TITLE: The variation in morphology of perennial ryegrass cultivars throughout the grazing season and effects on organic matter digestibility

AUTHOR(S): Beecher, M., Hennessy, D., Boland, T. M., McEvoy, M., O'Donovan, M. and Lewis, E.

The variation in morphology of perennial ryegrass cultivars throughout the grazing season and effects on organic matter digestibility

M. Beecher[1,2], D. Hennessy[1], T. Boland[2], M. McEvoy[1], M. O'Donovan[1] and E. Lewis[1*]

[1]Teagasc, Animal & Grassland Research and Innovation Centre, Moorepark, Fermoy, Co. Cork, Ireland

[2]School of Agriculture and Food Science, University College Dublin, Belfield, Dublin 4, Ireland

Corresponding author:

[*]Dr. Eva Lewis, Teagasc, Grassland Science Research Department, Animal & Grassland Research and Innovation Centre, Moorepark, Fermoy, Co. Cork, Ireland

Telephone: + 353 25 42673

Fax: + 353 25 42340

Email: eva.lewis@teagasc.ie

Keywords: *Lolium perenne L.*, morphology, leaf, pseudostem, true stem, digestibility

Abstract

The grass plant is comprised of leaf, pseudostem, true stem (including inflorescence) and dead. These components differ in digestibility and variations in their relative proportions can impact sward quality. The objective of this study was to determine the change in the proportion and organic matter digestibility (OMD) of leaf, pseudostem, true stem and dead components of four perennial ryegrass cultivars (two tetraploids: Astonenergy and Bealey and two diploids: Abermagic and Spelga) throughout a grazing season. The DM proportions and *in vitro* OMD of leaf, pseudostem, true stem and dead in all cultivars were determined during 10 grazing rotations between May 2011 and March 2012. There was an interaction between rotation and cultivar for leaf, pseudostem, true stem and dead proportions. In May and June, Astonenergy had the highest leaf and lowest true stem proportion ($P < 0.05$). From July onwards there was no difference in leaf or true stem proportion between cultivars. Bealey had the highest annual mean OMD (752 g/kg) and Spelga the lowest (696 g/kg; $P < 0.05$). The OMD followed the order leaf > pseudostem > true stem > dead. Bealey had the highest combined leaf and pseudostem proportion 0.92, which explains why it had the highest OMD. In this study the tetraploid cultivars had the highest leaf and pseudostem proportion and OMD. For accurate descriptions of a sward in grazing studies and to accurately determine sward morphological composition, pseudostem should be separated from true stem, particularly during the reproductive stage when true stem is present.

Introduction

The economic success of milk production in grass based production systems is dependant on the optimal utilisation of high quality grass. With a large choice of cultivars, each with different properties, selecting the correct cultivar to sow on-farm is of major importance to producers due to its potential influence on both animal and sward productivity (Gowen *et al.*, 2003). This has led to interest in assessing the differences between perennial ryegrass cultivars in terms of animal performance and sward productivity.

Perennial ryegrass cultivars differ in their chemical composition (O'Donovan and Delaby, 2005). O’Donovan and Delaby (2005) found that there was a 4-unit difference in OMD between intermediate heading tetraploid and diploid cultivars. The higher digestibility of tetraploids compared to diploids is potentially linked to tetraploids having larger epidermal and mesophyll cells and a higher ratio of cell contents to cell wall (Sugiyama, 2005; Stewart and Hayes, 2011). Organic matter digestibility (OMD) is a key driver of metabolisable energy supply as the main factors that affect metabolisable energy are those that influence digestibility (McDonald *et al.*, 2002a). Grass quality, as evidenced by OMD, is a key driver of animal performance in grazing systems and is associated with higher overall farm profit (Shalloo *et al.*, 2007).

Several morphological components make up the grass plant and these vary in digestibility (Stakelum and Dillon, 2007). The leaf is comprised of the leaf blade (leaf) and leaf sheath; the collection of leaf sheaths on a tiller make up the pseudostem. During the reproductive stage true stem emerges upwards from the base of the tiller through the pseudostem. Digestibility is inversely related to the degree of lignification, which in turn is linked to the morphological composition of the sward. The true stem has a higher lignin content (Laredo and Minson, 1975) and lower digestibility than the leaf (Wilson, 1994; Buxton, 1996). During the reproductive stage there is a proportionally lower leaf and higher true stem content than during the vegetative stage (Buxton and Redfearn, 1997), and so swards are expected to be less digestible during the reproductive stage than during the vegetative stage. Little research has been carried out on the pseudostem component of the sward, regarding both its proportion in the sward and its digestibility. In studies that determine sward morphology the pseudostem is usually combined with the true stem (Pritchard *et al.*, 1963; Kennedy *et al.*, 2007; O'Donovan and Delaby, 2008). There is some suggestion that the pseudostem is more digestible than the true stem (Terry and Tilley, 1964). Therefore, categorising the pseudostem with true stem during morphological separations may not be appropriate. Animal output is dependent on the amount of herbage ingested and the quality of that herbage (Shalloo *et al.*, 2007), but there is a physical limit on the height to which animals can graze (Illius and Gordon, 1987). Therefore it is the grazed horizon of the sward that is of interest when determining sward morphology. There is a need to determine the proportions of the morphological components and their contribution to the overall digestibility of the sward which has the potential to aid plant breeders in achieving their target of improving plant digestibility (O’Donovan *et al.*, 2011).

The objectives of this study were to determine the contribution of leaf, pseudostem, true stem and dead to the overall plant digestibility in four perennial ryegrass swards and to identify potential selection criteria for plant breeders to improve the digestibility of the cultivars.

Materials and Methods

Study Area and Experimental Design

The study was conducted at the Teagasc, Animal & Grassland Research and Innovation Centre, Moorepark, Fermoy, Co. Cork, Ireland (50º 09'N; 8º16'W) where four paddocks were used. The soil type was a free draining acid brown earth of sandy loam-to-loam texture. In 2009 the paddocks (average size 0.8 ha) were sown with perennial ryegrass. Each of the four paddocks was sown with a different perennial ryegrass cultivar. Two tetraploid cultivars were used: Bealey and Astonenergy, with heading dates of 24 May and 31 May, respectively. Two diploid cultivars were used: Spelga and Abermagic, with heading dates of 22 May and 28 May, respectively. Soil analysis indicated that all four paddocks had a similar pH (6.5) and nutrient status (6.5 to 10 mg/l for phosphorus and 140 mg/l for potassium). The pH and nutrient status of the soils were considered adequate for intensive grassland production (Coulter and Lalor, 2008).

The four paddocks described here were part of a larger grazing study described by Wims *et al.* (2012). Swards were grazed at a targeted herbage mass of 1500 kg DM ha^{-1} and to a targeted post grazing sward height of 4 cm.

