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

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

46 **M. Beecher^{1,2}, D. Hennessy¹, T. Boland², M. McEvoy¹, M.**
47 **O'Donovan¹ and E. Lewis^{1*}**

48

49 *¹Teagasc, Animal & Grassland Research and Innovation Centre,*
50 *Moorepark, Fermoy, Co. Cork, Ireland*

51 *²School of Agriculture and Food Science, University College Dublin, Belfield, Dublin*
52 *4, Ireland*

53

54 **Corresponding author:**

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

57

58 **Telephone:** + 353 25 42673

59 **Fax:** + 353 25 42340

60 **Email:** eva.lewis@teagasc.ie

61

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

63 **Abstract**

64 The grass plant is comprised of leaf, pseudostem, true stem (including inflorescence)
65 and dead. These components differ in digestibility and variations in their relative
66 proportions can impact sward quality. The objective of this study was to determine the
67 change in the proportion and organic matter digestibility (OMD) of leaf, pseudostem,
68 true stem and dead components of four perennial ryegrass cultivars (two tetraploids:
69 Astonenergy and Bealey and two diploids: Abermagic and Spelga) throughout a
70 grazing season. The DM proportions and *in vitro* OMD of leaf, pseudostem, true stem
71 and dead in all cultivars were determined during 10 grazing rotations between May
72 2011 and March 2012. There was an interaction between rotation and cultivar for leaf,
73 pseudostem, true stem and dead proportions. In May and June, Astonenergy had the
74 highest leaf and lowest true stem proportion ($P < 0.05$). From July onwards there was
75 no difference in leaf or true stem proportion between cultivars. Bealey had the highest
76 annual mean OMD (752 g/kg) and Spelga the lowest (696 g/kg; $P < 0.05$). The OMD
77 followed the order leaf > pseudostem > true stem > dead. Bealey had the highest
78 combined leaf and pseudostem proportion 0.92, which explains why it had the highest
79 OMD. In this study the tetraploid cultivars had the highest leaf and pseudostem
80 proportion and OMD. For accurate descriptions of a sward in grazing studies and to
81 accurately determine sward morphological composition, pseudostem should be
82 separated from true stem, particularly during the reproductive stage when true stem is
83 present.

84

85 **Introduction**

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

93

94 Perennial ryegrass cultivars differ in their chemical composition (O'Donovan and
95 Delaby, 2005). O'Donovan and Delaby (2005) found that there was a 4-unit
96 difference in OMD between intermediate heading tetraploid and diploid cultivars. The

97 higher digestibility of tetraploids compared to diploids is potentially linked to
98 tetraploids having larger epidermal and mesophyll cells and a higher ratio of cell
99 contents to cell wall (Sugiyama, 2005; Stewart and Hayes, 2011). Organic matter
100 digestibility (OMD) is a key driver of metabolisable energy supply as the main factors
101 that affect metabolisable energy are those that influence digestibility (McDonald *et*
102 *al.*, 2002a). Grass quality, as evidenced by OMD, is a key driver of animal
103 performance in grazing systems and is associated with higher overall farm profit
104 (Shalloo *et al.*, 2007).

105

106 Several morphological components make up the grass plant and these vary in
107 digestibility (Stakelum and Dillon, 2007). The leaf is comprised of the leaf blade
108 (leaf) and leaf sheath; the collection of leaf sheaths on a tiller make up the
109 pseudostem. During the reproductive stage true stem emerges upwards from the base
110 of the tiller through the pseudostem. Digestibility is inversely related to the degree of
111 lignification, which in turn is linked to the morphological composition of the sward.
112 The true stem has a higher lignin content (Laredo and Minson, 1975) and lower
113 digestibility than the leaf (Wilson, 1994; Buxton, 1996). During the reproductive
114 stage there is a proportionally lower leaf and higher true stem content than during the
115 vegetative stage (Buxton and Redfearn, 1997), and so swards are expected to be less
116 digestible during the reproductive stage than during the vegetative stage. Little
117 research has been carried out on the pseudostem component of the sward, regarding
118 both its proportion in the sward and its digestibility. In studies that determine sward
119 morphology the pseudostem is usually combined with the true stem (Pritchard *et al.*,
120 1963; Kennedy *et al.*, 2007; O'Donovan and Delaby, 2008). There is some suggestion
121 that the pseudostem is more digestible than the true stem (Terry and Tilley, 1964).
122 Therefore, categorising the pseudostem with true stem during morphological
123 separations may not be appropriate. Animal output is dependent on the amount of
124 herbage ingested and the quality of that herbage (Shalloo *et al.*, 2007), but there is a
125 physical limit on the height to which animals can graze (Illius and Gordon, 1987).
126 Therefore it is the grazed horizon of the sward that is of interest when determining
127 sward morphology. There is a need to determine the proportions of the morphological
128 components and their contribution to the overall digestibility of the sward which has
129 the potential to aid plant breeders in achieving their target of improving plant
130 digestibility (O'Donovan *et al.*, 2011).

