reduced with increased starch content of the concentrate, the latter likely due to a reduction of cellulolytic activity. The absence of an effect on fibre digestibility in the current study may be due to the fact that the diet was offered as a TMR. Supporting this suggestion, Keady et al. (1998) found no effect of starch level on fibre digestibility when concentrates where offered in small amounts during the day. Furthermore, the apparent digestibility of ADF was lower in the previous studies than the current study where more easily digested as a whole.

Effect of straw inclusion

Straw inclusion reduced total DMI by 0.7 kg/day (Table 3). The inclusion of straw in the diet will increase rumen retention time and reduce the rate of passage of digesta through the digestive tract leading to satiety and reduced DMI (Nandra et al., 1993). Despite the reduction in DMI, milk yield was not significantly affected by straw inclusion, although milk protein content was reduced by 0.4 g/kg with the straw treatments (Table 3). The latter is likely due to the dilution of ME in the diet when straw is included, in agreement with previous studies (Blair et al., 1974; Ferris et al., 2000). Milk fat content was unaffected by straw inclusion to the diet, which agrees with the findings of Ferris et al. (2000), who offered straw at levels between 0 - 3 kg/cow/d. In contrast, Owen et al. (1969) and Blair et al. (1974) observed an increase in milk fat content with the addition of milled straw to the diet (at 24% and 47.5% of the total diet); however, the overall diets offered and straw inclusion levels adopted were very different from those in the current study. The concentrates offered in the current study were balanced to contain optimum levels of structural fibre, and this may have negated

any possible effects of straw inclusion on milk fat. Although milk yield was unaffected by straw inclusion, both milk fat yield and milk protein were reduced, with this due to the numerically lower milk yield (0.8 kg/d) and milk fat content (0.5 g/kg), and significantly lower protein content (0.4 g/kg) with the straw treatment.

As straw inclusion was expected to promote rumen acetate production, and *de novo* FA synthesis, the increasing concentrations of C4:0 - C15:0, C16:0 with the No-straw treatment was unexpected (Table 3). The C18:0, C18:1 and C20:0 fats in milk are mostly derived from stearic acid in the diet (Moate et al., 2008), and their higher concentrations with the straw treatment reflects the fact that straw contains a high proportion of stearic acid (42% of total FA; Tyagi et al., 2010). In general, straw inclusion resulted in a small improvement in the fatty acid profile of the milk which could be considered as beneficial concerning human health (Vafeiadou et al., 2015), as the concentrations of SFA decreased and concentrations of MUFA increased.

There was no effect of straw inclusion on BW or BCS (Table 3), while serum β HB and NEFA, and plasma glucose concentrations, all of which can provide an indication of energy status, were also unaffected (Table 4). The tendency (P = 0.053) for a reduction in serum urea concentration when cows were offered straw reflects the dilution of total diet protein content associated with straw. However, the interaction between concentrate type and straw inclusion suggests that a starch-based concentrate promoted a rumen environment that was more effective at utilising rumen ammonia, while the reverse occurred when straw was offered alongside a fibre-based concentrate.

Surprisingly, straw inclusion had no effect on faecal scores or digestive efficiency during the nutrient utilisation study. Ferris et al. (2000) observed that the inclusion of increasing levels of straw in the diet actually decreased the digestibility of DM, N and energy, although the highest inclusion level in that study, was considerably higher than in the current study (3 kg/cow/d). While straw inclusion may have been expected to improve nutrient utilisation by stabilising the rumen environment and reducing the rate of passage of digesta, nutrient utilisation was not improved in either the study by Ferris et al. (2000) or the current study (Table 5). Total N intake was reduced when straw was included in the diet within the nutrient utilisation study, a consequence of the lower

DMI observed and the low protein content of straw, and this was associated with a corresponding reduction in output of faecal and manure N (Table 6). However, this did not impact on N utilisation efficiency, perhaps due to a reduction in the ability of rumen bacteria to capture ammonia due to straw inclusion in the diet. There was also a trend for reduced energy intake when straw was offered and a corresponding decrease in urinary energy, but no impact on faecal, urine or milk energy as a proportion of GE intake (Table 7).

The results of this experiment have a number of practical implications. For example, this study has demonstrated that modern dairy cow rationing programs can be used to formulate a high starch concentrate which can be used to supplement a very high quality grass silage, with no adverse effects on performance, while actually promoting intakes and milk protein content. In addition, this can be achieved with moderate yielding cows without the need to include straw in the diet. While there may have been an expectation that that supplementing a starch-based concentrate with straw would improve digestibility while maintaining intakes, and supplementing a fibre-based concentrate with straw would reduce intakes and milk yield, the absence of interactions in this study does not support these expectations. Furthermore, in common with the findings of earlier research, this study has failed to demonstrate any practical benefits of including straw in dairy cow diets, irrespective of concentrate type, provided that the concentrate fraction of the diet is designed appropriately and the diet is offered as a total mixed ration.

Conclusion

In the present study, neither concentrate type nor straw inclusion had a significant impact on milk yield or milk fat + protein yield. A High-starch concentrate, increased DMI and milk protein content compared to a High-fibre concentrate, and had no negative effects on faecal scores or nutrient utilisation when offered alongside a high quality silage. Straw inclusion reduced DMI and milk protein content, and had no beneficial effect on milk fat content or nutrient utilisation. Therefore, there is little evidence that straw inclusion in the diet of dairy cows is beneficial, and a carefully formulated High-starch diet can be fed alongside a high quality silage, without the use of straw as an additional fibre source.

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	High- starch	High- fibre
Maize meal	54.2	22.7
Wheat		10.9
Soyabean meal (high protein)	4.5	5.9
Rapeseed meal	4.5	4.4
Soya hulls (toasted)	11.3	18.2
Sugar beet pulp (dry)	9.0	19.1
Maize gluten	9.0	11.4
Protected protein (Sopralin ^a)	4.5	2.7
Protected fat (Maxfat CS ^a)		1.8
Mineral/Vitamin mix (Maxcare Dairy ^a)	1.8	1.8
Rumen buffer (Acid Guard ^a)	1.1	1.1
Yeast (Actisaf ^b)	0.1	0.1

Table 1: Ingredient composition of the High-starch and High-fibre concentrates offered (%, fresh basis).

^a Trouw Nutrition, Belfast, Northern Ireland,UK ^bLesaffre, Marcq-en-Baroeul , France

				Conce		(- 1)		
	Grass silage	(s.d)	High- starch	(s.d)	High- fibre	(s.d)	Straw	(s.d)
Oven dry matter (g/kg)	404	(23.4)	891	(15.8)	898	(13.8)	859	(29.6)
VCODM (g/kg)	418	(24.3)						
Crude protein (g/kg DM)	170	(7.0)	163	(3.8)	164	(1.7)	34	(0.6)
Ash (g/kg DM)	95	(1.8)	69	(3.8)	76	(4.3)	44	(7.2)
Acid detergent fibre (g/kg DM)	237	(3.6)	112	(8.0)	169	(6.8)	528	(0.3)
Neutral detergent fibre (g/kg DM)	364	(7.7)	258	(13.6)	339	(15.4)	864	(0.4)
Starch (g/kg DM)			373	(12.4)	237	(8.9)		
Gross energy (MJ/kg DM)	19.8	(2.85)	18.0	(0.09)	18.0	(0.10)	18.8	(0.01)
Metabolisable energy ^a (MJ/kg DM)	12.1	(0.21)						
pH	4.2	(0.07)						
Lactic acid (g/kg DM)	83	(4.1)						
Acetic acid (g/kg DM)	8.4	(1.91)						
Ethanol (g/kg DM)	11.4	(4.90)						
Ammonia (g/kg total N)	60	(8.7)						

Table 2: Chemical composition of the grass silage, concentrates and straw offered during the 12 week experimental period.

^a Predicted using NIRS; VCODM: Volatile corrected oven dry matter

Table 3: Effects of concentrate type and straw inclusion on the feed intake, milk production and composition, the fatty acid content of milk, and body tissue reserves as measured during final week of each experimental period.

	Concentrate						
	ty	type Straw inclusion			P-Valu	ie	
	High-	High-	No-				
	starch	fibre	straw	Straw	SED	Concentrate	Straw
Silage DMI (kg/d)	14.7	14.2	15.1	13.7	0.21	0.001	< 0.001
Concentrate DMI (kg/d)	10.9	10.7	10.9	10.7	0.16	0.027	0.114
Total DMI (kg/d)	26.1	25.3	26.0	25.4	0.37	0.006	0.014
Milk yield (kg/d)	32.9	33.3	33.5	32.7	0.82	0.562	0.161
Fat (g/kg)	44.9	45.0	45.2	44.7	0.77	0.879	0.319
Protein (g/kg)	38.1	37.3	37.9	37.5	0.29	< 0.001	0.036
Lactose (g/kg)	46.7	46.8	46.7	46.7	0.26	0.822	0.999
Fat yield (kg/d)	1.46	1.49	1.51	1.44	0.042	0.398	0.035
Protein yield (kg/d)	1.25	1.24	1.27	1.22	0.026	0.635	0.023
Fat+protein yield (kg/d)	2.71	2.73	2.77	2.66	0.064	0.692	0.403
Milk FA concentrations (g	/100g tota	al FA identi	ified)				
Total C4:0 to C15:0	29.4	28.6	29.4	28.6	0.23	0.004	< 0.001
C16:0	37.0	37.5	37.7	36.8	0.36	0.037	0.002
C18:0	8.4	8.4	8.0	8.7	0.17	0.749	< 0.001
C18:1 <i>cis</i> -9	16.7	16.9	16.3	17.3	0.29	0.259	< 0.001
CLA,18:2cis-9, trans-							
11	0.43	0.46	0.44	0.45	0.010	< 0.001	0.117
C18:2cis-9, trans-12	1.7	1.8	1.6	2.0	0.13	0.970	0.144
C20:0	0.13	0.14	0.13	0.14	0.003	0.455	< 0.001
Total Saturated	74.6	74.3	74.9	73.9	0.43	0.399	0.002
Total MUFA	20.3	20.5	20.0	20.9	0.31	0.461	< 0.001
Total PUFA	3.1	3.0	3.0	3.1	0.16	0.104	0.316
Total n-3 PUFA	0.8	0.8	0.8	0.8	0.02	0.199	0.084
Total n-6 PUFA	2.3	2.2	2.2	2.3	0.14	0.960	0.165
Total n-7 PUFA	2.4	2.3	2.3	2.3	0.06	0.341	0.492
Total n-9 PUFA	16.7	17.0	16.3	17.4	0.29	0.263	< 0.001
Saturated:Unsaturated							
ratio	3.2	3.2	3.3	3.1	0.04	0.672	< 0.001
Body weight (kg)	679	680	681	678	4.3	0.828	0.605
Body condition score	2.3	2.3	2.3	2.3	0.02	0.236	0.126

DMI, dry matter intake; FA, fatty acids, MUFA, monounsaturated fatty acids, PUFA poly-unsaturated fatty acids

	Concentrate type		Straw inclusion			P-Value	
	High- starch	High- fibre	No- straw	Straw	SED	Concentrate	Straw
Serum BHB (mM)	0.51	0.35	0.50	0.35	0.227	0.295	0.345
Plasma glucose (mM)	3.65	3.57	3.62	3.61	0.055	0.065	0.665
Serum NEFA (mM)	0.12	0.12	0.12	0.12	0.018	0.759	0.726
Serum urea (mM) ^a	2.83	3.27	3.14	2.95	0.138	< 0.001	0.053

Table 4: Effects of concentrate type and straw inclusion on the blood metabolites of dairy cows

^a There was an interaction between concentrate type and straw inclusion for serum urea, with mean values for High-starch/No-straw, High-starch/Straw, High-fibre/No-straw and High-fibre/Straw being 3.13, 2.53, 3.16, 3.37 mM, respectively (SED = 0.138; P < 0.001).