Management of each grazing single cultivar sward

There were 10 grazing rotations between May 2011 and March 2012. Rotations 1 to 8 took place in 2011 on 17 to 18 May (mid May), 6 to 8 June (start June), 25 to 26 June (end June), 9 to 10 July (mid July), 27 to 28 July (end July), 17 to 18 August (mid Aug), 15 to 16 September (mid Sept) and 11 to 16 October (mid Oct). Rotations 9 and 10 took place in 2012 on 15 to 18 February (mid Feb) and 28 to 30 March (end Mar), respectively. During the experimental period each sward received 224 kg N/ha which was applied at a similar time for all cultivars. No phosphorus or potassium fertiliser was applied.

Sward Morphology

Immediately prior to grazing, the morphological composition was determined in each of the four single cultivar grazing swards. In each sward a subsection of the paddock was identified and divided into four replicates, each measuring 361 m^2. Within each of the four replicates grass samples were taken along a diagonal at eight points. The grass samples were cut to ground level using a scissors and the vertical structure of the sward was preserved using elastic bands. The grass samples were weighed before separation into the upper and lower sward horizon: > 4 cm and < 4 cm (measured from ground level). Average fresh weight of the > 4 cm total sample collected was 238 g; average fresh weight of < 4 cm sample was 102 g. The upper layer (> 4 cm) was mixed before separating into two fractions, one of which was left intact (average fresh weight was 73 g) (from here onwards this is referred to as the “whole” sample) and the other which was manually separated into leaf, pseudostem, true stem and dead (average fresh weight was 130 g). Leaf blades were detached from the base of the pseudostem or true stem. Leaf sheaths were separated from the true stem and defined and included in the pseudostem fraction. Inflorescences, if present, were included as true stem. Dead matter was defined as any senesced material that was yellow/brown in colour. The leaf, pseudostem, true stem and dead samples, the whole samples and both the < 4 cm samples (from the whole samples and separated > 4 cm samples) were weighed fresh, and after oven-drying to determine DM content. Samples were separated within 72 hours of collection. Whilst awaiting separation grass samples were stored in a cold room (4ºC), laid out on paper towel, in open plastic bags, in order to absorb any surface moisture and avoid decay.

Chemical Analysis

The leaf, pseudostem, true stem and dead samples > 4 cm, the whole samples > 4 cm and the < 4 cm samples were oven dried at 40ºC for 48 hours in a Binder FED 720 drying oven (Binder GmbH, Tuttlingen, Germany) to determine the dry matter (DM) content. The dried grass samples were milled through a 1-mm screen using a Cyclotech 1093 Sample Mill (Foss, DK-3400 Hillerød, Denmark). The dried milled grass samples were retained for chemical analysis. There was an individual leaf sample for each replicate during each grazing rotation period (rotation). Due to insufficient sample quantity the pseudostem, true stem and dead replicate samples were bulked by morphological component using the total amount of sample collected for the first four rotations of 2011 (mid May to mid July), the last four rotations of 2011 (end July to mid Oct) and the two 2012 rotations (mid Feb and end Mar). A similarly-bulked leaf sample was also created for comparison based on the proportions of the pseudostem, true stem and dead bulked samples.

The leaf, pseudostem, true stem and dead samples and the whole samples were analysed for ash content by placing samples into a Gallenkamp muffle furnace size 3 (Thermo Fisher Scientific INC., Waltham, MA, USA) for 16 h at 500°C. The crude protein (CP) concentration of the grass samples was analysed using a Leco N analyser (Leco FP-428; Leco Corporation, St. Joesph, MI, USA). The NDF and ADF samples were analysed for neutral detergent fibre (NDF) and acid detergent fibre (ADF) with an Ankom Fibre Analyser (Ankom Technology Corporation, Macedon, NY, USA) using the method of Van Soest *et al.* (1991). Amylase and sulfite were used in the NDF process and the values of ADF and NDF are expressed excluding ash. Sample OMD was analysed using the using the *in vitro* neutral detergent cellulase method of Morgan *et al.* (1989) (Fibertec™ Systems, FOSS, Dublin, Ireland). Whole sample OMD was determined at every rotation.

Statistical Analysis

Data (leaf, pseudostem, true stem and dead proportion expressed on a DM basis; and whole samples and leaf chemical composition) were analysed using the mixed procedure (PROC MIXED) of SAS (2002). The model (outlined below) included terms for cultivar, rotation number and replicate and the interaction of cultivar and rotation number:

$$Y = \mu + C_i + R_j + C_i \times R_j + P_k(C_i) + e$$

Where: μ = mean; C_i = cultivar (i= 1…4); R_j = rotation number (j= 1…10); $C_i \times R_j$ = the interaction of cultivar and rotation number; P_k (C_i) = random effect of replicate (k= 1…4) within cultivar; e = residual error term.

Rotation number was the repeated measure. For all data the random statement specified the compound symmetry structure. The Tukey Kramer multiple range test was used for mean separation (P < 0.05). All data were first analysed for normality (PROC UNIVARIATE) in SAS (2002). The pseudostem, true stem and dead proportion data were non-parametric and were transformed using log10, exponential and sin functions respectively. The chemical composition of the bulked leaf, pseudostem, true stem and dead samples were not statistically analysed as there was only one sample for each cultivar.

Results

Morphological component proportions

Figures 1 to 4 show the > 4 cm DM proportions of leaf, pseudostem, true stem and dead in Astonenergy, Abermagic, Bealey and Spelga during the 10 grazing rotations. There was an interaction between rotation number and cultivar for leaf, pseudostem, true stem and dead proportions. Figure 1 shows that Astonenergy had a higher leaf proportion than Abermagic (mid May), Bealey (start June) and Spelga (start June and end June; $P < 0.05$). In end July Bealey had a higher pseudostem proportion than Astonenergy ($P < 0.05$; Figure 2). In mid Feb Bealey had a higher pseudostem proportion than all other cultivars ($P < 0.001$; Figure 2), Astonenergy also had a higher pseudostem proportion than Spelga ($P < 0.01$). In end March Bealey had a higher pseudostem proportion than Spelga ($P < 0.001$). Figure 3 shows that true stem proportion was lower in Astonenergy than Abermagic (mid May and end June), Bealey (start June) and Spelga (mid May, start June and end June; $P < 0.05$). From mid July onwards there were no differences between cultivars in leaf or true stem proportion. In mid July and end July Spelga had a higher dead proportion than Astonenergy and Abermagic (P < 0.05; Figure 4).

Chemical Composition

Organic Matter Digestibility

Whole sample OMD refers to the average OMD for the 10 rotations. For the whole samples there was a cultivar effect ($P < 0.05$) and a rotation effect ($P < 0.05$) on OMD, but no cultivar by rotation interaction. Bealey had a higher OMD (752 g/kg ± 10.4) than Spelga (696 g/kg ± 10.4). Astonenergy (724 g/kg ± 10.4) and Abermagic (715 g/kg ± 10.4) were intermediate. Organic matter digestibility was higher in mid May, start June, end June, end July and mid Aug than in mid Oct and mid Feb. Higher

OMD values were recorded in mid Sept than mid Feb (Figure 5). Higher OMD values were recorded in Start June and end June had a higher OMD than mid July (Figure 5).