131

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

136

137 **Materials and Methods**

138 *Study Area and Experimental Design*

139 The study was conducted at the Teagasc, Animal & Grassland Research and
140 Innovation Centre, Moorepark, Fermoy, Co. Cork, Ireland (50° 09'N; 8°16'W) where
141 four paddocks were used. The soil type was a free draining acid brown earth of sandy
142 loam-to-loam texture. In 2009 the paddocks (average size 0.8 ha) were sown with
143 perennial ryegrass. Each of the four paddocks was sown with a different perennial
144 ryegrass cultivar. Two tetraploid cultivars were used: Bealey and Astonenergy, with
145 heading dates of 24 May and 31 May, respectively. Two diploid cultivars were used:
146 Spelga and Abermagic, with heading dates of 22 May and 28 May, respectively. Soil
147 analysis indicated that all four paddocks had a similar pH (6.5) and nutrient status (6.5
148 to 10 mg/l for phosphorus and 140 mg/l for potassium). The pH and nutrient status of
149 the soils were considered adequate for intensive grassland production (Coulter and
150 Lalor, 2008).

151

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

155

156 *Management of each grazing single cultivar sward*

157 There were 10 grazing rotations between May 2011 and March 2012. Rotations 1 to 8
158 took place in 2011 on 17 to 18 May (mid May), 6 to 8 June (start June), 25 to 26 June
159 (end June), 9 to 10 July (mid July), 27 to 28 July (end July), 17 to 18 August (mid
160 Aug), 15 to 16 September (mid Sept) and 11 to 16 October (mid Oct). Rotations 9 and
161 10 took place in 2012 on 15 to 18 February (mid Feb) and 28 to 30 March (end Mar),
162 respectively. During the experimental period each sward received 224 kg N/ha which
163 was applied at a similar time for all cultivars. No phosphorus or potassium fertiliser
164 was applied.

165

166 *Sward Morphology*

167 Immediately prior to grazing, the morphological composition was determined in each
168 of the four single cultivar grazing swards. In each sward a subsection of the paddock
169 was identified and divided into four replicates, each measuring 361 m². Within each
170 of the four replicates grass samples were taken along a diagonal at eight points. The
171 grass samples were cut to ground level using a scissors and the vertical structure of
172 the sward was preserved using elastic bands. The grass samples were weighed before
173 separation into the upper and lower sward horizon: > 4 cm and < 4 cm (measured
174 from ground level). Average fresh weight of the > 4 cm total sample collected was
175 238 g; average fresh weight of < 4 cm sample was 102 g. The upper layer (> 4 cm)
176 was mixed before separating into two fractions, one of which was left intact (average
177 fresh weight was 73 g) (from here onwards this is referred to as the “whole” sample)
178 and the other which was manually separated into leaf, pseudostem, true stem and dead
179 (average fresh weight was 130 g). Leaf blades were detached from the base of the
180 pseudostem or true stem. Leaf sheaths were separated from the true stem and defined
181 and included in the pseudostem fraction. Inflorescences, if present, were included as
182 true stem. Dead matter was defined as any senesced material that was yellow/brown
183 in colour. The leaf, pseudostem, true stem and dead samples, the whole samples and
184 both the < 4 cm samples (from the whole samples and separated > 4 cm samples)
185 were weighed fresh, and after oven-drying to determine DM content. Samples were
186 separated within 72 hours of collection. Whilst awaiting separation grass samples
187 were stored in a cold room (4°C), laid out on paper towel, in open plastic bags, in
188 order to absorb any surface moisture and avoid decay.

189

190 *Chemical Analysis*

191 The leaf, pseudostem, true stem and dead samples > 4 cm, the whole samples > 4 cm
192 and the < 4 cm samples were oven dried at 40°C for 48 hours in a Binder FED 720
193 drying oven (Binder GmbH, Tuttlingen, Germany) to determine the dry matter (DM)
194 content. The dried grass samples were milled through a 1-mm screen using a
195 Cyclotech 1093 Sample Mill (Foss, DK-3400 Hillerød, Denmark). The dried milled
196 grass samples were retained for chemical analysis. There was an individual leaf
197 sample for each replicate during each grazing rotation period (rotation). Due to
198 insufficient sample quantity the pseudostem, true stem and dead replicate samples

199 were bulked by morphological component using the total amount of sample collected
200 for the first four rotations of 2011 (mid May to mid July), the last four rotations of
201 2011 (end July to mid Oct) and the two 2012 rotations (mid Feb and end Mar). A
202 similarly-bulked leaf sample was also created for comparison based on the
203 proportions of the pseudostem, true stem and dead bulked samples.