 β HB, beta-hydroxybutyrate; NEFA, non-esterified fatty acids

	Concentrate type		Straw inclusion			P-Value			
	High- starch	High- fibre	No- straw	Straw	SED	Concentrate	Straw		
Silage DMI (kg/d)	12.5	12.5	13.4	11.5	0.58	0.825	0.007		
Concentrate DMI (kg/d)	9.9	9.9	10.4	9.5	0.47	0.865	0.078		
Total DMI (kg/d)	22.8	22.8	23.8	21.8	1.08	0.885	0.885		
Milk yield (kg/d)	26.8	27.6	28.2	26.3	1.90	0.754	0.356		
Digestibility coefficients (g/g)									
Dry matter	0.749	0.737	0.748	0.738	0.0119	0.291	0.406		
Organic matter	0.748	0.742	0.749	0.740	0.0134	0.630	0.507		
Nitrogen	0.604	0.601	0.600	0.605	0.0188	0.855	0.755		
Gross energy	0.744	0.741	0.748	0.737	0.0138	0.720	0.441		
ADF	0.757	0.757	0.769	0.755	0.0130	0.459	0.303		
NDF	0.716	0.737	0.730	0.723	0.0151	0.185	0.621		

Table 5: Effects of concentrate type and straw inclusion on dry matter intake and milk yield during the nutrient utilisation study, and on total ration digestibility coefficients.

DMI, dry matter intake; ADF, acid detergent fibre; NDF, Neutral detergent fibre

	Concentrate type		Straw in	Straw inclusion		P-Value		
	High - starch	High- fibre	No- straw	Straw	SED	Concentrate	Straw	
N intake/output (g/d)								
Total N intake	574	599	622	551	27.1	0.479	0.009	
Faecal N	225	236	246	216	14.1	0.533	0.028	
Urine N	158	176	170	164	11.3	0.124	0.587	
Manure N	384	411	415	380	16.8	0.157	0.054	
Milk N	154	155	162	146	9.5	0.978	0.122	
N utilisation (g/g)								
Faecal N/N intake	0.396	0.399	0.400	0.395	0.0188	0.855	0.755	
Urine N/N intake	0.280	0.297	0.276	0.300	0.0196	0.299	0.234	
Manure N/N intake	0.676	0.695	0.675	0.695	0.0196	0.267	0.370	
Milk N/N intake	0.271	0.262	0.264	0.270	0.0127	0.510	0.645	
Faecal N/manure N	0.587	0.573	0.593	0.567	0.0236	0.466	0.293	
Urine N/manure N	0.413	0.427	0.407	0.433	0.0236	0.466	0.293	

Table 6: Effect of concentrate type and straw inclusion on nitrogen (N) intake, nitrogen output and nitrogen utilisation efficiency of dairy cows.

	Concentrate type		Straw in	Straw inclusion		P-Value		
	High- starch	High- fibre	No- straw	Straw	SED	Concentrate	Straw	
Energy intake and output (MJ/d)								
GE intake	407	416	429	393	19.3	0.752	0.086	
Faecal energy	103	106	106	102	6.2	0.637	0.404	
Urinary energy	13	14	15	13	0.7	0.107	0.050	
Milk energy	95	99	101	94	5.6	0.510	0.285	
Energy utilisation (M	IJ/MJ)							
Faecal E/GEI	0.256	0.259	0.252	0.263	0.0138	0.720	0.441	
Urine E/GEI	0.033	0.035	0.034	0.034	0.0016	0.108	0.613	
Milk E/GEI	0.238	0.242	0.237	0.243	0.1172	0.652	0.592	

Table 7: Effect of concentrate type and straw inclusion on energy intake, energy output and energy utilisation efficiency in dairy cows.

GE, gross energy; E, energy; GEI, gross energy intake

Chapter 8

Removal of autumn growth herbage using sheep: effects on yield and quality of first cut silage and subsequent cow performance

Introduction

The nutritive value of grass silage is a key determinant of cow performance during the housed period. Offering high quality silage can improve dry matter intake (DMI), milk yield (Keady et al., 2013; Huhtanen et al., 2013), milk protein content (Rinne et al., 1999) and allow for concentrate sparing (Ferris et al., 2003; Randby et al., 2012; Huhtanen, 2018). Silage nutritive value is influenced by many factors, including the quality of the herbage ensiled (Keady et al., 2013). Herbage quality may be influenced by the presence of grass which grows during the autumn period which is subsequently harvested with spring growth herbage the following year. The relatively mild maritime climate in western areas of the United Kingdom (UK) and Ireland allows grass growth to continue throughout the autumn and winter, albeit at a slow rate, with growth promoted on many farms by the application of slurry to swards after final harvest of silage in September. The perceived impact of ensiling this autumn growth herbage was highlighted in a survey of Northern Ireland (NI) dairy farmers, with 30% of respondents indicating that they believed autumn growth herbage had a large or very large negative impact on the quality of first cut silage produced the following spring (Ferris et al., 2022). For this reason, many farmers remove autumn growth herbage by grazing with sheep or youngstock (Ferris et al., 2022), although this practice is not always adopted.

The impact of removing this autumn growth herbage by grazing livestock, on grass availability the following spring, has been examined in a number of studies. For each additional day of delaying closing of grazing swards in autumn, there was a reduction in spring herbage accumulation, ranging from 10 - 16 kg DM d⁻¹ (O'Donovan *et al.*, 2002; Lawrence *et al.*, 2017; Claffey *et al.*, 2020). Swards closed earlier tended to have a higher herbage mass in spring (Moloney *et al.*, 2017), although they also contained a greater proportion of senescent material (Hennessy *et al.*, 2006) compared to later closed swards. The presence of senescent herbage within a sward has been shown to reduce herbage quality and digestibility (Hennessy *et al.*, 2006; Lawrence *et al.*, 2017). As leaf senescence, and the associated decline in herbage mass and quality, are moderated by soil and air temperatures and solar radiation (Herrmann *et al.*, 2005) there is considerable year to year variation.

Few studies have investigated the impact of removing late season growth herbage on silage quality the following spring. In one exception, Moloney *et al.* (2017) found that the dry matter digestibility of silage produced from swards harvested in May was improved when swards were defoliated in December compared to those defoliated in late October. However, the impact on animal performance of offering silages produced from swards which were either defoliated or not defoliated, does not appear to have been examined. The objective of the current study was therefore to investigate the effects of either leaving grass swards ungrazed following harvest of third-cut silage in September, or grazing the sward with sheep in autumn on: 1) herbage yield and composition 2) the nutritive quality of silages produced from herbage harvested from these swards, and 3) cow performance when these silages were offered.

Materials and Methods

Two experiments were conducted at the Agri-Food and Biosciences Institute (AFBI), Hillsborough, NI (54°27'N; 06°04'W). Cows were housed and cared for under an establishment licence granted by the Department of Health, Social Services & Public Safety for NI and in accordance with the Animals (Scientific Procedures) Act 1986.

Sward management

Re-growth herbage from perennial ryegrass (*Lolium perenne*) based swards was harvested for third-cut silage from three adjoining blocks of land (total area of 4.7 ha) on 13 September 2018 and 15 September 2020 (Experiments 1 and 2, respectively). Following harvest, dairy cattle slurry was applied at approximately 35 m³ ha⁻¹ over the entire area, using a trailing shoe application system, providing approximately 27 kg available N ha⁻¹ (based on an assumed slurry DM content, N content and N availability of 60 g kg⁻¹ and 2.6 kg per m³, and 30%, respectively: RB209, 2020). Each land block was then divided into two similar sized areas, giving six sub-blocks. Within each block one sub-block was grazed (from 14 November and 30 November in 2018 and 2020, respectively) by non-pregnant ewes at a stocking rate of 34 (2018) to 37 (2020) ewes ha⁻¹ (grazing continued until 11 December and 21 December in 2018 and 2020, respectively). The second sub-block within each block was left ungrazed.

After the sheep were removed, mean residual sward heights were measured using a rising plater meter (Jenquip, Feilding, New Zeland), with 100 sward height measurements taken in each sub-block in a 'W' formation. Sward measurements were

calculated using the JenQuip EC10 standard equation (Herbage mass (kg DM ha-1) = (Compressed sward height [cm] x 140) + 500). Post grazing sward heights for the grazed swards were 4.9 and 4.6 cm (Experiment 1 and 2, respectively) while the ungrazed sward heights were 12.0 and 9.4 cm. Residual herbage masses of 1964 and 1779 kg DM ha⁻¹ (Experient 1 and 2 respectively) were calculated for the grazed subplots, and 3865 and 3144 kg DM ha⁻¹ for the ungrazed sub-plots.

The following spring 45 m³ ha⁻¹ of dairy cow slurry was applied to the entire experimental area (on 25 February 2019 and 3 March 2021 in Experiments 1 and 2, respectively) using a trailing shoe application system. This slurry was assumed to supply 41 kg available N ha⁻¹ (assumed DM, N content and N availability of 60 g kg⁻¹, 2.6 kg per m³, and 35%, respectively (RB209, 2020)). In 2019, inorganic fertiliser was applied, in a split dressing, comprising 58 kg N ha⁻¹ in the form of urea (proportionally 0.46 N) on 26 February, and 72 kg N ha⁻¹ as compound fertiliser (N, P and K, 26 : 0 : 6) on 9 April. In 2021 58 kg N ha⁻¹ was applied in the form of urea (proportionally 0.38 N) on 23 March.

On 11 May 2019 and 17 May 2021, herbage from both the grazed and ungrazed subblocks blocks was cut using a Class 3200 mower (Harsewinkel, Ostwestfalen-Lippe, Germany), tedded and allowed to wilt for approximately 24 hours, placed into rows using a Class 3100 grass rake (Harsewinkel, Ostwestfalen-Lippe, Germany), and harvested using a John Deere 7450 precision-chop forage harvester (Moline, IL, USA). Grass was treated at harvest with a bacterial inoculant (ULV50, Biotal, UK) at approximately 20 ml t⁻¹ of fresh herbage, with herbage from the grazed and ungrazed areas ensiled in separate bunker silos (nominal capacity of 70 t), covered in polythene sheeting, and the sheet weighed down with rubber mats. The fresh weight of herbage harvested from each treatment was determined by weighing all trailer loads of grass on a commercial weighbridge prior to ensiling. A sample of herbage was taken from throughout each load for oven DM determination, and the yield of DM harvested from each treatment area was subsequently determined.

Animals, experimental design and housing

Silages produced in 2019 and 2021 were offered in separate experiments to latelactation multiparous Holstein cows (Experiment 1: 20 cows, mean 196 d calved, Experiment 2: 16 cows, mean 240 d calved) in balanced change-over design feeding studies. Each experiment involved two 28 d periods, comprising a 21 d feed adaptation phase followed by a 7 d measurement period. Cows were blocked according to milk fat plus protein yield during the week prior to the start of experiment, and cows within each block randomly allocated to one of the two treatments: GS (silage from the 'Grazed' sub-blocks) and UGS (silage from the 'Ungrazed' sub-blocks). Silages were offered daily between 09.00 and 10.00 hours, while uneaten silage was removed the following day at approximately 08.00 hours. Silages were offered using a feeder wagon (Vari-Cut 12, Redrock, Armagh, UK). The appropriate silage was placed in the feeder wagon, and mixed for approximately five minutes, and then transferred from the feeder wagon to a series of feed-boxes mounted on weigh scales. Cows accessed these boxes via an electronic identification system, enabling individual cow intakes to be recorded daily (Controlling and Recording Feed Intake, Bio-Control, Rakkestad, Norway). Silages were offered ad libitum at 107 % of the previous day's intake. Cows were offered a commercial concentrate via in-parlour feeders during both experiments. In Experiment 1 cows were offered 8.0 kg of concentrates d⁻¹ throughout the experimental period (4.0 kg per milking), while in Experiment 2 cows were offered 4.0 kg of concentrates d⁻¹ (2.0 kg per milking) due to their later stage of lactation and lower milk yields. The ingredient list and chemical composition of the concentrates offered are presented in Table 1.