There was a cultivar effect on annual mean leaf OMD. Bealey had a higher leaf OMD (780 g/kg ± 7.7) than Astonenergy (737 g/kg ± 7.4; $P < 0.01$). Spelga (753 ± 7.7) and Abermagic (755 ± 8.8) were intermediate to Bealey and Astonenergy. There was also a rotation effect on leaf OMD. Leaf OMD was higher in mid May (803 g/kg) than in mid Oct (725 g/kg; $P < 0.05$). All other rotations recorded intermediate values to mid May and mid Oct. There was no cultivar by rotation interaction on leaf OMD.

Figure 6 shows the OMD of the bulked samples of leaf, pseudostem, true stem and dead for rotations 1 to 4 (mid May to mid July), rotations 5 to 8 (end July to mid Oct) and rotations 9 to 10 (mid Feb and end Mar). During rotations 1 to 4, Abermagic had the highest leaf OMD. During rotations 5 to 8, both Bealey and Abermagic had the highest leaf OMD (Figure 6). During rotations 9 to 10, Astonenergy, Abermagic and Bealey had the highest leaf OMD. For all rotations Spelga consistently had a lower leaf OMD than all other cultivars (Figure 6). During rotations 1 to 4 and 5 to 8, Astonenergy and Spelga respectively, had the highest pseudostem OMD. For rotations 9 to 10, there was not enough pseudostem in any cultivar to analyse OMD. During rotations 1 to 4, Abermagic and Bealey had the highest true stem OMD, and Astonenergy and Spelga had the lowest (Figure 6). During rotations 5 to 8 and 9 to 10 there was no true stem present for any cultivar. During rotations 1 to 4 and 5 to 8, Abermagic and Spelga respectively, had the highest dead OMD. During rotations 9 to 10, Astonenergy had the highest dead OMD.

Acid Detergent Fibre

For the whole samples ADF, there was an interaction between cultivar and rotation ($P < 0.05$). In mid Sept Abermagic (290 g/kg ± 13.6) had a lower ADF than Bealey (359 g/kg ± 13.6) and Spelga (363 g/kg ± 13.6; $P < 0.05$). There was also an interaction between cultivar and rotation for leaf ADF. In mid July Spelga (320 g/kg ± 13.6) had a higher leaf ADF than Abermagic (242 g/kg ± 13.6; $P < 0.001$).

Neutral Detergent Fibre

There was an interaction between cultivar and rotation for whole sample NDF (P<0.05). In start June Astonenergy had a lower NDF (433 g/kg ± 20.2) than Spelga (589 g/kg ± 20.2; $P < 0.05$).

Crude Protein

For the whole samples CP there was an interaction between cultivar and rotation. In mid Oct Bealey had a higher CP (305 g/kg ± 7.4) than Abermagic (260 g/kg ± 7.4; $P < 0.05$). There was an interaction between cultivar and rotation for leaf CP ($P < 0.05$; Figure 7). In end June Abermagic had a higher leaf CP value than Spelga ($P < 0.05$). Bealey recorded a higher leaf CP value than both Astonenergy and Abermagic in mid Sept ($P < 0.05$). In mid Oct Bealey recorded a higher leaf CP than Abermagic ($P < 0.05$), but in mid Feb Abermagic recorded a higher leaf CP than Bealey ($P < 0.05$).

Discussion

Ensuring good grassland management and the correct choice of perennial ryegrass cultivar is fundamental to achieving increased grass utilisation, quality and milk production. Differences between cultivars may indicate that different management strategies are required for different cultivars in order to maximise leaf proportion and OMD. Increased leaf proportion and OMD results in increased herbage DM intake and increased milk production (Stakelum and Dillon, 2004).

Morphological components

The physiological state of the plant affects the proportions of leaf and true stem (Beever *et al.*, 2003). During the grass reproductive stage there is a reduction in leaf and an increase in pseudostem and true stem proportion (Beever *et al.*, 1986; Minson, 1990; McDonald *et al.*, 2002b). From mid May to start June there was an increase in the true stem proportion, while the leaf proportion remained static (and low compared to later in the year). The true stem proportion declined from start June onwards as the swards returned to the vegetative stage. Simultaneously the proportion of leaf increased. This agrees with Jewiss (1981), who found that temperate grasses produce little or no true stem during the vegetative stage. During the reproductive stage true stem production limits leaf production (Jewiss, 1981), but during the vegetative stage there is a morphological limit on leaf production. Lower leaf production and growth is also seen in Timothy during the reproductive stage (Gustavsson and Martinsson, 2004). The present study and previous research by Wilson (1994) show that the leaf is the most digestible component of the grass plant and the true stem is less digestible. Astonenergy had the highest leaf and lowest true stem proportion and was intermediate to Bealey and Spelga regarding overall OMD agreeing with The Northern Ireland DARD Grass and Clover Recommended List (2012) which reports Astonenergy as being a highly digestible cultivar (DARD, 2012). O'Donovan *et al.* (2011) identified that grass breeding needs to focus on improving the digestibility during the mid season and to ensure that sward canopy structure is appropriate for grazing. These differences in the digestibility of the plant components offer an opportunity to plant breeders to improve the digestibility of all plant components and to select cultivars with a higher leaf and lower true stem proportion to target maximum animal intake from grass.

Pseudostem grows as the plant moves into the reproductive stage and remains short when in the vegetative stage (Parsons and Chapman, 1980). The decreasing pseudostem proportion was associated with a decrease in true stem and increase in leaf. Similarly, Terry and Tilley (1964) found that pseudostem decreased over the year. Likewise, Wims *et al.* (2012) also found that stem (defined as pseudostem + true stem) decreased from early summer (April to June) to late summer (July to September). Development of true stem can begin anytime from March onwards (Hurley *et al.*, 2008). As a consequence, there was a slight increase in pseudostem content in end March compared to mid Feb in all cultivars except Bealey in which pseudostem content increases in mid Feb. Bealey seems to prepare for the reproductive stage before the other cultivars and is also reputed to have early spring growth (O'Donovan *et al.*, 2009). Bealey had the highest pseudostem proportion of all cultivars and therefore, despite its relatively high true stem proportion Bealey was highly digestible, as pseudostem is the next most digestible component after leaf. Using the same cultivars McEvoy *et al*. (2012b) found that dairy cows grazing Bealey and Astonenergy had the highest milk solids and milk protein yield of the four cultivars experimented. Despite this McEvoy *et al.* (2012a) found that Bealey, Abermagic and Spelga had the highest stem proportion, which would be considered a negative characteristic given the low digestibility of stem. In that experiment stem was defined as pseudostem + true stem. Pseudostem is regularly defined as "stem" as the true stem is encapsulated within the pseudostem (Langer, 1972; Robson *et al.*, 1988; Buxton and Redfearn, 1997). The pseudostem is highly digestible, however classifying pseudostem under the "stem" category can be misleading.