204

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

217

218 ***Statistical Analysis***

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

224

$$225 \quad Y = \mu + C_i + R_j + C_i \times R_j + P_k(C_i) + e$$

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

229

230 Rotation number was the repeated measure. For all data the random statement
231 specified the compound symmetry structure. The Tukey Kramer multiple range test

232 was used for mean separation ($P < 0.05$). All data were first analysed for normality
233 (PROC UNIVARIATE) in SAS (2002). The pseudostem, true stem and dead
234 proportion data were non-parametric and were transformed using log10, exponential
235 and sin functions respectively. The chemical composition of the bulked leaf,
236 pseudostem, true stem and dead samples were not statistically analysed as there was
237 only one sample for each cultivar.

238

239 **Results**

240

241 **Morphological component proportions**

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

257

258 **Chemical Composition**

259 ***Organic Matter Digestibility***

260 Whole sample OMD refers to the average OMD for the 10 rotations. For the whole
261 samples there was a cultivar effect ($P < 0.05$) and a rotation effect ($P < 0.05$) on
262 OMD, but no cultivar by rotation interaction. Bealey had a higher OMD (752 g/kg \pm
263 10.4) than Spelga (696 g/kg \pm 10.4). Astonenergy (724 g/kg \pm 10.4) and Abermagic
264 (715 g/kg \pm 10.4) were intermediate. Organic matter digestibility was higher in mid
265 May, start June, end June, end July and mid Aug than in mid Oct and mid Feb. Higher

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

268

269 There was a cultivar effect on annual mean leaf OMD. Bealey had a higher leaf OMD
270 (780 g/kg \pm 7.7) than Astonenergy (737 g/kg \pm 7.4; $P < 0.01$). Spelga (753 \pm 7.7) and
271 Abermagic (755 \pm 8.8) were intermediate to Bealey and Astonenergy. There was also
272 a rotation effect on leaf OMD. Leaf OMD was higher in mid May (803 g/kg) than in
273 mid Oct (725 g/kg; $P < 0.05$). All other rotations recorded intermediate values to mid
274 May and mid Oct. There was no cultivar by rotation interaction on leaf OMD.

275

276 Figure 6 shows the OMD of the bulked samples of leaf, pseudostem, true stem and
277 dead for rotations 1 to 4 (mid May to mid July), rotations 5 to 8 (end July to mid Oct)
278 and rotations 9 to 10 (mid Feb and end Mar). During rotations 1 to 4, Abermagic had
279 the highest leaf OMD. During rotations 5 to 8, both Bealey and Abermagic had the
280 highest leaf OMD (Figure 6). During rotations 9 to 10, Astonenergy, Abermagic and
281 Bealey had the highest leaf OMD. For all rotations Spelga consistently had a lower
282 leaf OMD than all other cultivars (Figure 6). During rotations 1 to 4 and 5 to 8,
283 Astonenergy and Spelga respectively, had the highest pseudostem OMD. For rotations
284 9 to 10, there was not enough pseudostem in any cultivar to analyse OMD. During
285 rotations 1 to 4, Abermagic and Bealey had the highest true stem OMD, and
286 Astonenergy and Spelga had the lowest (Figure 6). During rotations 5 to 8 and 9 to 10
287 there was no true stem present for any cultivar. During rotations 1 to 4 and 5 to 8,
288 Abermagic and Spelga respectively, had the highest dead OMD. During rotations 9 to
289 10, Astonenergy had the highest dead OMD.

290

291 ***Acid Detergent Fibre***

292 For the whole samples ADF, there was an interaction between cultivar and rotation (P
293 < 0.05). In mid Sept Abermagic (290 g/kg \pm 13.6) had a lower ADF than Bealey (359
294 g/kg \pm 13.6) and Spelga (363 g/kg \pm 13.6; $P < 0.05$). There was also an interaction
295 between cultivar and rotation for leaf ADF. In mid July Spelga (320 g/kg \pm 13.6) had
296 a higher leaf ADF than Abermagic (242 g/kg \pm 13.6; $P < 0.001$).

297

298 ***Neutral Detergent Fibre***

299 There was an interaction between cultivar and rotation for whole sample NDF
300 ($P < 0.05$). In start June Astonenergy had a lower NDF ($433 \text{ g/kg} \pm 20.2$) than Spelga
301 ($589 \text{ g/kg} \pm 20.2$; $P < 0.05$).