Throughout each of the 8 wk experiments cows were housed in a free-stall house with concrete flooring, and had access to individual cubicles that were fitted with rubber mats and bedded with sawdust. The cubicle-to-cow ratio was \geq 1:1 at all times, meeting the recommendations of FAWC (1997). The floor area was cleaned every 3 h using an automated scraper system.

Sward measurements

The yield of herbage on each sub-block was recorded on three occasions during each experiment (8 February, 2 April and 10 May during 2019: 14 January, 31 March and 17 May during 2021). On each occasion, within each sub-block, five strips (each approximately 5 m long: actual length recorded) were harvested at five random locations using a Tracmaster power scythe (BCS 630 Crusader) with the knife bar set at 4 cm. The weight of herbage harvested from each strip (15 strips per treatment on each date) was recorded, and each sample thoroughly mixed. A sub-sample of

herbage harvested from each strip was dried at 85°C for DM determination, while a second fresh sub-sample was scanned using NIRS and its metabolisable energy (ME) content predicted as per Park *et al.* (1998), using a methodology for fresh grass. In May a further sub-sample of herbage harvested from each strip was characterised for sward morphology (n = 15 samples per treatment in each year). Each sample was thoroughly mixed on a bench, and large weeds removed by hand. The sample was then 'halved' a number of times to leave a subsample of approximately 50 g for separation. This herbage sample was hand separated into 'live' and 'dead' tissue, and the live tissue then separated into leaf, stem and pseudostem fractions. Leaf blades were detached at the collar, and leaf sheaths separated from the true stem and included in the pseudostem fraction. For each sample, the separated fractions were dried and the weight of the dried fractions was determined.

Animal measurements

Cows were milked twice daily (between 06.00 and 08.00 hours and between 15.00 and 17.00 hours) throughout the experiment using a 50-point rotary milking parlour (Boumatic, Madison, USA). Milk yields were automatically recorded at each milking, and a total daily milk yield for each cow for each 24 h period calculated. Milk samples were taken during six consecutive milkings at the end of each measurement period, treated with a preservative tablet (lactab Mark III, Thompson and Cooper Ltd., Runcorn, UK), and stored at 4°C until analysed (normally within 48 h) for fat, protein and lactose concentrations using an infrared milk analyser (Milkoscan CombifossTM7; Foss Electric, Hillerød, Denmark). A weighted concentration of each constituent was determined for the 24 h sampling period and a mean composition over the three day sampling period was subsequently calculated for each cow. Results for period 2 in Year 2 are based on a single 24 h sampling period due to deterioration of the other samples. Milk gross energy content (GE) was calculated using the following equation (Tyrell and Reid, 1965):

Milk GE = $(fat \times 0.0384) + (protein \times 0.0223) + (lactose \times 0.0199) - 0.108.$

Energy corrected milk (ECM) yield (kg d⁻¹) was calculated assuming the GE content of 1 kg 'standard milk' to be 3.1 MJ kg⁻¹ (i.e., for milk containing 40 g kg⁻¹ fat, 32 g kg⁻¹ ¹ crude protein, and 48 g kg⁻¹ lactose, as described by Muñoz *et al.* (2015)) as follows:

ECM (kg d^{-1}) = (milk yield (kg d^{-1}) × GE (MJ kg⁻¹))/3.1

Body weight (BW) was recorded twice daily (immediately after each milking) using an automated weighbridge, and a mean weekly BW for each cow was determined. The body condition score (BCS) of each cow was estimated by a single trained technician on the final day of each period according to Edmonson *et al.* (1989) on a 5-point (including quarter points) scale.

Feed analysis

A sample of the grass silage offered was taken daily throughout each experiment and dried (Experiment 1 at 85°C for 18 h; Experiment 2 at 60°C for 48 h) to determine oven DM content. Twice weekly a sample of grass silage was dried at 60°C and bulked for each 14 d period with the bulked sample milled through a sieve with 0.85 mm aperture, and analysed for neutral detergent fibre (NDF), acid detergent fibre (ADF) and ash concentrations. Each week a fresh silage sample was analysed using NIRS for ME concentration according to Park *et al.* (1998). A further fresh silage sample was taken weekly and analysed for GE, nitrogen (N), pH, ammonia-N and volatile components. A sample of concentrate was taken weekly, and one sub-sample dried at 85°C for 24 h to determine oven DM content. An additional sub-sample was dried at 60°C for 48 h, bulked for each 14 d period, milled through a 0.85 mm sieve, and subsequently analysed for N, NDF, ADF, ash and starch concentrations. All chemical analysis of the feed stuffs offered where undertaken as described by Purcell *et al.* (2016).

Statistical analysis

Data within each of Experiments 1 and 2 were analysed separately. Herbage yield and ME concentrations, as recorded across the three pre-harvest sampling occasions, were analysed using a repeated measures analysis, with data from each strip harvested treated as an experimental unit blocked within each sub-block. Cow performance data recorded during the final week of each experimental period (DMI, milk yield, milk composition, BW and BCS) were analysed using a linear mixed model methodology according to the two-period change-over experimental design, with constant + treatment as the fixed model, and cow × period as the random model. In all cases the method of residual maximum likelihood (REML) was used as the estimation method. All data were analysed using GenStat (21st edition; VSN International).

Results

Grass quality and morphology

Across the three pre-harvest sampling periods, herbage DM yield as measured in the 5 m strips (Figure 1a and 2a) increased over time (P < 0.001) and was greater in the ungrazed swards (P < 0.001). There was a significant treatment × time interaction observed in both Experiment 1 (P = 0.045) and Experiment 2 (P = 0.017). The yield gap between the grazed and ungrazed swards decreased over time and did not differ significantly (P > 0.05) at the time of the May sampling (undertaken at the time of the field harvest) in either Experiment. At the time of field harvest the total yield of herbage harvested across the three sub-blocks, based on the total weight of each trailer load of grass harvested, and the oven DM of samples taken from each load, were 6.2 and 7.0 t DM ha⁻¹ in Experiment 1 and 6.7 and 7.7 t DM ha⁻¹ in Experiment 2 (grazed vs. ungrazed, respectively).

In Experiment 1, the ME content of herbage as measured in the 5 m strips was greater in the grazed swards (P < 0.001) and decreased from February through to May (Figure 1b; P < 0.001) with both treatments. There was a significant treatment × time interaction (P < 0.001), with herbage ME content not significantly different between treatments at either of the April or May measurement periods. The ME content of herbage harvested from the strips in Experiment 2 (Figure 2b) was greater in the grazed swards (P = 0.008). Herbage ME content increased between the January and March measurement period with both treatments, and then decreased between the March and May measurement period in the grazed treatment (Treatment × time, P < 0.001), with ME concentrations not significantly different between treatments at the third measurement in May.

Morphology of the grazed and ungrazed swards at the time strips were harvested in May was similar in Experiment 1, with swards comprising similar amounts of dead material (17% and 18%), green leaf (36% and 34%), green pseudostem (24% and 24%) and stem (24% and 24%), respectively (Figure 3a). In Experiment 2 there was a greater percentage of dead tissue in the ungrazed sward (14%) compared to the grazed sward (6%). The grazed sward also contained a higher percentage of green leaf (50%) compared to the ungrazed sward (40%). However, pseudostem (30% and 31%) and stem (14% and 15%) fractions of the live tissue were similar between the

grazed and ungrazed swards, respectively (Figure 3b). Samples from Experiment 2 were more variable as indicated by the error bars.

The mean chemical composition of the silages made from the grazed and ungrazed swards, based on weekly analysis of silages as offered throughout the experiments, are presented in Table 2. While the sampling methodology precludes a statistical comparison of the silages, silages made from both swards within each experiment were generally similar in composition in Experiment 1, although silage ME content was 0.2 MJ kg⁻¹ DM greater with the grazed sward. Differences between silages were greater in Experiment 2, with silage produced from the ungrazed sward having 7% greater NDF content, and 0.5 MJ kg⁻¹ DM lower ME content. The concentrations of lactic acid concentration in silage from the grazed sward was 29% higher than in silage from the ungrazed sward, while acetic acid concentrations were twice as high in the silage from the ungrazed sward. Ammonia N concentrations were 27% higher in silage from the ungrazed sward.

Cow performance

In Experiment 1, silage DMI and total DMI did not differ between treatments (Table 3) while cows offered GS produced 0.8 kg more milk day⁻¹ (P < 0.001; Table 3) than those offered UGS. Milk fat and milk protein content where not affected by treatment, but cows offered GS produced milk which contained 0.4 g kg⁻¹ more lactose (P = 0.014) and had a 0.03 kg d⁻¹ higher milk protein yield (P = 0.014). However, fat plus protein yield and ECM did not differ between treatments. Bodyweight and BCS were also unaffected by silage type.

In Year 2, cows offered GS had greater silage DMI and total DMI (+1.5 kg d⁻¹; P <0.001; Table 4) compared to UGS. However, milk yield was not affected by treatment (P > 0.05). Milk fat content tended (P = 0.056) to be greater in cows offered GS, while milk lactose content was 0.8 g kg⁻¹ greater in cows offered UGS (P = 0.047). Fat yield (+1.5 kg d⁻¹; P = 0.047) was greater in cows offered GS, and there was a tendency for fat plus protein yield (0.17 kg d⁻¹; P = 0.063) and ECM (2.2 kg d⁻¹; P = 0.068) to be greater in cows offered GS. There was no difference between treatments in BW or BCS.
Discussion

This study investigated the impact of autumn management of grass swards on herbage yield and ME content, and examined the impact on cow performance when silages made from these swards were offered to dairy cows. In view of year-to-year variability in weather conditions between September and the following May when silage was harvested, the study was repeated during two separate years.

Effect of autumn management on herbage yield and silage quality

Grazing autumn growth herbage with sheep reduced the yield of herbage removed at the time of field scale harvesting by 0.8 and 1.0 t DM ha⁻¹ (Experiments 1 and 2, respectively), although yield differences based on harvesting of 5 m strips were not significantly different (numerically 0.2 and 0.9 t DM ha⁻¹ lower with grazed swards in Experiments 1 and 2, respectively). Similarly, studies primarily focused on grazing swards have observed that a delayed closing date in autumn resulted in lower herbage yields the following spring (Hennessy et al., 2006; Ryan et al., 2010; Looney et al., 2021). The reduction in herbage yield following autumn grazing is likely due to a number of factors, including direct removal of herbage during grazing, less photosynthetic material available to initiate growth the following spring, damage to swards by grazing livestock delaying spring growth and lower soil temperatures in spring as a result of removal of the insulating layer of herbage. Nevertheless, Figures 1a and 1b demonstrate that differences in yield between grazed and ungrazed swards decreased as the growing season progressed, and it is likely that if swards had been harvested for silage in late May or early June, yield differences would have been negligible.

Removing autumn growth herbage was expected to improve the nutritional quality of silage produced the following spring by reducing the amount of senesced material within the sward, and by perhaps increasing the proportion of leaf relative to stem. However, sward morphology data suggests that that both grazed and ungrazed swards in Experiment 1 were morphologically very similar in terms of proportion of dead material. In contrast, the greater proportion of dead material, and a lesser proportion of leaf material in the grazed sward in Experiment 2 was as expected, with an earlier closing associated with an increased proportion of dead material previously (Hennessy *et al.*, 2008). It is likely that the proportion of dead material will be

influenced by weather conditions over the winter period and the severity of grazing (Parsons *et al.*, 1988).