Senescent material accumulates over the winter period (Hennessy *et al.*, 2008; Ryan *et al.*, 2010). This resulted in an increase in dead proportion in mid Feb compared to mid Oct with the proportion of dead in mid Feb similar to that reported by Hennessy *et al.* (2008) for a closing date in October. This was associated with a decrease in leaf proportion and lower OMD in mid Feb than at several other times suggesting that the dead was an accumulation of senescent leaves. According to Frame and Hunt (1971) up to 0.40 of the leaf that is ungrazed eventually senesces causing a decrease in leaf proportion. The overall dead proportion in the sward was lower than previously reported for perennial ryegrass/white clover swards (Holmes *et al.*, 1992; Hoogendoorn *et al.*, 1992) and for *Dichanthium* swards (Boval *et al.*, 2007). A tolerable amount of dead in sward is any amount that does not negatively impact on animal production. The proportion of dead in the present study was less than previously reported, indicating that there was not an excessive amount of dead in the sward (Tuñon *et al.*, 2013; Wims *et al.*, 2012). The dead proportion in all cultivars remained stable throughout the year apart from an increase for Spelga in mid July and end July. In the present study, Spelga had the highest dead proportion of all cultivars. This may be partly attributed to its higher post grazing sward height which leads to increased true stem and dead proportion (Stakelum and Dillon, 2007; Stakelum and O'Donovan, 2000). In the current study Spelga had the highest post grazing sward height compared to the other cultivars (data not presented) but pre-grazing height was not significantly different between cultivars (data not presented). Likewise, Wims *et al.* (2012) found that Spelga had a significantly higher post grazing sward height than Astonenergy and Bealey but was similar to Abermagic, which contributed to it having the highest dead proportion of the four cultivars experimented.

All cultivars are classified in the same heading category (intermediate) with a narrow range of heading dates. It has been shown that cultivars within the same heading category differ morphologically (Smit *et al.*, 2005). This was also true in the present study as the cultivars behave differently during the reproductive stage with differences evident regarding leaf and true stem proportion. Wims *et al.* (2012) found that Abermagic had the highest stem (defined as pseudostem + true stem) proportion and

Spelga and Abermagic had the lowest leaf proportion between April and June agreeing with the present study which also found that Spelga had a low leaf proportion. Abermagic was intermediate regarding leaf and pseudostem proportion and had a high true stem proportion compared to the other cultivars.

Organic matter digestibility

Although the bulked OMD samples were not statistically analysed as there was not enough sample quantity, the values obtained from the chemical analysis will be discussed below. The morphological components of perennial ryegrass differ biologically in terms of OMD. Wilson (1994) found that OMD was highest in the leaf component and lowest in the dead component, following the order leaf > pseudostem > true stem > dead, agreeing with the present study. This suggests that digestibility decreases from the top of the sward to the base, which is also found in cocksfoot (Duru, 2003), although the individual plant components of perennial ryegrass are more digestible than in cocksfoot and timothy (Terry and Tilley, 1964). Other studies have also shown that differences exist in digestibility between the pseudostem and true stem (Terry and Tilley, 1964; Buxton and Redfearn, 1997), indicating that the pseudostem should be separated from the true stem, especially during the reproductive stage when the true stem is at its highest proportion. The higher structural components in the true stem make it less digestible than the leaf, agreeing with Stone (1994) and Buxton (1996).

Wilman and Rezvani Moghaddam (1998) and McEvoy *et al.* (2010) found that OMD was lower in July to August compared to earlier in April to June, agreeing with the present study when in mid July OMD was lower than at other times. O'Donovan and Kennedy (2007) showed that OMD was lowest in August, but they used late heading cultivars. Late heading cultivars enter the reproductive stage at the start of June whereas intermediate cultivars, which were used in the present study, enter the reproductive stage at the end of May (Frame, 1991). This difference in heading dates explains why O'Donovan and Kennedy (2007) found the lowest OMD in August while in the present study it was in mid July. The post grazing sward heights at end June may also have contributed to the low OMD in mid July. In end June all cultivars had a significantly higher post grazing height (data not presented) than in mid May. A higher post grazing sward height is associated with a reduction in digestibility in subsequent rotations (Stakelum and Dillon, 2007; Baudracco *et al.*, 2010). Higher post grazing sward height may create swards with higher herbage mass and a greater proportion of true stem (Minson, 1990) and dead (Hoogendoorn *et al.*, 1992) resulting in lower digestibility. The low OMD in mid Oct could be due to the low leaf OMD at that time. This shows the influence of leaf OMD on the overall digestibility of the sward and Stakelum and O'Donovan (2000) showed that a 5.5 percentage unit change in leaf content was equal to a 1 percentage unit change in digestibility. The low OMD in mid Feb could be due to the plant preparing itself for the reproductive stage and diverting energy away from leaf production and towards pseudostem and true stem development, which may also have resulted in an increase in dead. The combination of low leaf OMD and high dead proportion resulted in herbage in mid Feb having a low OMD.

The OMD of leaf and true stem were similar in the bulk sample of rotations 1 to 4 for Bealey, most likely due to the swards being well managed by imposing a short 3-week rotation. If longer regrowth intervals were used greater differences in OMD between morphological components may have occurred. According to Minson (1990) and McDonald *et al.* (2002b) there were no major differences in OMD between morphological components when the forage was maintained in a young vegetative state by regular cutting or grazing. Regular cutting or grazing reduces true stem elongation and flowering which reduces the decline in digestibility (Minson, 1990). Another possible explanation is that the true stem of Bealey is actually highly digestible. Indeed, other studies, with cocksfoot, have shown that young stem is as digestible or more digestible than leaf (Terry and Tilley, 1964). There have been no other studies that have separated the pseudostem from the true stem and determined the OMD of both components.

In the present study the two tetraploid cultivars performed best regarding combined leaf and pseudostem proportion and OMD. Bealey had the highest OMD of all four cultivars agreeing with Palladino *et al.* (2009) who found that Bealey had the highest DMD compared to a number of diploid and tetraploid, and late and intermediate heading cultivars. Bealey had the highest leaf + pseudostem proportion and hence the highest OMD. This shows the influence of leaf + pseudostem on overall OMD. The low fibre content and high leaf proportion in Astonenergy and low NDF coupled with high leaf CP in Bealey, agrees with these two cultivars being highly digestible. Acid detergent fibre is the cell wall portion of the grass plant and has a negative relationship with digestibility (Beever *et al.*, 2003). The cell wall increases as grasses mature (Stone, 1994; Buxton, 1996; Beever *et al.*, 2003). This agrees with the present study where Spelga was more mature than Astonenergy in start June as Spelga had a higher NDF content and higher true stem proportion than Astonenergy. Spelga had a low OMD and was characterised by low leaf and high true stem proportions. Abermagic had a high leaf OMD and leaf CP content, but because the leaf proportion was not as high, the OMD value was only intermediate. Wims *et al.* (2012) found that both Spelga and Abermagic had a lower OMD than Astonenergy and Bealey during the reproductive phase. McEvoy *et al.* (2012b) found that cows grazing Astonenergy and Bealey produced a higher milk yield and milk solids content than Abermagic and Spelga suggesting that milk production at farm level could be improved by using these cultivars.