302

303 **Crude Protein**

304 For the whole samples CP there was an interaction between cultivar and rotation. In
305 mid Oct Bealey had a higher CP ($305 \text{ g/kg} \pm 7.4$) than Abermagic ($260 \text{ g/kg} \pm 7.4$; P
306 < 0.05). There was an interaction between cultivar and rotation for leaf CP ($P < 0.05$;
307 Figure 7). In end June Abermagic had a higher leaf CP value than Spelga ($P < 0.05$).
308 Bealey recorded a higher leaf CP value than both Astonenergy and Abermagic in mid
309 Sept ($P < 0.05$). In mid Oct Bealey recorded a higher leaf CP than Abermagic ($P <$
310 0.05), but in mid Feb Abermagic recorded a higher leaf CP than Bealey ($P < 0.05$).

311

312 **Discussion**

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

319

320 **Morphological components**

321 The physiological state of the plant affects the proportions of leaf and true stem
322 (Beever *et al.*, 2003). During the grass reproductive stage there is a reduction in leaf
323 and an increase in pseudostem and true stem proportion (Beever *et al.*, 1986; Minson,
324 1990; McDonald *et al.*, 2002b). From mid May to start June there was an increase in
325 the true stem proportion, while the leaf proportion remained static (and low compared
326 to later in the year). The true stem proportion declined from start June onwards as the
327 swards returned to the vegetative stage. Simultaneously the proportion of leaf
328 increased. This agrees with Jewiss (1981), who found that temperate grasses produce
329 little or no true stem during the vegetative stage. During the reproductive stage true
330 stem production limits leaf production (Jewiss, 1981), but during the vegetative stage
331 there is a morphological limit on leaf production. Lower leaf production and growth is
332 also seen in Timothy during the reproductive stage (Gustavsson and Martinsson,

333 2004). The present study and previous research by Wilson (1994) show that the leaf is
334 the most digestible component of the grass plant and the true stem is less digestible.
335 Astonenergy had the highest leaf and lowest true stem proportion and was
336 intermediate to Bealey and Spelga regarding overall OMD agreeing with The
337 Northern Ireland DARD Grass and Clover Recommended List (2012) which reports
338 Astonenergy as being a highly digestible cultivar (DARD, 2012). O'Donovan *et al.*
339 (2011) identified that grass breeding needs to focus on improving the digestibility
340 during the mid season and to ensure that sward canopy structure is appropriate for
341 grazing. These differences in the digestibility of the plant components offer an
342 opportunity to plant breeders to improve the digestibility of all plant components and
343 to select cultivars with a higher leaf and lower true stem proportion to target
344 maximum animal intake from grass.

345

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

367 1988; Buxton and Redfearn, 1997). The pseudostem is highly digestible, however
368 classifying pseudostem under the “stem” category can be misleading.

369

370 Senescent material accumulates over the winter period (Hennessy *et al.*, 2008; Ryan
371 *et al.*, 2010). This resulted in an increase in dead proportion in mid Feb compared to
372 mid Oct with the proportion of dead in mid Feb similar to that reported by Hennessy
373 *et al.* (2008) for a closing date in October. This was associated with a decrease in leaf
374 proportion and lower OMD in mid Feb than at several other times suggesting that the
375 dead was an accumulation of senescent leaves. According to Frame and Hunt (1971)
376 up to 0.40 of the leaf that is ungrazed eventually senesces causing a decrease in leaf
377 proportion. The overall dead proportion in the sward was lower than previously
378 reported for perennial ryegrass/white clover swards (Holmes *et al.*, 1992;
379 Hoogendoorn *et al.*, 1992) and for *Dichanthium* swards (Boval *et al.*, 2007). A
380 tolerable amount of dead in sward is any amount that does not negatively impact on
381 animal production. The proportion of dead in the present study was less than
382 previously reported, indicating that there was not an excessive amount of dead in the
383 sward (Tuñon *et al.*, 2013; Wims *et al.*, 2012). The dead proportion in all cultivars
384 remained stable throughout the year apart from an increase for Spelga in mid July and
385 end July. In the present study, Spelga had the highest dead proportion of all cultivars.
386 This may be partly attributed to its higher post grazing sward height which leads to
387 increased true stem and dead proportion (Stakelum and Dillon, 2007; Stakelum and
388 O'Donovan, 2000). In the current study Spelga had the highest post grazing sward
389 height compared to the other cultivars (data not presented) but pre-grazing height was
390 not significantly different between cultivars (data not presented). Likewise, Wims *et al.*
391 (2012) found that Spelga had a significantly higher post grazing sward height than
392 Astonenergy and Bealey but was similar to Abermagic, which contributed to it having
393 the highest dead proportion of the four cultivars experimented.