Trends in predicted herbage ME content during the growing season were inconsistent between years. However, at the time of harvest in May herbage from both the grazed and ungrazed swards had a similar predicted ME content, particularly in Experiment 1. This is perhaps not unsurprising given the similar sward morphologies observed with both grazed and ungrazed swards in Experiment 1. Although the ratio of dead material : leaf differed in Experiment 2, it is unclear if NIRS was able to differentiate between these, especially as the dead material was observed to be primarily 'dead leaf'. Furthermore, while not comparable statistically, the ME content of silage produced from the grazed swards tended to be higher than that from the ungrazed swards (0.2 and 0.5 MJ kg⁻¹ DM higher in Experiment 1 and 2, respectively). These trends broadly reflect the trends in silage NDF contents, which did not differ between treatments in Experiment 1, but which were 35 g/kg DM higher with the ungrazed sward in Experiment 2. This may have been driven in part by the greater proportion of dead material and subsequent decrease in leaf tissue within the ungrazed sward, and by the later harvest date in Experiment 2 compared to Experiment 1. The higher silage CP content in Experiment 1, compared to Experiment 2 reflects a higher than planned application of inorganic fertiliser that year.

Senesced material is known to have a detrimental effect on silage fermentation (Hennessy *et al.*, 2006), and this might explain the lower lactic acid concentration (33 g/kg DM lower) and higher acetic acid (23 g/kg DM higher) and ammonia N concentrations (14 g/kg DM higher) with the ungrazed sward in Experiment 2. Concentrations of lactic acid, acetic acid and ammonia N in Experiment 1 were almost identical, perhaps reflecting the similar proportion of senesced material in swards in this study.

Effect of autumn management on cow performance

Silage DMI (and total DMI) in Experiment 1 were high considering the late lactation stage of cows on this study, and likely reflect the early harvest date, the associated low silage NDF content, the optimum DM concentration of around 300 g/kg, the high CP content and the well preserved nature of the silage. Comparable intakes have also been recorded by Randby *et al.* (2012) and Craig *et al.* (2023) with highly digestible

silages. However, the absence of a treatment effect on silage DMI can be attributed to the similar silage compositions, especially the similar NDF concentrations. While GS increased milk yield by 0.8 kg d⁻¹ compared to UGS, milk fat and protein content were unaffected, though both were numerically higher with UGS. As a consequence, neither fat plus protein yield or ECM differed between treatments, which agrees with the absence of a treatment effect on intake and body tissue reserves. Therefore, from an animal performance point of view, Experiment 1 offers no indication of benefits associated with removal of autumn growth herbage over the winter.

The 1.6 kg lower silage intake with the UGS treatment in Experiment 2 was most likely driven by the higher NDF of this silage (Oba and Allen, 1999). However, despite the increase in DMI, and subsequent increase in energy intake, milk yield was not affected. There was a trend (P = 0.056) for greater milk fat content in cows offered the GS, although the high SED highlights the variability in milk fat in later lactation. The higher milk fat content within the GS drove a greater milk fat yield (+0.15 kg d⁻¹) and a tendency for greater fat plus protein and ECM yields. In view of the higher silage DMI with GS, and the 0.5 MJ/kg DM higher ME content of the silage offered, cows on this treatment consumed approximately 25 MJ d⁻¹ more ME than cows on UGS. Based on the assumption that the production of 1 kg ECM requires approximately 5.3 MJ ME, this would equate to an extra 4.7 kg of ECM, considerably more than 2.2 kg ECM actually observed. Given the similar BCS and BW between treatments there is nothing to suggest that the extra energy was diverted to body reserves, although measurements were only taken over a four-week period.

While individual cow performance is important, the overall impact of autumn management on milk solids output per ha should also be considered. Total fat plus protein yield ha⁻¹ was calculated within each of Experiments 1 and 2 based on total herbage DM yield ha⁻¹ for each treatment on a field basis (assumed in-silo and feed out losses of 15%), divided by mean silage DMI with each treatment to determine a total 'cow-feeding-days' ha⁻¹, which was used to calculate total fat plus protein yield ha⁻¹ (based on actual performance). Total yield of fat plus protein ha⁻¹ were 734 and 843 kg for the GS and UGS respectively (Experiment 1) and 893 and 1,072 kg (Experiment 2). Therefore, when expressed on a per ha basis, winter grazing by sheep of autumn regrowth reduced total fat plus protein output by 13 and 17% in Experiments 1 and 2, respectively.

While Moloney *et al.* (2015) examined the impact of closing date on grass silage ensilibility, we are not aware of any study which has examined the impact of offering silages produced from swards with different closing dates on animal performance. However, studies which investigated the effects of autumn closing date on grazing cow performance also found no difference in cow performance the following spring (Roche *et al.*, 1996: Fenger *et al.*, 2021). Furthermore, in agreement with the current study, Claffey *et al.* (2019) found that the higher herbage yield of earlier closed swards increased milk yield in grazing cows compared to later closed swards (Claffey *et al.*, 2019).

Practical considerations

In most parts of the UK and Ireland herbage growth throughout the autumn and winter is encouraged by applications of slurry following final silage harvest, an increasingly common practice due in part to intensification on many farms. The practice of using sheep or other livestock to remove autumn grass has arisen from farmers experience of the negative impact of ensiling poor quality late season growth on subsequent silage quality. Under certain conditions winter growth herbage can 'die-off' creating mats of highly senesced material. However, winter conditions within the current study were not particularly harsh, with swards remaining green and 'healthy' over the winter.

The impact of winter grazing on herbage yield is likely to be influenced by post-grazing sward height and date that grazing livestock are removed from the swards. Earlier removal of sheep, and less intensive grazing than adopted in the current study are likely to limit the potential loss of yield the following spring. However, if swards are grazed, it is important that swards are grazed out cleanly, as 'trampled' herbage is likely to be much susceptible to senescence. If swards were grazed by cattle in late summer, rather than being cut for silage, the resultant higher residuals and selective grazing mean that these swards are likely to benefit from sheep grazing to a greater extent than swards following silage harvest.

While autumn grazing certainly may have an impact on yield the following spring, for farmers with a sheep enterprise a positive trade-off arises due to meeting the nutrient requirements of sheep (approximately 4 weeks grazing for pregnant ewes stocked at 36 ewes/ha). However, within the UK many dairy farmers allow sheep farmers to 'clean up' swards in the autumn at no or very little cost. In addition, while the early cutting

dates in the current study meant that yield of herbage harvested was reduced following winter grazing, the yield differential between the two systems was decreasing, and may well have been negligible if harvest had been delayed by one to two weeks. It should also be considered that removing herbage during the winter by grazing can leave soil exposed with increased risk of surface runoff and associated nutrient losses, while removal of the herbage mat can also make it more difficult for farm machinery (spreading slurry and sowing fertiliser) to traffic ground in the spring.

Conclusion

While this study provided some evidence of an improvement in quality of silage produced from swards grazed in early winter, improvements in individual cow performance were limited, if actually observed. Given the tendency for herbage yields the following spring to be reduced following winter grazing, total milk fat plus protein yield output per ha was also reduced.

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	Experiment 1	Experiment 2
Ingredients		
Wheat	17.4	9.5
Maize	17.9	16.0
Maize distillers	10.0	7.6
Maize gluten	9.9	
Wheat feed		8.2
Sugar beet pulp		9.9
Rapeseed meal	4.3	9.9
Soyabean meal (high protein)	10.1	12.1
Soya hulls	17.5	15.1
Molaferm ¹	7.5	7.5
Palm fatty acid distillate	10.0	0.6
Megalac ²	1.4	0.5
Limestone	0.9	0.7
Calcined magnesite	0.2	0.8
Salt	0.6	0.9
Rumitech ³	0.7	
Vitamin/mineral mix ³	0.5	0.7
Chemical composition		
Oven dry matter (g kg ⁻¹)	894	887
Crude protein (g kg ⁻¹ DM)	195	191
Ash (g kg ⁻¹ DM)	87	83
Acid detergent fibre (g kg ⁻¹ DM)	145	164
Neutral detergent fibre (g kg ⁻¹ DM)	293	293
Starch (g kg ⁻¹ DM)	276	209
Gross energy (MJ kg ⁻¹ DM)	18.2	18.1

Table 1 Ingredient composition (% fresh) and chemical composition of concentratesoffered during Experiments 1 and 2.

¹United Molasses (Ireland) Ltd, Duncrue Street, Belfast, BT3 9AQ. ²Volac Wilmar Feed Ingredients Ltd, Hertfordshire, UK, SG8 5QX. ³John Thompson and Sons Ltd, York Road, Belfast, BT15 3GW.

	Experiment 1		Experin	Experiment 2	
	Silage from	Silage	Silage from	Silage	
	grazed	from	grazed	from	
	sward	ungrazed	sward	ungrazed	
		sward		sward	
Oven dry matter (g kg ⁻¹)	278	310	208	212	
VCODM (g kg ⁻¹)	295	326	222	229	
Crude protein (g kg ⁻¹ DM)	176	179	123	128	
Ash (g kg ⁻¹ DM)	102	101	85	90	
Acid detergent fibre (g kg ⁻¹ DM)	266	263	274	288	
Neutral detergent fibre (g kg ⁻¹	469	471	487	522	
DM)					
Metabolisable energy (MJ kg ⁻¹	11.1	10.9	11.8	11.3	
DM)					
pH	4.1	4.1	3.7	3.8	
Lactic acid (g kg ⁻¹ DM)	94	99	148	115	
Acetic acid (g kg ⁻¹ DM)	12	11	23	46	
Ethanol (g kg ⁻¹ DM)	25	22	42	34	
Ammonia (g kg ⁻¹ total N)	62	61	52	66	

Table 2 Chemical composition of silages produced from swards that were eithergrazed by sheep during the winter or left ungrazed, and as offered to dairy cows duringExperiments 1 and 2.

VCODM; volatile corrected oven dry matter

	GS	UGS	SED	P Value
Silage DMI (kg d ⁻¹)	16.8	16.3	0.35	0.160
Total DMI (kg d ⁻¹)	23.6	23.1	0.35	0.160
Milk yield (kg d ⁻¹)	27.0	26.2	0.21	< 0.001
Fat $(g kg^{-1})$	49.8	50.8	0.73	0.171
Protein (g kg ⁻¹)	37.1	37.2	0.22	0.799
Lactose (g kg ⁻¹)	46.4	46.0	0.14	0.014
Fat yield (kg d ⁻¹)	1.34	1.33	0.020	0.793
Protein yield (kg d ⁻¹)	1.00	0.97	0.009	0.014
Fat plus protein yield (kg d ⁻¹)	2.34	2.31	0.025	0.250
Energy corrected milk yield (kg d ⁻¹)	30.8	30.3	0.32	0.131
Bodyweight (kg)	667	667	2.6	0.893
Body condition score	2.3	2.3	0.02	0.492

Table 3 Performance of dairy cows offered silages produced from swards which were either grazed by sheep during the winter (GS) or left ungrazed (UGS) (Experiment 1).

Table 4. Performance of dairy cows offered silages produced from swards which were either grazed by sheep during the winter (GS) or left ungrazed (UGS) (Experiment 2).

	GS	UGS	SED	P Value
Silage DMI (kg d ⁻¹)	13.2	11.6	0.20	< 0.001
Total DMI (kg d ⁻¹)	16.7	15.2	0.20	< 0.001
Milk yield (kg d ⁻¹)	21.4	20.8	0.66	0.338
Fat $(g kg^{-1})$	57.1	52.1	2.38	0.056
Protein (g kg ⁻¹)	38.7	38.8	0.44	0.742
Lactose (g kg ⁻¹)	45.9	46.7	0.34	0.047
Fat yield (kg d ⁻¹)	1.24	1.09	0.066	0.047
Protein yield (kg d ⁻¹)	0.83	0.80	0.026	0.276
Fat plus protein yield (kg d ⁻¹)	2.07	1.90	0.087	0.063
Energy corrected milk yield (kg d ⁻¹)	27.0	24.8	1.27	0.068
Bodyweight (kg)	659	657	8.4	0.850
Body condition score	2.6	2.6	0.05	0.743

Figure 1. Impact of removing autumn herbage by grazing sheep, compared to leaving swards ungrazed on 1a) herbage yield (kg DM ha⁻¹, measured above 4 cm) and 1b) herbage metabolizable energy (MJ kg⁻¹ DM) content, as measured on three occasions during the following year (Experiment 1).