Implications

True stem development can begin any time between March and May (Hurley *et al.*, 2008). This highlights the importance of early grazing to keep true stem proportion to a minimum as it has a low OMD and a high proportion of true stem in the sward could lead to reduced animal performance. Differences in morphological proportions suggest that different cultivars should be managed differently during the reproductive stage to maximise the leaf proportion and OMD and minimise the true stem and dead proportion. Future work should investigate the option of tailoring rotation length to cultivar by offering a set daily herbage allowance and only move the animals once a target post-grazing sward height has been achieved. Differences in the proportions of plant components and between cultivars are evident predominantly during the reproductive stage implying that it would be worthwhile for evaluation programmes to record cultivar characteristics such as leaf, pseudostem, true stem, dead proportion and OMD of these components at this time. In studies that determine the sward morphology, the pseudostem should be separated from the true stem in order to accurately characterise the sward. During the vegetative stage, there were no differences between cultivars in terms of leaf and true stem proportion. As there is no true stem in swards during the vegetative stage, a longer regrowth period can be implemented, facilitating the build-up of herbage mass. This can be used to extend the grazing season in autumn.

Conclusion

The four cultivars were a similar heading date category but differed in leaf, pseudostem, true stem, and dead proportion throughout the grazing season, particularly during the reproductive stage. Organic matter digestibility is highest in the leaf component and lowest in the dead component of the grass plant. The pseudostem is intermediate to the leaf and true stem regarding OMD. The high OMD of the pseudostem component compared to the true stem component, suggests that the two should not be considered "stem" but should be separated from one another especially during the reproductive stage. This will give an accurate representation of the sward morphology in grazing studies. In this study the greatest influence on OMD was found to be leaf + pseudostem and not just leaf as found by Stakelum and O'Donovan (2000). However because pseudostem constituted such a small proportion of the grazed sward, it is not worthwhile to concentrate on this character in perennial ryegrass breeding. Given that leaf is the dominant component of the plant throughout the year, and that leaf has the highest digestibility, perennial ryegrass breeders should focus on further improving digestibility by focusing on the leaf component.

Acknowledgements

The authors wish to acknowledge M. Feeney, C. Fleming and N. Galvin for their technical assistance.

References

BAUDRACCO J., LOPEZ-VILLALOBOS N., HOLMES C. W. and MACDONALD K. A. (2010) *Effects of stocking rate, supplementation, genotype and their interactions on grazing dairy systems: A review*. *New Zealand Journal of Agricultural Research*, **53**, 109-133.

BEEVER D. E., DHANOA M. S., LOSADA H. R., EVANS R. T., CAMMELL S. B. and FRANCE J. (1986) *The effect of forage species and stage of harvest on the processes of digestion occurring in the rumen of cattle*. *British Journal of Nutrition*, 439-454.

BEEVER D. E., OFFER N. and GILL M. (2003) The feeding value of grass and grass products. In: HOPKINS A. (ed.) *Grass: Its production and utilisation*, pp. 140-195. Devon, UK: British Grassland Society.

BUXTON D. R. (1996) *Quality-related characteristics of forages as influenced by plant environment and agronomic factors*. *Animal Feed Science and Technology*, **59**, 37-49.

BUXTON D. R. and REDFEARN D. D. (1997) *Plant limatations to fiber digestion and utilisation*. *Journal of Nutrition*, **127**, 8145-8185.

BOVAL M., ARCHIMÈDE H., CRUZ P. and DURU M. (2007) *Intake and digestibility in heifers grazing a dichanthium spp. Dominated pasture, at 14 and 28 days of regrowth*. *Animal Feed Science and Technology,* **134**, 18-31.

COULTER B. and LALOR S. (2008) *Major and micro nutrient advice for productive agricultural crops*. *Teagasc, Johnstown Castle Environment Research Centre: Wexford, Ireland*.

DARD (2012) Grass and clover: Recommended varieties for northern ireland 2012/2013.

DURU M. (2003) *Effect of nitrogen fertiliser rates and defoliation regimes on the vertical structure and composition (crude protein content and digestibility) of a grass sward*. *Journal of the Science of Food and Agriculture*, **83**, 1469-1479.

FRAME J. (1991) *Improved grassland management*. Ipswich: Farming Press Books.

FRAME J. and HUNT I. (1971) *The effects of cutting and grazing systems on herbage production from grass swards*. *Grass and Forage Science*, **26**, 163-172.

GOWEN N., O'DONOVAN M., CASEY I., RATH M., DELABY L. and STAKELUM G. (2003) *The effect of grass cultivars differing in heading date and ploidy on the performance and dry matter intake of spring calving dairy cows at pasture*. *Animal Research*, **52**, 321-336.

GUSTAVSSON A.-M. and MARTINSSON K. (2004) *Seasonal variation in biochemical composition of cell walls, digestibility, morphology, growth and phenology in timothy*. *European journal of agronomy*, **20**, 293-312.

HENNESSY D., O'DONOVAN M., FRENCH P. and LAIDLAW A. (2008) *Factors influencing tissue turnover during winter in perennial ryegrass dominated swards*. *Grass and Forage Science*, **63**, 202-211.

HOLMES C. W., HOOGENDOORN C. J., RYAN M. P. and CHU A. C. P. (1992) *Some effects of herbage composition, as influenced by previous grazing management, on milk production by cows grazing on ryegrass/white clover* *pastures. 1. Milk production in early spring: Effects of different regrowth intervals during the preceding winter period*. *Grass and Forage Science*, **47**, 309-315.

HOOGENDOORN C. J., HOLMES C. W. and CHU A. C. P. (1992) *Some effects of herbage composition, as influenced by previous grazing management, on milk production by cows grazing on ryegrass/white clover pastures. 2. Milk production in late spring/summer: Effects of grazing intensity during the preceding spring period*. *Grass and Forage Science*, **47**, 316-325.

HURLEY G., GILLILAND T. and O'DONOVAN M. (2008) *Relationship between reproductive initiation and ear emergence development in lolium perenne l. The Journal of Agricultural Science*, **146**, 655.

ILLIUS A. W. and GORDON I. J. (1987) *The allometry of food intake in grazing ruminants*. *Journal of Animal Ecology*, **56**, 989-999.

JEWISS O. R. (1981) Shoot development and number. In: DAVIES A., BAKER R. W., GRANT S. A. and LAIDLAW A. S. (eds.) *Sward measurement handbook*. Reading, UK: BGS.

KENNEDY E., DONOVAN M. O., MURPHY J. P., DELABY L. and MARA F. P. O. (2007) *Effect of spring grazing date and stocking rate on sward characteristics and dairy cow production during midlactation*. *Journal of Dairy Science*, **90**, 2035-2035.

LANGER R. H. M. (1972) The grass plant *How grasses grow*: Edward Arnold (Publishers) Limited.

LAREDO M. A. and MINSON D. J. (1975) *The voluntary intake and digestibility by sheep oe leaf and stem fractions of lolium perenne*. *Grass and Forage Science*, **30**, 73-77.

MCDONALD P., EDWARDS R. A., GREENHALGH J. F. D. and MORGAN C. A. (2002a) *Evaluation of foods: Energy content of foods and the partition of food energy within the animal*. Essex. England: Pearson Education Limited.