394

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

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

405

406 **Organic matter digestibility**

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

422

423 Wilman and Rezvani Moghaddam (1998) and McEvoy *et al.* (2010) found that OMD
424 was lower in July to August compared to earlier in April to June, agreeing with the
425 present study when in mid July OMD was lower than at other times. O'Donovan and
426 Kennedy (2007) showed that OMD was lowest in August, but they used late heading
427 cultivars. Late heading cultivars enter the reproductive stage at the start of June
428 whereas intermediate cultivars, which were used in the present study, enter the
429 reproductive stage at the end of May (Frame, 1991). This difference in heading dates
430 explains why O'Donovan and Kennedy (2007) found the lowest OMD in August
431 while in the present study it was in mid July. The post grazing sward heights at end
432 June may also have contributed to the low OMD in mid July. In end June all cultivars
433 had a significantly higher post grazing height (data not presented) than in mid May. A
434 higher post grazing sward height is associated with a reduction in digestibility in

435 subsequent rotations (Stakelum and Dillon, 2007; Baudracco *et al.*, 2010). Higher post
436 grazing sward height may create swards with higher herbage mass and a greater
437 proportion of true stem (Minson, 1990) and dead (Hoogendoorn *et al.*, 1992) resulting
438 in lower digestibility. The low OMD in mid Oct could be due to the low leaf OMD at
439 that time. This shows the influence of leaf OMD on the overall digestibility of the
440 sward and Stakelum and O'Donovan (2000) showed that a 5.5 percentage unit change
441 in leaf content was equal to a 1 percentage unit change in digestibility. The low OMD
442 in mid Feb could be due to the plant preparing itself for the reproductive stage and
443 diverting energy away from leaf production and towards pseudostem and true stem
444 development, which may also have resulted in an increase in dead. The combination
445 of low leaf OMD and high dead proportion resulted in herbage in mid Feb having a
446 low OMD.

447

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

461

462 In the present study the two tetraploid cultivars performed best regarding combined
463 leaf and pseudostem proportion and OMD. Bealey had the highest OMD of all four
464 cultivars agreeing with Palladino *et al.* (2009) who found that Bealey had the highest
465 DMD compared to a number of diploid and tetraploid, and late and intermediate
466 heading cultivars. Bealey had the highest leaf + pseudostem proportion and hence the
467 highest OMD. This shows the influence of leaf + pseudostem on overall OMD. The
468 low fibre content and high leaf proportion in Astonenergy and low NDF coupled with

469 high leaf CP in Bealey, agrees with these two cultivars being highly digestible. Acid
470 detergent fibre is the cell wall portion of the grass plant and has a negative
471 relationship with digestibility (Beever *et al.*, 2003). The cell wall increases as grasses
472 mature (Stone, 1994; Buxton, 1996; Beever *et al.*, 2003). This agrees with the present
473 study where Spelga was more mature than Astonenergy in start June as Spelga had a
474 higher NDF content and higher true stem proportion than Astonenergy. Spelga had a
475 low OMD and was characterised by low leaf and high true stem proportions.
476 Abermagic had a high leaf OMD and leaf CP content, but because the leaf proportion
477 was not as high, the OMD value was only intermediate. Wims *et al.* (2012) found that
478 both Spelga and Abermagic had a lower OMD than Astonenergy and Bealey during
479 the reproductive phase. McEvoy *et al.* (2012b) found that cows grazing Astonenergy
480 and Bealey produced a higher milk yield and milk solids content than Abermagic and
481 Spelga suggesting that milk production at farm level could be improved by using
482 these cultivars.

483

484 **Implications**

485 True stem development can begin any time between March and May (Hurley *et al.*,
486 2008). This highlights the importance of early grazing to keep true stem proportion to
487 a minimum as it has a low OMD and a high proportion of true stem in the sward could
488 lead to reduced animal performance. Differences in morphological proportions
489 suggest that different cultivars should be managed differently during the reproductive
490 stage to maximise the leaf proportion and OMD and minimise the true stem and dead
491 proportion. Future work should investigate the option of tailoring rotation length to
492 cultivar by offering a set daily herbage allowance and only move the animals once a
493 target post-grazing sward height has been achieved. Differences in the proportions of
494 plant components and between cultivars are evident predominantly during the
495 reproductive stage implying that it would be worthwhile for evaluation programmes to
496 record cultivar characteristics such as leaf, pseudostem, true stem, dead proportion
497 and OMD of these components at this time. In studies that determine the sward
498 morphology, the pseudostem should be separated from the true stem in order to
499 accurately characterise the sward. During the vegetative stage, there were no
500 differences between cultivars in terms of leaf and true stem proportion. As there is no
501 true stem in swards during the vegetative stage, a longer regrowth period can be

502 implemented, facilitating the build-up of herbage mass. This can be used to extend the
503 grazing season in autumn.