Figure 2. Impact of removing autumn herbage by grazing with sheep, compared to leaving swards ungrazed on 2a) herbage yield (kg DM ha⁻¹, measured above 4 cm) and 2b) metabolizable energy (MJ kg⁻¹ DM) content as measured on three occasions during the following year (Experiment 2).



Figure 3. Impact of removing autumn herbage by grazing with sheep, compared to leaving swards ungrazed, on percentage of dead material, leaf, psudostem and stem within the sward as measured pre-harvest in Experiment 1 (3a) and Experiment 2 (3b).



Chapter 9

Performance of dairy cows offered either zerograzed grass or grass silage prepared from the same sward

Introduction

Grazed grass is the most cost effective feed source for dairy cows in temperate regions (O'Brien and Hennessy, 2017; Hanrahan et al., 2018). Nevertheless, within the United Kingdom (UK) and many other parts of Europe there has been a move towards systems were cows are confined during the summer, with this due in part to expanding herd sizes, and the ability to improve the nutritional management of higher yielding dairy cows when housed (Reijs et al., 2013; March et al., 2014).

On many farms cows that are confined over the summer are offered conserved forages, particularly grass silage. However, grass harvested for silage is normally more mature than grass which is grazed and will have a lower nutritive value than grazed grass. As a result, there has been growing interest in the implementation of 'zero-grazing' systems in which fresh herbage is harvested and offered daily when its nutritive value is still high (Meul et al., 2012). Although results from studies comparing conventional grazing and zero-grazing are inconsistent, in general, zero-grazing increases dry matter intake (DMI) compared to grazing (Dohme-Meier et al., 2014), and can improve grass utilisation efficiency (Meul et al., 2012; Decruyenaere et al., 2014). However, despite these benefits, zero-grazing can be labour intensive, with grass often harvested twice daily throughout the grass growing season. In addition, given the variability in grass growth rates throughout the season, it can be challenging to ensure a daily supply of grass at the optimum growth stage.

Some of these challenges might be overcome by harvesting herbage for silage production at the same growth stage as herbage which is used for zero-grazing. For example, compared to zero-grazing, silage production can be less labour intensive as large quantities of grass can be ensiled on a single day, while day-to-day variation in silage dry matter (DM) content and nutritive value can be reduced by harvesting on a single date. However, in order to ensure the silage produced has a high nutritive value, there is a relatively short time-window during which harvesting must take place. Furthermore, weather conditions must be sufficiently favourable during this time-window to facilitate a period of field wilting as immature grass crops can be difficult to ensile.

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While a number of studies have compared the performance of cows offered fresh grass or grass silage, in some of these studies the forages offered were produced from different swards or harvested at a different growth stages (Keady et al., 1996; Keady and Murphy, 1998; Mohammed et al., 2009). Furthermore, these earlier studies involved low yielding cows in late lactation. A small number of studies have however attempted to compare performance when cows were offered either grass silage or fresh grass produced from the same sward. For example, Cushnahan and Mayne (1995) demonstrated that neither DMI nor milk yields differed when cows were offered fresh grass and grass silage harvested from the same sward at a similar time, while in two separate studies, intakes of cows offered silage were lower than those of cows offered fresh grass (Mayne and Cushnahan, 1995; Cushnahan et al., 1996). However, in all of these studies herbage was harvested at a more mature growth stage than would be normally used in zero-grazing, while Cushnahan and Mayne (1995) offered silage produced following a one-week ensiling period.

Thus, the objective of the current study was to examine the effect of offering either fresh grass of a quality that would be typically offered to zero-grazed cows, or grass silage prepared from the same sward at a similar time, on forage quality and cow performance over a single season. We hypothesized that the quality (DM, CP, fiber content, ME) of the forages offered (zero-grazed grass or grass silage) would be similar due to the harvesting regime implemented and we expected no difference in performance of cows offered fresh zero-grazed grass or resulting grass silage. Preliminary results from this study have been published previously in abstract form and presented at the 78th Annual Conference of the British Society of Animal Science, Nottingham, UK, 2022 (Lavery and Ferris, 2022).

Material and methods

This study was conducted at the Agri-Food and Biosciences Institute (AFBI), Hillsborough, Northern Ireland (54°27'N; 06°04'W).

Housing, experimental animals and treatment overview

Throughout the experiment cows were housed in a free-stall house with concrete flooring and had access to individual cubicles fitted with rubber mats and bedded with sawdust. The cubicle-to-cow ratio was >1.1 at all times. The floor area was scraped every 3 hours using an automated system.

This two-treatment experiment (Zero-grazing [ZG] and Silage [SIL]) involved 36 dairy cows (28 multiparous and 8 primiparous: mean lactation number 2.5, SD 1.28). Cows were a mean of 178 (SD 15.1) days calved when they started the experiment. The experimental design involved fresh grass being harvested daily and offered to one treatment group (ZG) for a 12-week period (16 June to 8 September), while the same sward was harvested once weekly, ensiled for a five-week period, and the resultant silage offered to the second treatment group (SIL) for a 12-week period (21 July to 13 October). Thus, the feeding part of the study was conducted over a 17-week period.

This study was a continuous study which required two groups of cows which were 'balanced' at the time they started to consume their experimental diets (16 June and 21 July). To achieve this, a larger group of fifty-four cows were identified for possible use in the study and these were randomly split into two groups based on calving date: group 1 (n = 27) had a mean calving date of 26 December 2019, while group 2 (n = 27) had a mean calving date of 23 January 2020. Cows in both groups were offered the same pre-experimental diet (a non-experimental grass silage plus 10 kg of concentrate per cow/day) for a three-week period prior to being offered their experimental diets. Group 1 cows started the study on 16 June 2020 and were offered their experimental diets (from 21 July onwards) 18 cows were selected from each of Groups 1 and 2, with these two experimental groups balanced for lactation number, and for days-in-milk, mean milk yield, milk composition, body weight (BW) and body condition score (BCS) during the seven-day period prior to 16 June and 21 July 2020 (for groups 1 and 2, respectively).

Throughout the experimental feeding period all cows were offered 8.0 kg concentrate per day, with 1.0 kg/day of this offered via an in-parlour feeding system (0.5 kg at each milking) and the remaining 7.0 kg/day offered via an out-of-parlour feeding system. The ingredient composition of the concentrate was as follows (g/100 g): maize meal,

16.0: wheat, 9.5; sugar beet pulp, 13.0; wheat feed, 2.4; rapeseed meal, 13.0; maize gluten, 12.9; soya hulls, 12.4; maize distillers, 9.8; molasses, 6.5; palm fatty acid distillate, 0.6; protected fat (*Megalac, Volac Wilmar, Hertfordshire, UK*), 0.5; mineral and vitamin pre-mix, 3.4. Cows had access to fresh water at all times.

Sward management, grass cutting and silage production

A 21-ha land block, comprising a perennial ryegrass (*Lolium perenne L.*) based sward, was used within the experiment. It was planned that the study would start April 2020 with primary growth herbage, however restrictions in place during spring 2020 due to the global Covid pandemic resulted in the start date being delayed by approximately 10 weeks. As a result, primary growth herbage was harvested from across the entire land block on 4 May 2020 (for non-experimental silage), with inorganic fertilizer applied as calcium ammonium nitrate (CAN) at a rate of 250 kg per ha post-harvest. The land block was then managed to create a 'grass wedge', to ensure that herbage was at the target cutting height during the early phase of the study. To achieve this, the land block was divided into four equal sized sub-blocks and commencing approximately 16 days after the harvest of the primary growth herbage, herbage from blocks 2, 3 and 4 were harvested at approximately one-week intervals and removed. Following these harvests, inorganic fertilizer was applied as CAN at a rate of 20, 30 and 40 kg N per ha (blocks 2, 3 and 4, respectively).

Cows on the ZG treatment were offered fresh 'zero-grazed' grass. Herbage was harvested daily between 09.00 h and 10.00 h using a zero-grazer (*Grass Tech GT80, Future Grass Technology, Carlow, Ireland*), with herbage harvested from adjacent strips each day (approximately 0.2 ha harvested daily) moving across the block of land. Harvested herbage was deposited on a clean silo floor and then approximately half of the harvested herbage was transferred into a diet feeder with no cutting knives in place (*Redrock Varicut, Redrock, Armagh, Northern Ireland*). Grass was then transferred directly from the diet feeder and offered to the group via a series of feed-boxes mounted on weight scales (at between 09.30 h and 10.30 h), with individual cows accessing fresh grass in these boxes via an electronic identification system, thus enabling individual cow intakes to be recorded daily (*Controlling and Recording Feed*)

Intake, Bio-control, Rakkestad, Norway). The remaining grass was offered at approximately 15.00 h when cows were absent from the house during milking. Allocating the fresh grass on two occasions each day was necessary as the feed-boxes were unable to hold sufficient fresh grass for a 24 h period due to its bulkiness. Uneaten grass was removed the following day at approximately 08.00 h. To ensure ad-libitum consumption, grass was offered at 107% of the previous day's intake.

On one day each week (normally Tuesday, but occasionally delayed by 1 – 2 days due to adverse weather) during the 12-week period during which zero-grazing took place, a block of approximately 1.4 ha was mown from the area alongside that being zerograzed, using a grass mower (Claas Disco 3200C, Suffolk, UK) between 10.00 h and 13.00 h (depending on weather). Following a period of field wilting (average 15 h, SD 13.7 h: target herbage DM content of 300 g/kg DM), grass was ensiled in round bales (Krone round pack 1250 Multi cut baler, Leeds, UK). The baler did not permit application of a silage additive. Grass silage bales were stored on a concrete apron for a five-week period prior to feeding. This approach of making silage weekly was adopted to ensure that the experiment objective, that herbage harvested for ensilage should be similar in composition to that harvested with the ZG treatment, was achieved. These bales were then offered to cows on SIL. Each day sufficient silage was placed in the diet feeder, mixed for 5 minutes to ensure consistency, and then transferred directly from the diet feeder and offered to the group via the feed boxes mounted on weight-scales. Individual cows were able to access silage in these boxes via an electronic identification system, enabling individual cow intakes to be recorded daily. Silage was offered once daily between 09.30 h and 10.30 h at 107% of the previous day's intake, while uneaten grass silage was removed the following day at approximately 08.00 h. Silage bales were offered in the same order that they were produced, with bales produced during the first week offered during the first seven days of the silage feeding period etc. Excess bales not required in any week were discarded.

Harvesting for both zero-grazing and silage production commenced on block 1, followed by block 2, 3 and 4, with the rotation then returning to block 1. Target preharvest herbage mass was 4000 – 4400 kg DM/ha above ground level (approximately 2400 - 2800 kg DM/ha above 4.0 cm). If pre-harvest herbage mass was in excess of 4600 kg DM/ha, this herbage was removed and not offered within this experiment. On one occasion, a slow rate of regrowth necessitated herbage being harvested from a 'non-experimental' land block for one seven-day period. Following each harvest, inorganic fertiliser (CAN) was applied at a rate of 40 kg N per ha.

Sward measurements

An assessment of pre-cutting herbage mass was made on one occasion each week on the same day that herbage was cut for silage production. Three 4.8 m × 1.2 m strips were cut from randomly selected areas in the swards prior to harvesting zero grazed grass or mowing grass for ensilage. These strips were mown at a height of approximately 4 cm using a reciprocating knife-bar mower (*Agria, Moeckmuehl, Germany*), and herbage collected and weighed. A subsample of grass collected from each strip was oven dried for 48 h at 60 °C for determination of DM content, and the dry grass discarded.