MCDONALD P., EDWARDS R. A., GREENHALGH J. F. D. and MORGAN C. A. (2002b) *Grass and forage crops*. Essex, England,: Pearson Education Limited.

MCEVOY M., DELABY L., MURPHY J. P., BOLAND T. M. and O'DONOVAN M. (2010) *Effect of herbage mass and allowance on sward characteristics, milk production, intake and rumen volatile fatty acid concentration*. *Grass and Forage Science*, **65**, 335-347.

MCEVOY M., O'DONOVAN M. and DELABY L. (2012a) Effect of cultivar on sward structural characteristics in a rotational grazing system during the spring and summer period *Agricultual Research Forum*, p. 83. Tullamore, Ireland.

MCEVOY M., O'DONOVAN M., MURPHY J. P. and DELABY L. (2012b) Cultivar influences milk production of grazing dairy cows *Agricultural Research Forum*, p. 76. Tullamore, Ireland.

MINSON D. J. (1990) *Digestible energy of forage*. Florida: Academic Press INC.

MORGAN D. J., STAKELUM G. and DWYER J. (1989) *Modified neutral detergent cellulase digestibility procedure for use with the 'fibertec' system*. *Irish Journal of Agricultural Research*, **28**, 91-92

O'DONOVAN M. and DELABY L. (2005) *A comparison of perennial ryegrass cultivars differing in heading date and grass ploidy with spring calving dairy cows grazed at two different stocking rates*. *Animal Research*, **54**, 337-350.

O'DONOVAN M. and DELABY L. (2008) *Sward characteristics, grass dry matter intake and milk production performance is affected by timing of spring grazing and subsequent stocking rate*. *Livestock Science*, **115**, 158-168.

O'DONOVAN M. and KENNEDY E. (2007) Using grass to reduce feed costs *National Diary Conference 'Exploiting the Freedom to Milk'*, pp. 63-80. Lyrath Hotel, Kilkenny, Breaffy House Hotel, Castlebar, Co.Mayo.

O'DONOVAN M., KENNEDY E. and MCEVOY M. (2009) Effect of winter closing date and spring opening date on the dry matter yield of perennial ryegrass cultivars. Proceedings of the Agricultual Research Forum, Tullamore, Ireland, p. 97.

O'DONOVAN M., LEWIS E. and O'KIELY P. (2011) *Requirements of future grass based ruminant production systems in Ireland. Irish Journal of Agricultural and Food Research*, **50**, 1-21.

PALLADINO R. A., O'DONOVAN M., KENNEDY E., MURPHY J. J., BOLAND T. M. and KENNY D. A. (2009) *Fatty acid composition and nutritive value of twelve cultivars of perennial ryegrass*. *Grass and Forage Science*, **64**, 219-226.

PARSONS A. J. and CHAPMAN D. F. (1980) The principles of pasture growth and utilisation. In: HOPKINS A. (ed.) *Grass its production and utilisation*, pp. 31-79. Oxford UK: British Grassland Society.

PRITCHARD G. I., FOLKINS L. P. and PIGDEN W. J. (1963) *The in vitro digestibility of whole grasses and their parts at progressive stages of maturity*. *Canadian Journal of Plant Science*, **43**, 79-87.

ROBSON M. J., RYLE G. J. A. and WOLEDGE J. (1988) The grass plant - its form and function. In: JONES M. B. and LAZENBY A. (eds.) *The grass crop - the physiological basis of production*, pp. 25-83. New York: Chapmand and Hall Ltd, London.

RYAN W., HENNESSY D., MURPHY J. and BOLAND T. (2010) *The effects of autumn closing date on sward leaf area index and herbage mass during the winter period*. *Grass and Forage Science*, **65**, 200-211.

SHALLOO L., DILLON P., O'LOUGHLIN J., RATH M. and WALLACE M. (2004) *Comparison of a pasture-based system of milk production on a high rainfall, heavy-clay soil with that on a lower rainfall, free-draining soil*. *Grass and Forage Science*, **59**, 157-168.

SHALLOO L., O'DONNELL S. and HORAN B. (2007) Profitable dairying in increased eu milk quota scenario. Proceedings of the Proceedings of the National Dairy Conference, 21 and 22 November, Kilkenny and Mayo, Ireland, pp. 20-44.

SMIT H. J., TAS B. M., TAWEEL H. Z. and ELGERSMA A. (2005) *Sward characteristics important for intake in six lolium perenne varieties*. *Grass and Forage Science*, **60**, 128-135.

STAKELUM G. and DILLON P. (2004) *The effect of herbage mass and allowance on herbage intake, diet composition and ingestive behaviour of dairy cows*. *Irish Journal of Agricultural and Food Research*, **43**, 17-30.

STAKELUM G. and DILLON P. (2007) *The effect of grazing pressure on rotationally grazed pastures in spring/early summer on subsequent sward characteristics*. *Irish Journal of Agricultural and Food Research*, **46**, 15-28.

STAKELUM G. and O'DONOVAN M. (2000) Grass utilisation and grazing management for dairying. Proceedings of the Proceedings of the National Dairy Conference, 16 November, Cork, Ireland, 16 November 2000, pp. 14-38.

STEWART A. and HAYES R. (2011) *Ryegrass breeding - balancing trait priorities*. *Irish Journal of Agricultural and Food Research*, **50**, 31-46.

STONE B. A. (1994) *Prospects for improving the nutritive value of temperate, perennial pasture grasses*. *New Zealand Journal of Agricultural Research*, **37**, 349-363.

SUGIYAMA S.-I. (2005) *Polyploidy and cellular mechanisms changing leaf size: Comparison of diploid and autotetraploid populations in two species of lolium*. *Annals of Botany*, **96**, 931-938.
TERRY R. A. and TILLEY J. M. A. (1964) *The digestibility of the leaves and stems of perennial ryegrass, cocksfoot, timothy, tall fescue, lucerne, and sainfoin, as measured by an in vitro procedure*. *Grass and Forage Science*, **19**, 363-372.
TUÑON G., KENNEDY E., HORAN B., HENNESSY D., LOPEZ-VILLALOBOS N., KEMP P., BRENNAN A. and O'DONOVAN M. (2013) *Effect of grazing severity on perennial ryegrass herbage production and sward structural characteristics throughout an entire grazing season*. *Grass and Forage Science*, n/a-n/a.
VAN SOEST P. J., J.B. R. and B.A. L. (1991) *Methods for dietary fibre, and non-starch polysaccharides in relation to animal nutrition. Journal of Dairy Science*, **74** 3583-3597.
WILMAN D. and REZVANI MOGHADDAM P. (1998) *In vitro digestibility and neutral detergent fibre and lignin contents of plant parts of nine forage species*. *Journal of Agricultural Science*, **131**, 51-58.
WILSON J. R. (1994) *Cell wall characteristics in relation to forage digestion by ruminants*. *The Journal of Agricultural Science*, **122**, 173-182.
WIMS C. M., MCEVOY M., DELABY L., BOLAND T. M. and O'DONOVAN M. (2012) *Effect of perennial ryegrass (lolium perenne l.) cultivars on the milk yield of grazing dairy cows*. *animal*, **7**, 410-421.