504

505

506 **Conclusion**

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

523

524

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528

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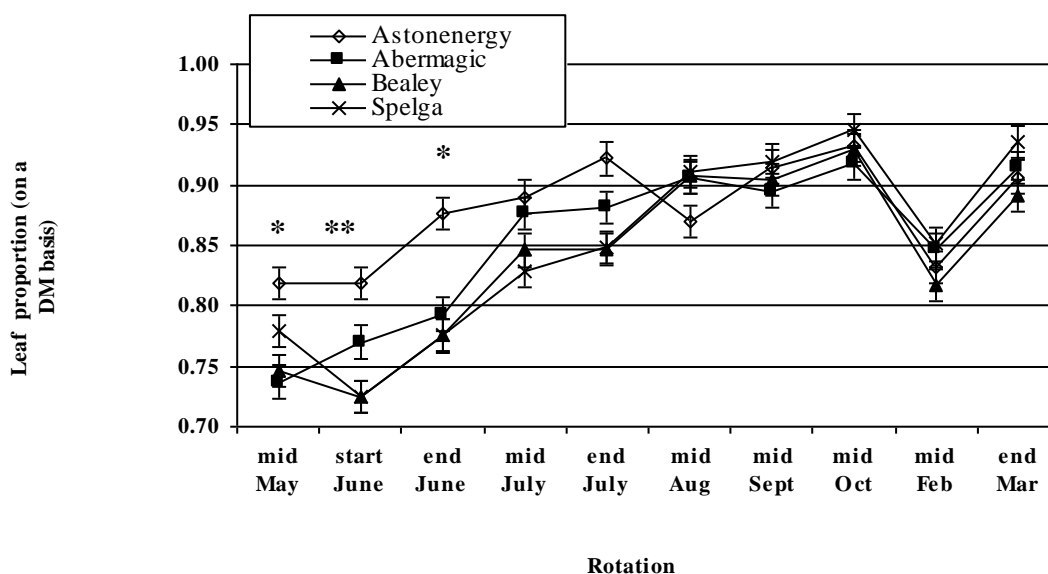
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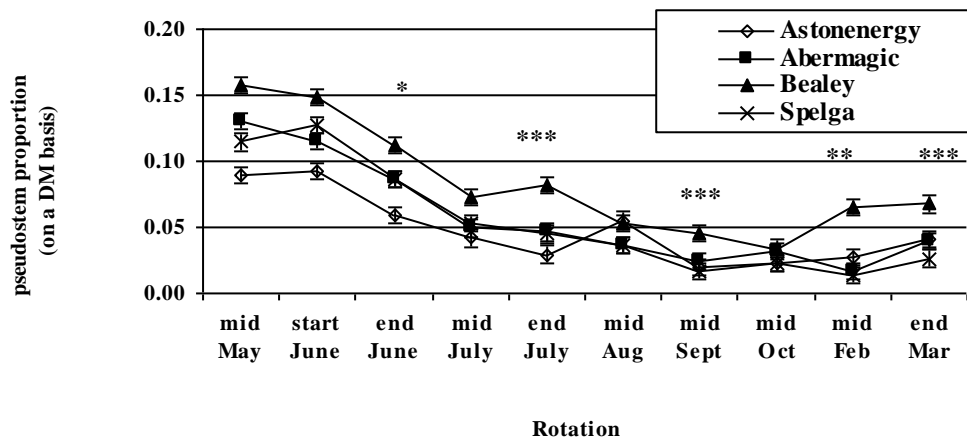
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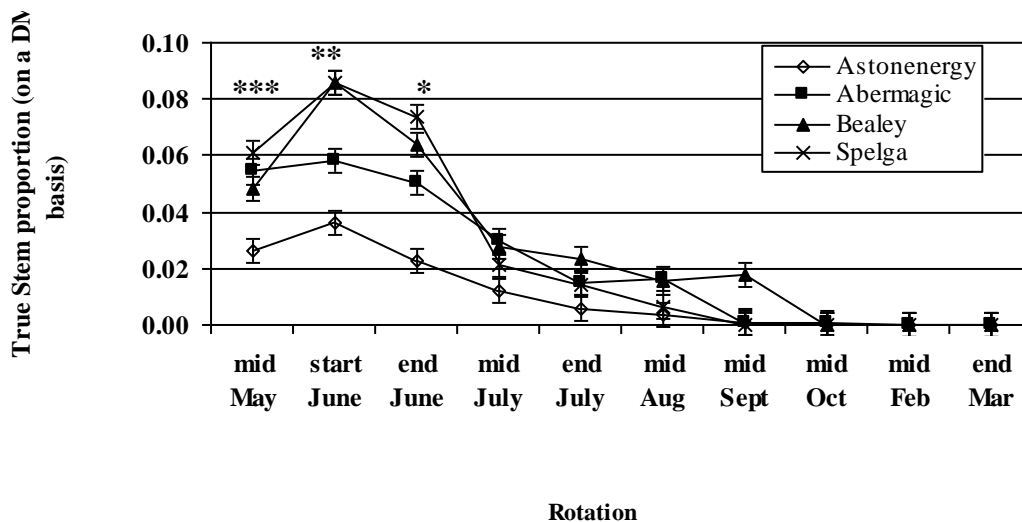