Cow measurements

All cows were milked twice daily (between 06.00 and 08.00 h and between 15.00 and 17.00 h) throughout the experiment using a 50-point rotary milking parlour (*Boumatic, Madison, USA*). Milk yields were automatically recorded at each milking and a total daily milk yield for each 24-hour period calculated. Milk samples were collected during two consecutive milkings each week, treated with a preservative tablet (*Lactab Mark III, Thompson and Cooper Ltd, Runcorn, UK*) and stored at 4°C until analysis (normally within 48 hours). Milk samples were analysed for fat, protein and lactose concentrations using mid-infrared spectroscopy (*Milkoscan CombifossTM7, Foss Electric, Hillerød, Denmark*), and a weighted concentration of each constituent determined for the 24-hour sampling period. The gross energy (GE) content of milk was calculated as described by Tyrrell and Reid (1965):

Energy correct milk yield (ECM; kg day) was calculated assuming the GE content of 1 kg 'standard milk' to be 3.1 MJ/kg (i.e., for milk containing 4.0% fat, 3.2% crude protein,

and 4.8% lactose), as described by Muñoz et al. (2015), according to the following equation:

Energy corrected milk (ECM, kg/day) = Milk yield (kg/day) × milk energy content (MJ/kg) / 3.1

In addition, a further milk sample was collected during weeks 4, 8 and 12 of each experimental period (am and pm samples combined to provide a representative daily sample). The sample was then stored at -20°C until analysis. Samples were analysed for milk fatty acids (FA), as follows: milk fat was extracted from 1.0 ml of homogenised milk using a chloroform methanol extraction method (Bligh and Dyer, 1959), and FA determined as methyl esters (FAME). The FA composition was determined using gasliquid chromatography, with an aliquot (1.0 ul) of the FAME extract injected onto a CP Sil88 capillary column (100 m x 0.25 mm id x 0.2 µm film thickness) in an Agilent 7890 gas chromatograph (both Agilent Technologies, Santa Clara, USA), equipped with a temperature programmable injector operated in the split mode and a flame ionisation detector. The oven was initially held at 50°C for 4 min then ramped at 8°C/min to 110°C, then 5°C/min to 170°C (hold time 10 min) and finally ramped at 2°C/min to 225 °C (hold time 30 min). Fatty acids were identified by their retention time with reference to commercially available FA standards (37 Supelco FAME mix) and individual standards for those not in the mix (SigmaAldrich Co. Ltd., Gillingham, UK), and were quantified using C13 FAME as an internal standard.

Individual cow BW was recorded twice daily (immediately after each milking) using an automated weighbridge, with a mean weekly BW for each cow determined. Throughout the experimental period the, BCS of each cow was assessed fortnightly by a trained technician, as described by Edmonson et al. (1989).

Blood samples were collected from the tail of each cow, prior to feeding, during week 4, 8 and 12 of each experimental period. Two samples were collected, one in a heparincoated tube and one in a fluoride oxalate tube and centrifuged at 3000 rpm for 15 mins to isolate plasma. Plasma samples were stored at -20 °C until analysis. Plasma β hydroxybutyrate (β HB) concentrations were determined according to McMurray et al. (1984), and plasma glucose concentrations were determined using the hexokinase method (*Roche Diagnostics Ltd.*). Plasma non-esterified fatty acid (NEFA) concentrations were determined using WaKo kits (*Wakop Chemicals GmbH, Neuss, Germany*). Plasma urea concentrations were analysed using Kinetic UV method (*Roche Diagnostics Ltd., Burgess Hills, UK*).

On day-11 of each of the two treatment periods, four cows from each treatment were administered with an indwelling rumen pH bolus (*smaXtec, Graz, Austria*). The bolus was calibrated to pH 7.0 at 37 °C as per the manufacturers' instructions approximately one hour prior to administration via the esophagus using an appropriate balling gun (*smaXtec, Graz, Austria*). These boluses measured and stored reticulo-rumen pH and temperature data at 10-minute intervals, creating 144 data points per 24 h period. Reticulo-ruminal pH and temperature measurements were collected until the end of the experimental period, with measurements during the first day following administration excluded from the analysis.

Feeding behaviour, including the duration of feeding box occupation (feeding time) was automatically recorded daily for each cow via the feed intake recording system - described earlier. An IceRobotics® IceQube® automatic activity sensor (IceRobotics Ltd., Edinburgh, Scotland, UK) was fitted to the lateral side of the right rear leg above the metatarsophalangeal joint of each cow. Data from these sensors (total lying/standing time, number of lying/standing bouts, and duration of lying/standing bouts and step count of each cow on a daily basis) was downloaded automatically each time the cow entered the milking parlour. Motion index, a demonstration of leg activity derived from the sum of the measured net acceleration in the three dimensions minus an offset for gravity (Maselyne et al., 2017), was also recorded. Leg mounted activity sensors were checked regularly throughout the experiment to ensure skin integrity remained intact.

Feed analysis

A sample of fresh grass (ZG) and grass silage (SIL) offered was taken daily throughout the experiment and dried at 60°C for 48 h to determine oven DM content. Samples dried on three days each week were combined to give a single weekly sample. The weekly grass samples were milled and analysed for neutral detergent fibre (NDF), acid detergent fibre (ADF), ash, GE, nitrogen (N) and water-soluble carbohydrate (WSC) content. Similarly, the weekly silage samples were analysed for NDF, ADF and ash concentrations. Each week a fresh silage sample was analysed for GE, N, pH, ammonia-N and volatile components. In addition, the metabolisable energy (ME) content of a fresh silage sample and fresh grass sample was predicted each week using near infrared reflectance spectroscopy (NIRS) as described by Park et al. (1998). The concentrate offered was sampled weekly, dried at 60°C for 48 h to determine ODM content, and samples combined for each two-week period. Combined samples were milled through a 0.8 mm sieve, and subsequently analysed for N, NDF, ADF, ash, and GE, while samples analysed starch concentrations were milled using a 0.5 mm sieve. Fatty acid content of dietary components were not measured in this study due to budgetary constraints. All chemical analysis of the feedstuffs offered were undertaken as described by Purcell et al. (2016).

Statistical analysis

For each individual cow, mean weekly data for DMI, milk yield, milk composition, BW, reticulo-rumen pH and reticulo-rumen temperature, fortnightly BCS data, and blood metabolite data and milk fatty acid data (week 4, 8, 12), were analysed using REML analysis using a repeated measures mixed model, with the model containing the following terms as fixed effects: pre-experimental data (covariate) + week of study (1 -12) + treatment + week of study × treatment, while cow and cow within week of study were included as random effects (full statistical models indicated in Supplementary Material S1). Appropriate pre-experimental variables (data collated in the two weeks prior to the start of the study period) were included as covariates when analysing corresponding dependent variables in the REML analysis which included preexperimental total DMI as a covariate in the analysis of forage and total DMI variables, and pre-experimental milk yield, fat yield and protein yield as covariates in the analysis milk constituents, while pre-experimental BW and BCS were used as covariates in BW and BCS analysis. Mean weekly pedometer data were analysed using a REML model with treatment included as a fixed effect in the model. Where differences detected were significant (P<0.05), these were subject to Fisher's protected least significant difference (PLSD) test. The adequacy of all models was assessed by visual inspection of the appropriate residual plots. Data were analysed using GenStat V.19.1 (VSN international, Oxford, UK).

Results

Meteorological data

Meteorological data was recorded at an on-site weather station. During the period when herbage was being harvested (16th June to 8th September 2020) mean daily temperatures were 13.7, 13.3, 14.0 and 12.3 °C for the months of June, July, August and September, respectively, while mean daily rainfall was 4.7, 3.3, 5.8 and 5.2 mm for June, July, August and September.

Herbage mass and composition

The mean pre-cutting herbage mass (above 4 cm) across the 12-week period was 2671 and 2884 kg DM/ha for the zero-grazing and silage treatments, respectively. Zero-grazed grass had a mean ODM content of 156 g/kg, compared to 273 g/kg for grass silage (Table 1). However, the mean DM content of the silage offered during the first 7 weeks of the study (311 g/kg DM) was numerically higher than that offered during weeks 8 - 12 of the study (189 g/kg DM). In contrast, the mean DM content of fresh grass offered was more consistent during the study period, averaging 161 g/kg DM during weeks 1 - 7, and 135 g/kg DM during weeks 8 – 12. Herbage protein levels were numerically lower with the grass silage treatment (157 vs 143 g/kg DM), while ADF levels were the same for both forages. Silage offered had an ME content that was on average 0.3 MJ higher than that of the zero-grazed grass (11.3 and 11.0 MJ/kg DM).

Animal performance

Average forage DMI and total DMI over the experimental period was higher for cows on ZG than SIL (P<0.001: Table 2), intakes of both parameters varied over time (P<0.001), while there was a significant treatment × week interaction (P<0.001) for both (Figure 1). During weeks 8 to 12 of the feeding period mean forage DM intakes

for cows on SIL were 4.8 kg/day less than for those on ZG (15.2 vs. 10.4 kg/day, respectively).

Cows on ZG had a higher milk yield, fat yield, protein yield, fat plus protein yield, milk protein content, milk energy content, ECM, and milk energy yield (P<0.001) than cows offered SIL (Table 2). Milk fat content was unaffected by treatment (P>0.05). Milk yield (P<0.001), fat yield (P=0.014), protein yield (P<0.001) and fat plus protein yield (P<0.001) all varied over time, while there were significant treatment × week interactions for each of milk yield, milk fat content, milk protein content and fat plus protein yield (Figure 2a-2d).

Total concentrations of C4:0 – C15:0, and total n-7 UFA in milk, were unaffected by treatment (P>0.05). Concentrations of C18:0, C18:1*cis*-9, conjugated linoleic acid (CLA), C18:2*cis*-9, *trans*-11 (P<0.001), C18:2*cis*-9,12 (P<0.001) and C20:0 (P=0.009) were greater in milk produced from cows on ZG (Table 2). Cows on ZG had higher concentrations of total monounsaturated fatty acids (MUFA, P<0.001), total polyunsaturated fatty acids (PUFA, P<0.001), total n-3 UFA (P<0.001), total n-6 UFA (P<0.001), and total n-9 UFA (P<0.001), compared to SIL. Milk produced from cows offered SIL had a greater concentration of C16:0 (P<0.001), and as a consequence these cows produced milk with a greater total saturated fatty acid (SFA) content and a greater saturated: unsaturated fatty acid ratio (P<0.001). The FA concentrations of milk produced varied over time (P<0.01) (except for C4:0 to C15:0, C18:1*cis*-9, total MUFA, total n-9 UFA: P>0.05). There were significant treatment × week interactions (P<0.05) for all milk FA measured with the exception of C16:0 and C18:0 (P>0.05, Table 2).

Treatment had no effect on mean BW (629 kg) and mean BCS (2.3) of cows over the experimental period. However, BCS did vary over time (P<0.001) and there was a significant treatment × week interaction (P=0.002, Table 2), with cows on SIL gaining body condition from earlier in the study (from approximately week 6 onwards) compared to those on ZG (approximately week 10 onwards).

Treatment had no effect on plasma glucose and plasma urea concentrations (Table 3: mean 3.78 and 4.38 mM, respectively). However, cows on ZG had greater plasma β HB and NEFA concentrations (P=0.023 and P<0.001, respectively) than cows on SIL.

Plasma β HB, NEFA and urea concentrations decreased during the experimental period (P<0.001). There was a significant treatment × week interaction for plasma β HB, with concentrations at weeks 4, 8 and 12 being 0.48, 0.41 and 0.44 mM for cows on ZG, and 0.44 and 0.43, 0.34 mM for cows on SIL, respectively (Table 3: P<0.001). There was also a significant treatment × week interaction for plasma urea, with concentrations for cows on ZG at weeks 4, 8 and 12 being 4.68, 5.79 and 3.58 mM, respectively, while values for cows on SIL were 5.42, 3.00 and 3.82 mM, respectively.

Cows on SIL had a higher mean reticulo-rumen pH and higher maximum reticulorumen pH (P=0.020 and P=0.038, respectively) compared to cows on ZG (Table 3). Minimum reticulo-rumen pH recorded did not differ between treatment groups (mean 5.84). Mean reticulo-rumen temperature was greater for cows on ZG (P=0.005), however minimum and maximum reticulo-rumen temperature recorded did not differ between treatment groups (mean 33.0 and 40.1 °C, Table 3).