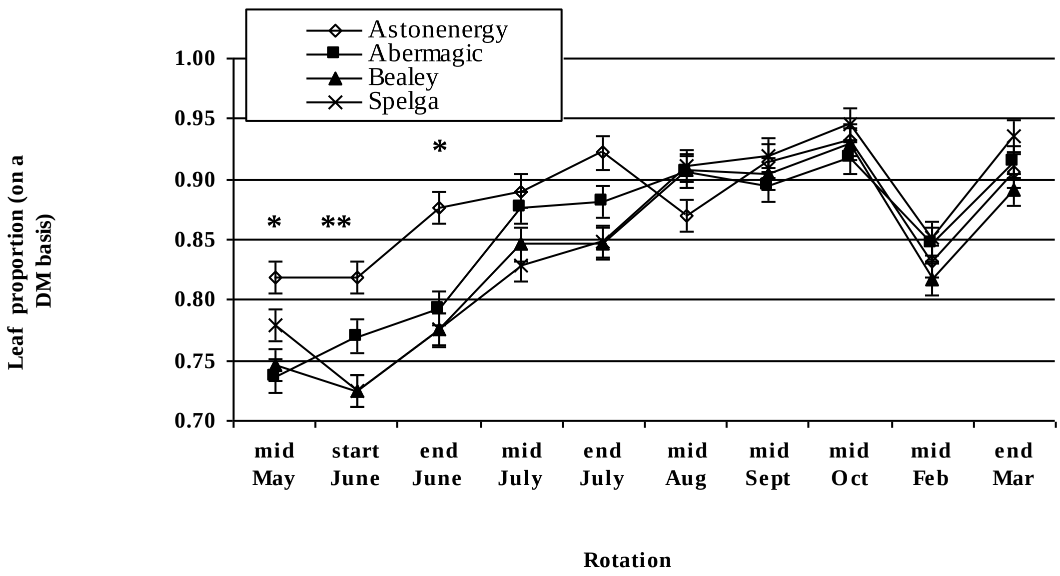

Figure 1. Leaf proportion above 4 cm (expressed on a DM basis) for four perennial ryegrass cultivars (Astonenergy, Abermagic, Bealey and Spelga), sown as single cultivar grazing swards, during 10 grazing rotations from May 2011 to March 2012 (mean±SEM; * = P < 0.05; ** = P < 0.01).

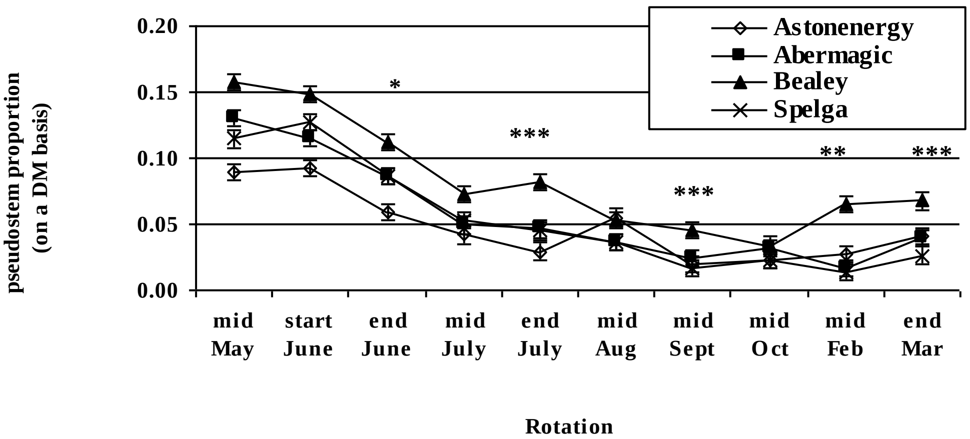

Figure 2. Pseudostem proportion above 4 cm (expressed on a DM basis) for four perennial ryegrass cultivars (Astonenergy, Abermagic, Bealey and Spelga), sown as single cultivar grazing swards, during 10 grazing rotations from May 2011 to March 2012 (mean±SEM; * P < 0.05, ** P < 0.01, *** P < 0.001)

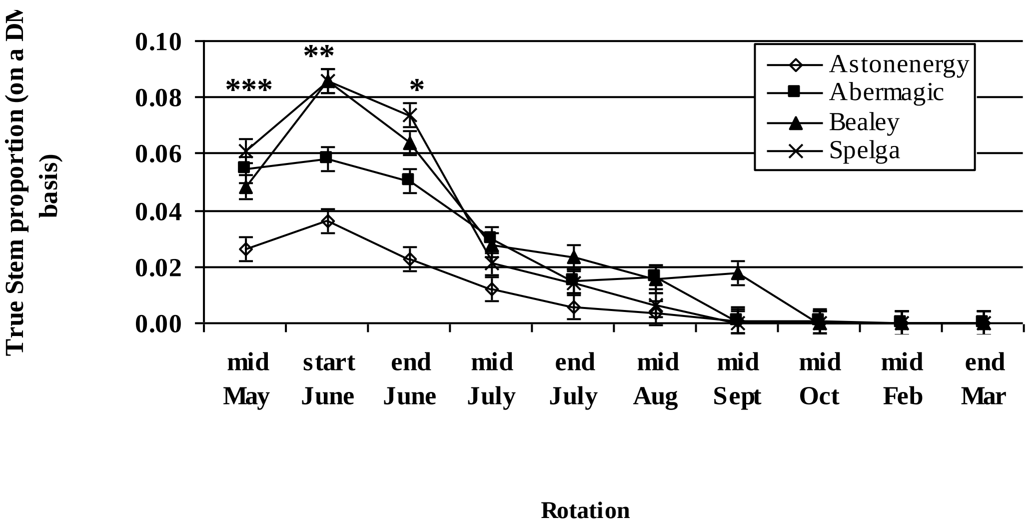

Figure 3. True stem proportion above 4 cm (expressed on a DM basis) for four perennial ryegrass cultivars (Astonenergy, Abermagic, Bealey and Spelga), sown as single cultivar grazing swards, during 10 grazing rotations from May 2011 to March 2012 (mean±SEM; * P < 0.05, ** P < 0.01, *** P < 0.001)