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697 **Figure 1.** Leaf proportion above 4 cm (expressed on a DM basis) for four perennial
698 ryegrass cultivars (Astonenergy, Abermagic, Bealey and Spelga), sown as single
699 cultivar grazing swards, during 10 grazing rotations from May 2011 to March 2012
700 (mean±SEM; * = P < 0.05; ** = P < 0.01).
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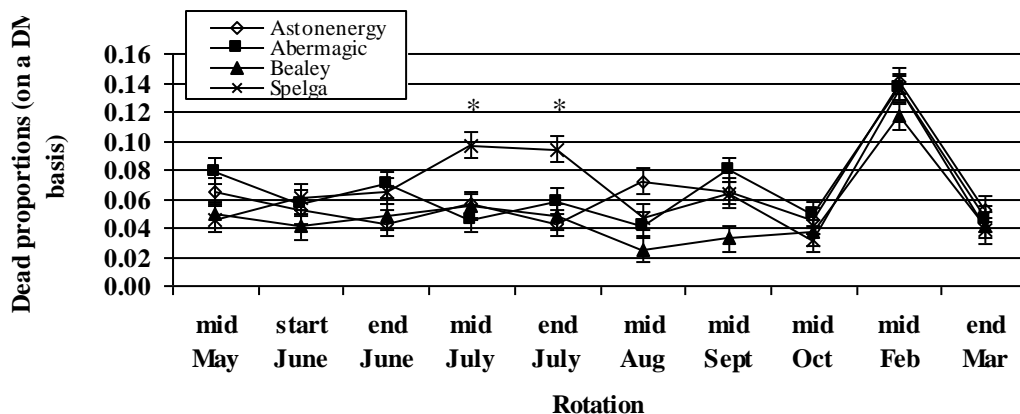
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Figure 2. Pseudostem proportion above 4 cm (expressed on a DM basis) for four perennial ryegrass cultivars (Astonenergy, Abermagic, Bealey and Spelga), sown as single cultivar grazing swards, during 10 grazing rotations from May 2011 to March 2012 (mean±SEM; * P < 0.05, ** P < 0.01, *** P < 0.001)



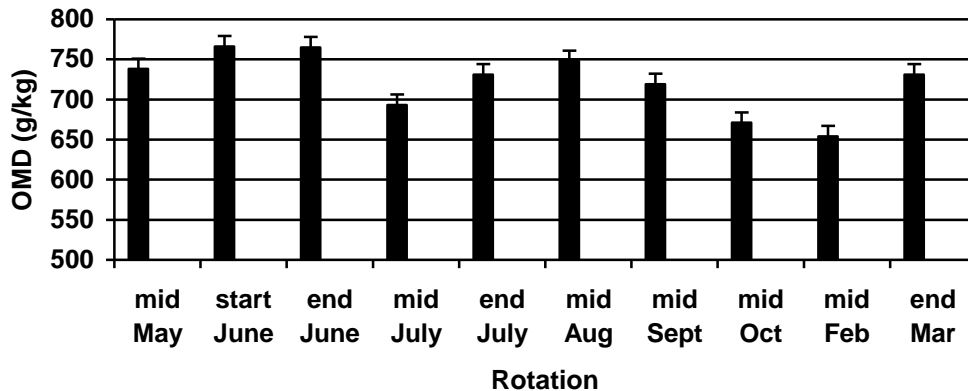
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Figure 3. True stem proportion above 4 cm (expressed on a DM basis) for four perennial ryegrass cultivars (Astonenergy, Abermagic, Bealey and Spelga), sown as single cultivar grazing swards, during 10 grazing rotations from May 2011 to March 2012 (mean±SEM;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