There was a tendency for cows on ZG to spend more time feeding compared to cows on SIL (P= 0.068, Table 4). Cows on SIL had a 7.9% greater daily lying time and 7.4% lower daily standing time than those on ZG (P=0.017 and P=0.011, respectively). There was a tendency for motion index to be lower for cows on SIL compared to cows on ZG (P=0.061). There were no significant differences in the number of transitions (P=0.105) or step count (P=0.174) between treatments groups.

Discussion

In many previous studies comparing the performance of cows offered either fresh grass or grass silage, the forages were harvested at different growth stages and from different swards (Keady et al., 1996; Keady and Murphy, 1998; Mohammed et al., 2009). However, the current study had a specific objective of comparing performance when cows were offered silage produced from herbage at the same growth stage and from the same sward, as herbage offered via zero-grazing. To facilitate this the silage was harvested weekly from within the same sward as zero-grazed grass was harvested, meaning that within any 'week' herbage for zero-grazing was normally harvested a maximum of either three days before or after herbage harvested for silage. This objective appears to have been achieved, with the zero-grazed grass and grass

silage having similar crude protein (157 vs 143 g/kg DM), ADF (264 g/kg DM for both) and ME (11.0 vs 11.3 MJ/kg DM) concentrations. That crude protein of the silage was marginally lower may reflect the loss of N components during ensilage, while the higher ME content of the silage may reflect the presence of higher energy volatile components. The mean lactic acid concentration of 78 g/kg DM suggests a lactic acid bacteria dominated fermentation, while an ammonia N concentration of 68 g/kg total N suggests protein had not been extensively degraded to ammonia. Nevertheless, the two forages differed in DM content. This reflects the fact that in general, zero-grazing is not weather dependent, and that fresh grass can be harvested and offered at a low DM content. In contrast, ensiling immature herbage with a low DM is extremely challenging, and for this reason silage was allowed to wilt prior to ensiling, with a target DM content of 25 – 30%. This was generally achieved prior to August, while thereafter decreasing day-length and reduced solar radiation made this more challenging, especially as grass was not tedded during the study.

Dry matter intake

While mean forage DMI over the entire experimental period was lower with SIL than ZG, Figure 1 highlights that there was a significant treatment x time interaction, with the difference in forage DMI only becoming evident from week 8 of lactation onwards (mean daily forage DM intakes during weeks 1 – 7 were 15.1 and 14.3 kg for ZG and SIL, respectively, while respective values during weeks 8 – 12 were 15.2 and 10.4 kg). In common with the results during weeks 1 - 7 of the current study, intakes of fresh grass and ensiled grass did not differ in a number of previous studies (Cushnahan and Mayne, 1995; Keady et al., 1996; Keady and Murphy, 1998). In contrast, Mohammed et al. (2009) observed intakes of grass silage to be 2.3 kg/day lower than intakes of zero-grazed grass, although in that study grass silage and zero-grazed grass were harvested after a five and three week regrowth interval, respectively. The lower silage intake later in the season in the current study is likely due to the lower DM content of the silage (299 vs 230 g/kg DM during weeks 1 – 7 and weeks 8 – 12, respectively), with silage DM a key determinant of silage DMI (Steen et al., 1998; Huhtanen et al., 2007). Silage digestibility is another important driver of silage DMI, with Keady et al. (2013) reporting that a 10 g/kg increase in D-value increased silage DMI by 0.22 kg/day. However, there was only a modest change in silage D-value between the early and later part of the study (714 vs 702 g/kg DM during week 1-7 and weeks 8-12, respectively) and therefore D-value is unlikely to have impacted silage DMI in this instance. The lower CP content of the silage in the latter part of the study (148 vs. 136 g/kg DM, during week 1-7 and 8-12 respectively) may also have contributed to a reduction in DMI (Steen et al., 1998).

Milk production and composition

The lower mean milk yield over the study period with SIL appears to be largely due to a reduction in milk yield from week eight onwards, mirroring the trends observed in silage DM intake. Previous studies have reported milk yield was reduced on average by 1.8 kg/day for cows offered grass silage compared to fresh grass, but with no associated difference in DMI (Keady et al., 1996; Keady and Murphy, 1998). These authors attributed the lower milk yield with the silage diets to, changes in nitrogenous components of the forage and decreased energy availability from volatile fatty acids for the rumen microflora. Similarly, Mohammed et al. (2009) reported a 4.0 kg/day reduction in milk yield when cows were offered grass silage compared to zero-grazed grass, with this attributed to the lower forage and total DMI with the silage treatment. In contrast, previous research has demonstrated that milk yield was unaffected when cows were offered either fresh grass or ensiled forage harvested from the same sward (Cushnahan and Mayne, 1995). The reduction in milk yield in the current study is likely a direct consequence of the lower DMI observed later in the season. It is possible that this reduction might have been reduced if herbage has been treated with a silage additive, with a recent meta-analysis reporting a 0.26 kg/day increase in DMI and a 0.37 kg/day increase in milk yield when inoculants were used (Oliveira et al., 2017). However, the round baler used in this study did not facilitate application of an additive. Nevertheless, a recent survey of silage-making practices on Northern Irish dairy farms found that only 47% of farms routinely used a silage additive (Ferris et al., 2022) meaning the silage offered in this study was not unreflective of local practice.

The lower milk protein content and associated reduction in milk protein yield with SIL agrees with the findings of previous studies in which cows were offered either grass silage or fresh grass-based diets (Keady et al., 1996; Younge et al., 2004; Mohammed et al., 2009). While a lower energy intake can contribute to a lower protein content, milk protein content with SIL was lower throughout the experiment, including during weeks 1-7 when intakes did not differ between treatments. This suggests that the

reduction is likely associated with the silage fermentation process. Although the protein content of the fresh grass and grass silage were relatively similar (157 and 143 g/kg DM, respectively), forage protein is extensively changed during ensilage by proteolysis and deamination. As a consequence the solubility of silage protein in the rumen is increased, leading to high concentration of ammonia-N in the rumen and increased urinary N losses, with an associated reduction in protein utilisation efficiency (Keady and Murphy, 1998; Charmley, 2001). Indeed, Tamminga et al. (1991) found that on average 61% of CP in grass silages was instantly solublised in the rumen, with only 9% of silage protein undegradable. In addition, WSC is converted to lactic acid and VFAs during the silage fermentation process, thus reducing energy available to rumen microbes for microbial protein synthesis (Jaakkola and Huhtanen, 1992; Keady et al., 1996; Keady and Murphy, 1998; Charmley, 2001). As a result, the flow of microbial protein to the small intestine is reduced, with metabolisable protein supply from grass silage based diets lower than from fresh grass based diets (Younge et al., 2004). Indeed, Keady and Murphy (1998) estimated a 0.10 proportional reduction in microbial CP synthesis in cows offered ensiled herbage rather than fresh grass.

In contrast to the findings of Keady et al. (1996) that milk fat concentration of cows offered an untreated silage compared to fresh grass was reduced by 6.1 g/kg, milk fat content was unaffected by treatment in the current study. The latter likely reflects the similar fibre concentration of the two forages, acetate produced from fibre fermentation being a lipogenic precursor. While lactic acid produced during ensiling may be metabolised to propionate rather than acetate or butyrate, thus reducing precursors for milk fat synthesis in the rumen (Charmley, 2001), this did not appear to be the case in this study. The decrease in milk fat yield of cows offered SIL in the current study reflects the reduction in milk yield with this treatment in the latter part of the study.

While milk lactose levels are generally not influenced by diet, concentrations were higher with ZG in this study, perhaps due to greater forage DMI and consequently energy intake of cows offered fresh grass. The high WSC content of fresh grass compared to silage (188 vs 35 g/kg DM, respectively) is likely to have promoted lactic acid production in the rumen, which can result in high milk lactose content (De La Torre-Santos et al., 2020).

Milk fatty acid composition

While total milk fat content was unaffected by treatment, the FA profile of milk produced differed (Table 2). C4:0 – C15:0 FA are formed through *de novo* synthesis, largely by chain elongation using acetate derived mainly from fiber breakdown (Grummer, 1991). That total concentrations of C4:0 – C15:0 did not differ between treatment groups is not surprising and likely reflects the similar fiber concentrations with ZG and SIL diets. However, C16:0 concentration (partly synthesized *de novo* and partly from preformed FA in the diet) was lower in milk of cows offered fresh grass, in agreement with the findings of Kalač and Samková (2010). This was the main driver of the decreased total SFA concentration of cows on ZG.

With both treatments the cutting process (either by the zero grazer (ZG) or by the grass mower (SIL)) will have exposed plant cells to air, causing oxidation and initiating lipolysis by plant lipases, and resulting in an immediate loss of PUFA (Dewhurst et al., 2006; Elgersma et al., 2006). While extensive wilting and field drying has been shown to decrease total FA and PUFA content (Dewhurst and King, 1998; Van Ranst et al., 2009a; Van Ranst et al., 2009b), a wilting period of less than 24hrs has been shown to not affect FA composition (Arvidsson et al., 2009), with the average wilting period in the current study 15 h. Nonetheless, extensive lipolysis takes place during ensiling (Kalač and Samková, 2010), while free-fatty acids formed during the fermentation process are oxidised at feed-out (Khan et al., 2009), with this likely explaining the lower concentration of linoleic acid, total MUFA and PUFA in the milk of cows offered SIL. Concentrations of CLA were greater in milk of cows offered fresh grass compared to silage, which may be attributable to increased rumen bio-hydrogenation of UFA in grass and fresh forage compared to ensiled forages (Palmquist et al., 2005; Couvreur et al., 2006; O'Callaghan et al., 2016). In agreement with previous studies, the proportion of UFA in the milk of cows offered fresh grass increased at the expense of SFA, while milk of cows offered silage had a greater proportion of SFA: UFA (Couvreur et al., 2006; Mendoza et al., 2016; O'Callaghan et al., 2016).

Blood metabolites, rumen function and behaviour

Increased concentrations of plasma β -hydroxybutyrate (β HB) and non-esterified fatty acids (NEFA), as observed with ZG, are often associated with dietary energy deficit and subsequent mobilization of body fat reserves (Whitaker et al., 1983; Whitaker et

al., 1993). While milk energy output was higher with ZG, DMI was also higher, while neither mean BW nor BCS differed between treatments. There was no evidence of loss of BCS with either treatment, although cows on SIL gained body condition faster than those on ZG. The fact that plasma concentrations of both NEFA and βHB for cows on both treatment groups were within the optimal range reported for lactating dairy cows under UK conditions (Macrae et al., 2006; Whitaker, 2004) suggests that the differences reported were of little biological importance. The absence of a treatment effect on plasma urea concentration reflects the similar mean diet protein concentrations with ZG and SIL (157 and 143 g/kg DM, respectively), elevated blood urea concentrations normally reflecting increased dietary protein levels (Kohn et al., 2005; Colmenero and Broderick, 2006). Plasma urea concentrations did decrease over time with both treatment groups, reflecting the reduction in forage CP content during the season. That urea concentrations of cows offered SIL decreased to a greater extent likely reflects the greater reduction in forage CP content with the SIL treatment and the substantial lower silage intake later in the season.

A number of rumen parameters differed between treatments (reticulo-rumen pH, maximum reticulo-rumen pH and reticulo-rumen temperature), although actual differences were small. The lower average reticulo-rumen pH observed with ZG may reflect the higher concentration of soluble sugars in the fresh grass compared to grass silage, and their rapid fermentation (Dijkstra et al., 2012). However, the minimum reticulo-rumen pH recorded (5.8) did not differ between treatment groups, indicating that neither group experienced sub-acute ruminal acidosis (SARA) (Rafferty et al., 2019).