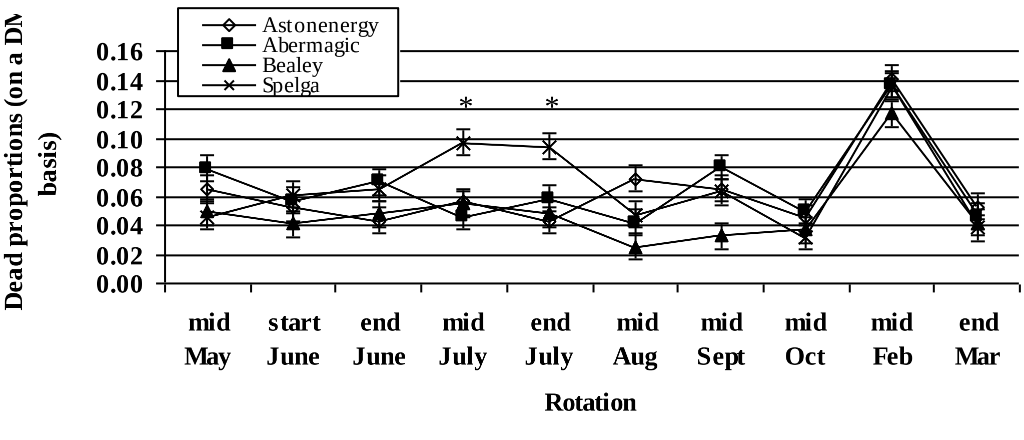

Figure 4. Dead proportion above 4 cm (expressed on a DM basis) for four perennial ryegrass cultivars (Astonenergy, Abermagic, Bealey and Spelga), sown as single cultivar grazing swards, during 10 grazing rotations from May 2011 to March 2012 (mean±SEM; * P < 0.05)

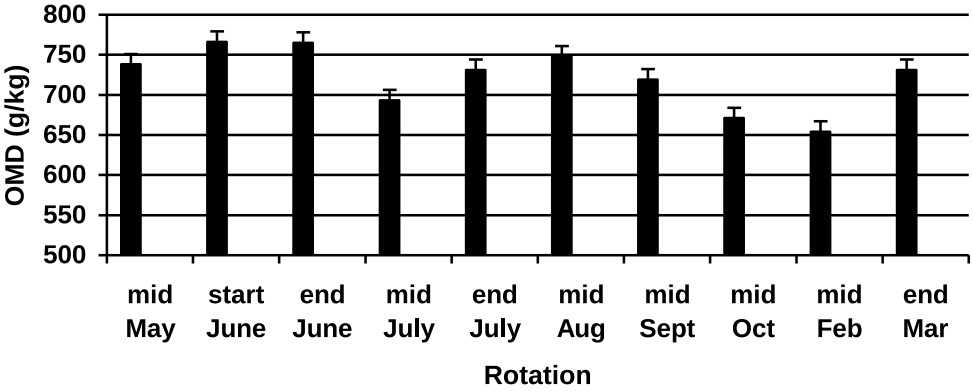

Figure 5. Mean whole plant above 4 cm organic matter digestibility (OMD) of four perennial ryegrass cultivars (Astonenergy, Abermagic, Bealey and Spelga), sown as single cultivar grazing swards, during 10 grazing rotations from May 2011 to March 2012 (mean±SEM)

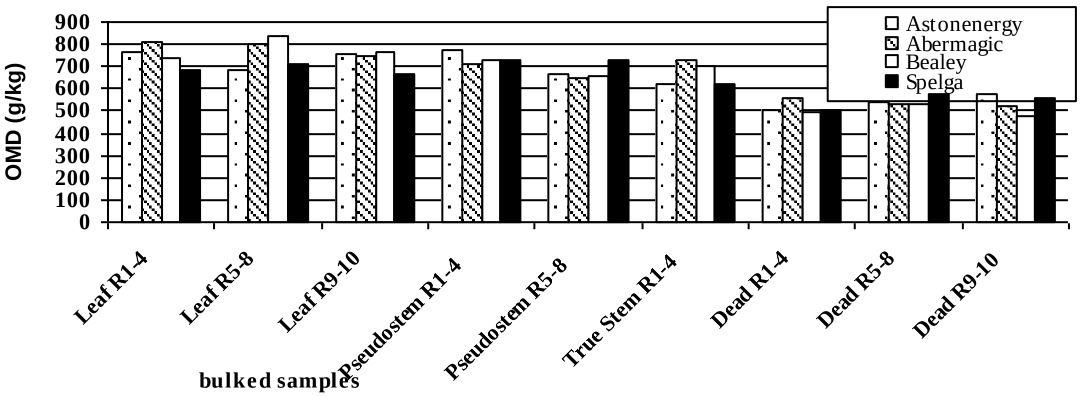

Figure 6. Organic matter digestibility (OMD) of leaf, pseudostem, true stem and dead morphological components above 4 cm of four perennial ryegrass cultivars (Astonenergy, Abermagic, Bealey and Spelga), sown as single cultivar grazing swards, bulked for rotations 1 (mid May) to 4 (mid July) (R1-4), rotations 5 (end July) to 8 (mid Oct) (R5-8) and rotations 9 (mid Feb) to 10 (end Mar) (R9-10)

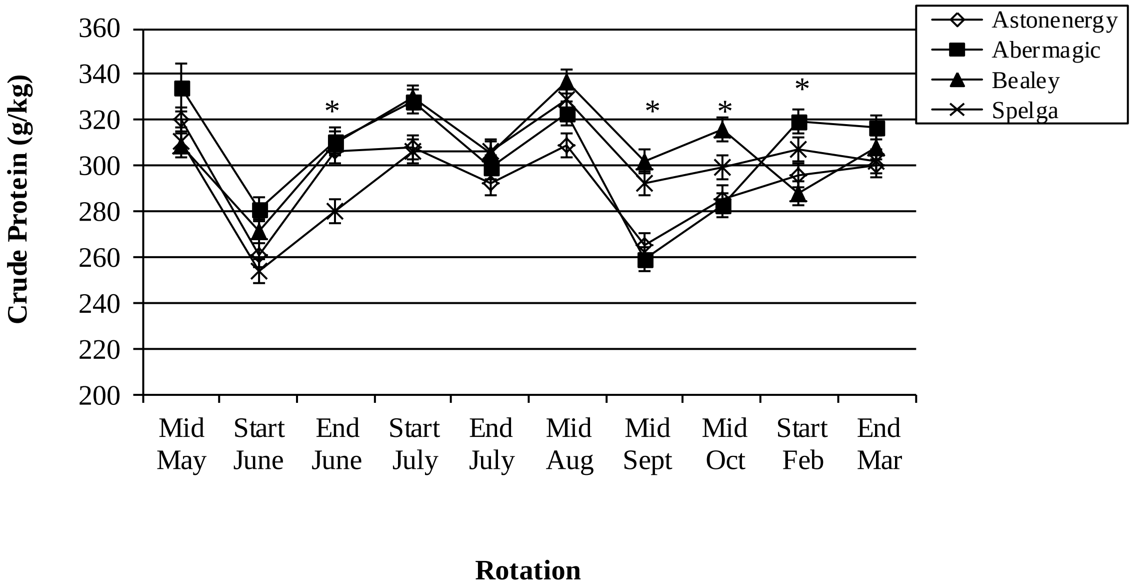

Figure 7. Leaf above 4 cm crude protein concentration (expressed on a DM basis) for four perennial ryegrass cultivars (Astonenergy, Abermagic, Bealey and Spelga), sown as single cultivar grazing swards, during 10 grazing rotations from May 2011 to March 2012 (mean±SEM; * $P < 0.05$)