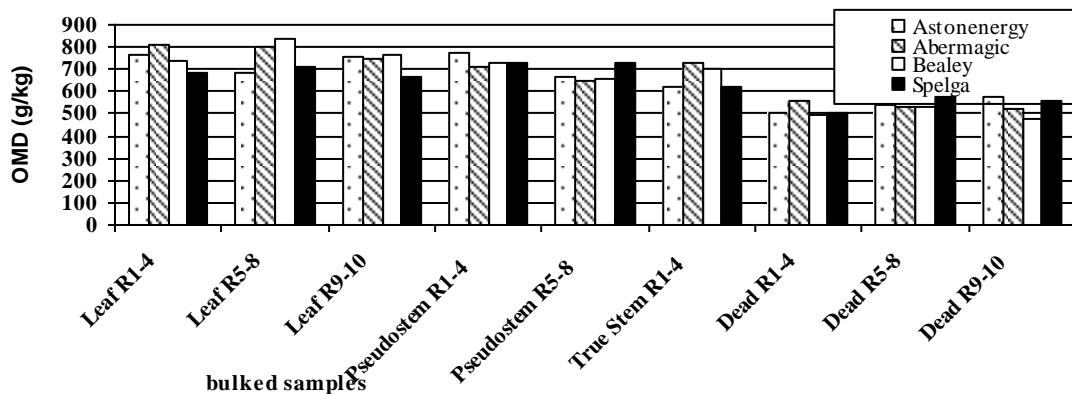


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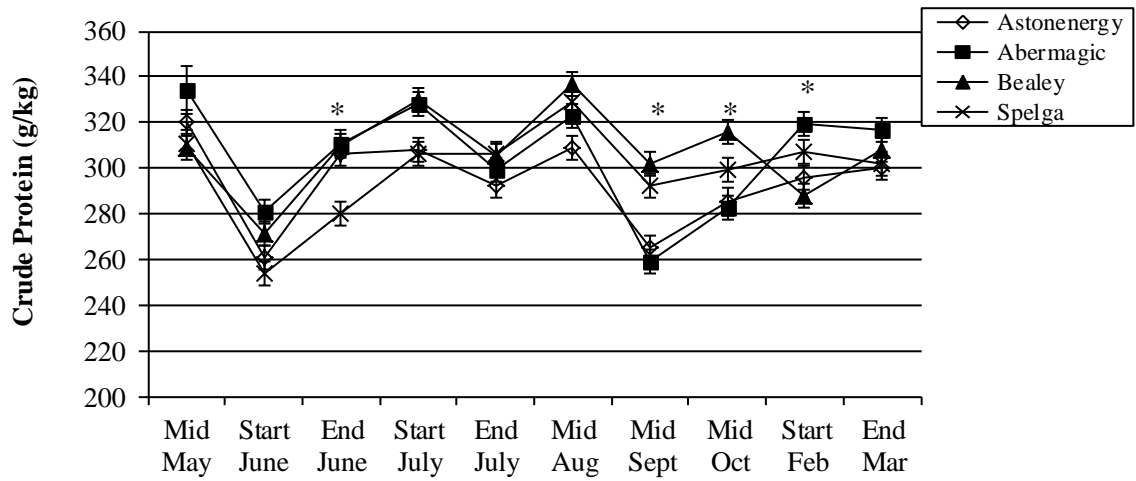
715 **Figure 4.** Dead proportion above 4 cm (expressed on a DM basis) for four perennial
 716 ryegrass cultivars (Astonenergy, Abermagic, Bealey and Spelga), sown as single cultivar grazing swards,
 717 during 10 grazing rotations from May 2011 to March 2012
 718 (mean±SEM; * P < 0.05)
 719



720
 721 **Figure 5.** Mean whole plant above 4 cm organic matter digestibility (OMD) of four
 722 perennial ryegrass cultivars (Astonenergy, Abermagic, Bealey and Spelga), sown as single cultivar grazing swards,
 723 during 10 grazing rotations from May 2011 to March
 724 2012 (mean±SEM)
 725



726
 727 **Figure 6.** Organic matter digestibility (OMD) of leaf, pseudostem, true stem and dead
 728 morphological components above 4 cm of four perennial ryegrass cultivars
 729 (Astonenergy, Abermagic, Bealey and Spelga), sown as single cultivar grazing
 730 swards, bulked for rotations 1 (mid May) to 4 (mid July) (R1-4), rotations 5 (end July)
 731 to 8 (mid Oct) (R5-8) and rotations 9 (mid Feb) to 10 (end Mar) (R9-10)
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Rotation

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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)