While daily feeding time tended to be greater with ZG, total forage intakes were also greater, meaning that actual intake rates were lower (19.7 and 21.9 minutes/kg DMI, with ZG and SIL, respectively). This is perhaps unexpected as fresh grass offered with ZG was un-chopped and was also more-bulky than the silage, with both of these likely to increase the time cows spent eating (Garmo et al., 2008; Grant and Ferraretto, 2018). The tendency towards a longer feeding time with ZG will account in part for the greater standing time and subsequently lower lying time. Lying is a priority behavior for dairy cows, and cows prefer to ruminate when lying down (Schirmann et al., 2012).

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Nevertheless, total lying time of both treatment groups were within the range reported for loose house cows by Maselyne et al. (2017).

Practical implications

The study was designed to examine cow performance when fresh grass harvested daily was replaced by the same grass following ensiling. While the performance benefits of zero-grazing are recognized, the process requires grass to be harvested daily (or twice daily on many farms), a labour-intensive process, and one that normally requires the farmer to have his own zero grazer. In contrast, grass at a similar growth stage as is used for zero-grazing could be harvested for ensilage approximately once every 4 weeks (herbage for SIL was harvested weekly in this study to ensure similar growth stages of both forages), a process that would require a significant labour input on 5 - 6 occasions during the harvest season. Furthermore, silage making is undertaken by contractors on many Northern Irish dairy farms, with over 60% of farms questioned in a recent survey indicating that they 'normally' used a contractor (Ferris et al., 2022). For many farmers this would eliminate the need to own their own harvesting equipment. Nevertheless, the silage made would still need to be removed from the pit and offered to cows on a daily basis, although with zero-grazing, the bulkiness of the feed may necessitate the fresh grass being placed in front of the cows twice daily.

Zero-grazing, like conventional grazing, requires the sward to be maintained at the optimum growth stage for cutting throughout the entire growing season, something that can be a particular challenge early in the season. In contrast, managing grass for ensilage does not require the same daily management decisions associated with zero grazing. Furthermore, zero-grazing is generally not weather dependent, and fresh grass can be harvested and offered during periods of wet weather. Silage production on the other hand, especially when it involves lush immature grass, is heavily weather dependent, with a dry matter of at least 25% targeted within this study. In addition, if harvesting was delayed due to a prolonged period of wet weather, herbage D-value will fall by an average of 0.5% units per day (Keady et al., 2013), with an associated impact on cow performance. Wet weather does not normally prevent zero-grazing from taking place.

Forage losses associated with the different systems are likely to differ. Field losses with zero-grazing, which combines cutting and pick-up of grass in a single operation, are likely to be low. However, once harvested and placed in a pile along a feed barrier, fresh grass can deteriorate rapidly particularly in warmer conditions, and as such requires excellent management to avoid this. Research regarding ME losses of zero-grazed grass is not available. In contrast, silage production may require the herbage to be tedded out, and rowed up, with in-field DM losses estimated to be between 4-11%, with further DM losses in the silo due to respiration and fermentation in the silo estimated to be 5-13%, and at feed out due to spoilage and aerobic deterioration estimated to be between 3-18%, although the extent of losses will be determined by management practices (Borreani et al., 2018). Silage may also be expected to lose about 0.5 MJ ME/kg DM between the field and feed-out (Wilkinson, 2015).

Conclusion

In the current study, the milk yield of dairy cows offered either fresh zero-grazed grass or ensiled forage harvested from the same sward at a same growth stage, did not differ early in the season. However, later in the season cows offered silage had a lower milk yield than those offered zero-grazed grass. This difference likely reflected the lower intake of silage later in the season. Thus in early season zero-grazing could be replaced by offering silage produced from herbage at the same growth stage, without loss in performance, although this is unlikely to be true in later season.

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	Zero- grazed grass	Range	(s.d.)	Grass silage	Range	(s.d.)	Concentrate	(s.d.)
Oven dry matter (g/kg)	156	(111-227)	32.7	273	(168-381)	67.5	893	6.0
VCODM (g/kg)	-	-	-	280	(172-387)	68.0	-	-
Crude protein (g/kg DM)	157	(133-204)	18.7	143	(120-181)	16.7	176	4.9
Ash (g/kg DM)	78.8	(58.2-98.9)	9.8	90	(69-114)	11.7	89	2.5
Acid detergent fibre (g/kg DM)	264	(224-303)	24.4	264	(220-286)	19.1	154	7.0
Neutral detergent fibre (g/kg DM)	505	(422-581)	48.1	476	(406-519)	30.7	311	12.5
Gross energy (MJ/kg DM)	18.7	(18.5-18.9)	0.1	18.8	(16.6-20.2)	0.99	18.0	0.10
WSC (g/kg DM)	188	(148-233)	26.7	35 ¹	(14-59)	16.5	-	-
Starch (g/kg DM)	-	-	-	-	-	-	198	18.4
D-value (g/kg DM)	690	(640-720)	23.5	708	(680-770)	24.4	-	-
Metabolisable energy (MJ/kg DM) ¹	11.0	(10.2-11.5)	0.38	11.3	(10.9-12.3)	0.39	-	-
рН	-	-	-	4.1	(3.9-4.3)	0.16	-	-
Lactic Acid (g/kg DM)	-	-	-	78	(43-139)	32.6	-	-
Acetic acid (g/kg DM)	-	-	-	14.2	(8.4-21.3)	4.2	-	-
Butyric acid (g/kg DM)	-	-	-	0.2	(0.0-2.8)	0.82	-	-
Ethanol (g/kg DM)	-	-	-	9.9	(0.9-25.4)	6.67	-	-
Ammonia (g/kg total N)	-	-	-	68	(48-97)	1.5	-	-

Table 1 Chemical composition of zero-grazed grass, grass silage and concentrates offered to cows on ZG and SIL.

VCODM = Volatile corrected oven dry matter; D-value = Digestible organic matter in the DM; WSC = water soluble carbohydrate ¹Predicted using NIRS, Metabolisable energy (ME)= D-value (%) \times 0.16

	Т	reatment		P-value				
					Treatment			
	ZG	SIL	SED	Treatment	Week	X		
Earaga DML (kg/d)	15 1	10.7	0.20	-0.001	-0.001			
Concentrate DML (kg/d)	10.1	12.7	0.29	<0.001	<0.001	<0.001		
	1.1	10.6	-	-0.001	-	-		
Nille vie Id (kg/d)	22.2	19.0	0.35	<0.001	<0.001	<0.001		
Milk yleid (kg/d)	27.1	25.1	0.55	< 0.001	<0.001	<0.001		
	45.0	44.7	1.01	0.447	0.014	<0.001		
Protein (g/kg)	37.2	34.5	0.52	<0.001	<0.001	<0.001		
Lactose (g/kg)	47.0	46.3	0.31	0.035	0.017	<0.001		
Fat yield (kg/d)	1.22	1.11	0.031	<0.001	<0.001	0.040		
Protein yield (kg/d)	0.99	0.87	0.025	<0.001	<0.001	<0.001		
Fat + protein yield (kg/d)	2.23	1.96	0.052	<0.001	<0.001	<0.001		
Milk energy (MJ/kg)	3.44	3.25	0.038	<0.001	<0.001	<0.001		
Energy Corrected Milk (kg/d)	29.6	26.5	0.64	<0.001	<0.001	<0.001		
Milk energy yield (MJ/d)	91.8	82.1	1.98	<0.001	<0.001	<0.001		
Milk FA concentrations (g/100 g total FA identified)								
Total C4:0 to C15:0	24.56	25.51	0.849	0.301	0.187	0.027		
C16:0	32.29	38.07	0.813	<0.001	<0.001	0.118		
C18:0	11.13	9.34	0.384	<0.001	0.002	0.217		
C18:1 <i>cis</i> -9	22.01	18.33	0.651	<0.001	0.243	0.007		
CLA, 18:2 <i>cis</i> -9, <i>trans</i> -11	1.24	0.87	0.075	<0.001	<0.001	<0.001		
C18:2 <i>cis</i> -9,12	1.63	1.35	0.064	<0.001	<0.001	<0.001		
C20:0	0.16	0.15	0.006	0.009	<0.001	<0.001		
Total Saturated	67.88	73.08	0.851	<0.001	<0.001	<0.001		
Total MUFA	25.32	21.87	0.652	<0.001	0.060	0.003		
Total PUFA	2.75	2.14	0.098	<0.001	<0.001	<0.001		
Total n-3 UFA	0.80	0.49	0.028	<0.001	<0.001	<0.001		
Total n-6 UFA	1.95	1.65	0.073	< 0.001	<0.001	< 0.001		
Total n-7 UFA	2.05	2.16	0.105	0.298	< 0.001	0.026		
Total n-9 UFA	22.07	18.38	0.652	<0.001	0 257	0.007		
Saturated: Unsaturated ratio	2 45	3.08	0.117	<0.001	0.001	<0.001		
Body weight (kg)	632	625	13.2	0 450	0.611	0,390		
Body condition score	2.3	2.3	0.031	0.929	<0.001	0.002		

Table 2 Effect of offering fresh zero-grazed grass (ZG) or grass silage produced from the same sward (SIL) on feed intake, milk production, milk composition, the fatty acid content of milk and body tissue reserves.

DMI = dry matter intake; FA = fatty acids; MUFA = monounsaturated fatty acids; PUFA = poly-unsaturated fatty acids; UFA = unsaturated fatty acids.

Table 3 Effect of offering fresh zero-grazed grass (ZG) or grass silage produced from the same sward (SIL) on mean blood metabolite concentrations, reticulo-rumen pH and reticulo-rumen temperature.

	Т	reatme	ent		P-values	6
	ZG	SIL	SED	Treatment	Week	Treatment × Week
Blood metabolites ¹						
Plasma βHB (mM)	0.44	0.41	0.023	0.037	<0.001	<0.001
Plasma glucose (mM)	3.66	3.90	0.177	0.185	0.195	0.366
Plasma NEFA (mM)	0.17	0.09	0.020	<0.001	<0.001	0.069
Plasma urea (mM)	4.69	4.08	0.141	0.167	<0.001	<0.001
Reticulo-rumen pH ²	6.25	6.34	0.031	0.020	<0.001	0.017
Max. pH	6.67	6.78	0.039	0.038	0.469	0.017
Min. pH	5.81	5.87	0.046	0.241	0.521	0.159
Reticulo-rumen temperature						
(°C) ²	39.1	38.9	0.052	0.005	0.021	0.001
Max. temperature (°C)	40.1	40.1	0.077	0.926	0.035	0.002
Min. temperature(°C)	33.1	32.8	0.226	0.148	0.093	0.004

 β HB = beta-hydroxybutyrate; NEFA = non-esterified fatty acid

¹Mean analysis of samples taken at week 4, 8 and 12 of the experimental period.

²Mean reticulo-rumen data collected from a sub-sample of cows (n=4/treatment) from week 3 to 12 of the experimental period.

		Treatment		
	ZG	SIL	SED	P-value
Total feeding time (min/day) ¹	298.7	277.2	11.43	0.068
Lying time (mins/day)	617.4	670.5	21.26	0.017
Standing time (min/day)	822.2	765.7	20.97	0.011
Number of transitions/day	19.77	22.38	1.567	0.105
Number of steps/day	1170	1064	76.2	0.174
Motion Index	44.16	38.21	3.074	0.061

Table 4 Total feeding time and daily activity measures of cows offered either zerograzed grass (ZG) or grass silage produced from the same sward (SIL).

¹Total duration of time spent at the feed box per day



Fig. 1. Mean weekly concentrate DMI (dashed line), forage DMI (dotted line) and total DMI (solid line) of cows offered fresh zero-grazed grass (ZG, \bullet) or grass silage produced from the same sward (SIL, \blacksquare).



Fig. 2. Mean weekly milk yield (kg/day) (a), milk fat content (g/day) (b), milk protein content (g/day) (c) and milk fat plus protein yield (kg/day) (d) of cows offered fresh grass zero-grazed (ZG, \bullet) or grass silage produced from the same sward (SIL, \blacksquare)