

1 **Production, intake and behaviour of dairy cows rotationally grazing on multi-**
2 **species swards**

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9 Short title: Grazing dairy cow intake on multi-species swards

10

11 **Abstract**

12 Increasing plant species diversity has been proposed as a means for enhancing
13 productivity and seasonal production stability of grazing lands facing drought stress.
14 Few studies have examined how botanical complexity of sown swards affects cow
15 performance. A two-year experiment was conducted to determine how sward
16 botanical complexity, from monocultures of grass to multi-species swards (MSS)
17 (grass-legume-forb), affect pasture chemical composition and nutritive value, milk
18 production and milk solids production, pasture dry matter (DM) intake and grazing
19 behaviour of cows. Five sward species: perennial ryegrass (L as *Lolium*), white clover
20 and red clover (both referred to as T as *Trifolium* because always sown together),
21 chicory (C as *Cichorium*) and tall fescue (F as *Festuca*) were assigned to four grazing
22 treatments by combining 1 (L), 3 (LT), 4 (LTC) or 5 (LTCF) species. Thereafter, the
23 LT swards were called mixed swards as a single combination of ryegrass and
24 clovers, while LTC and LTCF swards are called multi-species swards (MSS) as a
25 combination of at least four species from three botanical families. The experimental

26 area (8.7 ha) was divided into block replicates with a mineral nitrogen fertilisation of
27 75 kg N/ha/year in each treatment. The weather conditions were favorable for
28 pasture growth in both years, particularly in early summer. In total, 13 grazing
29 rotations were carried out by applying the same grazing calendar for all treatments
30 and the same pasture allowance of 19 kg DM/cow/day above 4 cm. Clover
31 represented 20% of DM in mixed and MSS swards; chicory represented 30% of DM
32 in MSS and tall fescue represented 10% of DM in LTCF swards. Greater milk
33 production (+1.1 kg/day) and milk solids production (+0.08 kg/day) were observed in
34 mixed swards than in ryegrass swards. Pasture quality and pasture DM intake were
35 unaffected by the inclusion of clover. Pasture DM ($P<0.001$), organic matter
36 ($P<0.001$) and neutral detergent fibre concentrations ($P<0.001$) were lower in MSS
37 than in mixed swards. Greater milk production (+0.8 kg/day), milk solids production
38 (+0.04 kg/day), pasture DM intake (+1.5 kg DM/day) and grazing time (+57 min/day)
39 were observed in MSS than in mixed swards. These positive effects of MSS swards
40 were observed in all seasons, but particularly during summer. In conclusion,
41 advantages of grazing MSS on cow performance were mainly due to the presence of
42 chicory, and to the cumulative effect of enhanced sward quality, increased pasture
43 DM intake that raised milk production and milk solids production.

44 **Keywords:** chicory, grass-legume mixtures, grazing, multi-species, milk production

45

46 **Implications**

47 Little is known about how increasing sward species complexity may affect milk
48 performance in grazing cows. The aim of this study was to determine how pastures
49 sown with 1, 3, 4 and 5 species - including grasses, legumes and chicory - affect
50 pasture chemical composition and nutritive value, milk production, pasture intake and

51 the grazing behaviour of cows. Pastures sown with greater sward species complexity
52 induced greater sward quality, pasture intake, milk production and milk solids
53 production than simpler pastures, in all seasons. Both legumes and chicory had
54 positive effects on sward quality and milk production.

55

56 **Introduction**

57 Grasslands cover approximately 40% of the agricultural area in Europe, and supply
58 most of the feed used by cattle and other ruminants (78 million livestock units)
59 (Huyghe *et al.*, 2014). Traditionally, sown grasslands have been based on a two-
60 species (grass-legume) mixture (Høgh-Jensen *et al.*, 2006). Legumes offer important
61 potential benefits when mixed with perennial ryegrass by (i) increasing pasture
62 production, (ii) substituting mineral N-fertiliser inputs by symbiotic N₂ fixation, (iii)
63 mitigating and facilitating adaptation to climate change, as elevated atmospheric
64 CO₂, warmer temperatures and drought-stress periods rise, (iv) increasing pasture
65 nutritive value and voluntary intake with a less marked decline of sward quality with
66 advancing maturity than grasses, leading to (v) higher cow performance (Lüscher *et*
67 *al.*, 2014). However, grass-legume swards may be limited by the intensive yet
68 relatively short growth period for legumes, mainly clovers, compared to other sward
69 species, adapted to a larger range of weather conditions (Høgh-Jensen *et al.*, 2006).
70 Increasing the botanical complexity of swards has been suggested as a means of
71 raising pasture productivity and seasonal production stability facing drought stress
72 (Sanderson *et al.*, 2005). Nevertheless, much of the research to date in Europe has
73 been focused on single mixed swards. Enhanced milk production (10% to 25%) of
74 grazing dairy cows in grass-legume swards compared to perennial ryegrass swards
75 is generally related to the increase in pasture intake rather than in pasture nutritive

76 value (Harris *et al.*, 1997; Ribeiro Filho *et al.*, 2003; Dewhurst *et al.*, 2009). There is a
77 rising interest to examine the effect of multi-species swards (MSS) on sward quality,
78 pasture intake (Sanderson *et al.*, 2006; Deak *et al.*, 2009; Sanderson, 2010) and milk
79 production in grazing dairy cows (Sanderson *et al.*, 2005; Soder *et al.*, 2006;
80 Chapman *et al.*, 2008). Evidence from these studies suggests that the yield benefit
81 mainly results from including drought-tolerant species (i.e., forbs such as chicory).
82 Skinner (2008) showed that including chicory in grass-legume swards improved
83 pasture production, especially in mid-summer when drought stress reduces
84 productivity of dominant cool-season sward species. However, chicory is
85 disadvantaged by its low persistency in swards beyond 3 or 4 years (Sanderson *et*
86 *al.*, 2003).

87 Chicory is well known as a highly productive species, with high nutritive value (Li and
88 Kemp, 2005). It has been found to enhance sward quality by improving the seasonal
89 availability of high quality pasture (Marley *et al.*, 2013). It shows a variable crude
90 protein concentration and contains higher water soluble carbohydrates (Hoskin *et al.*,
91 1995), greater digestibility, lower fibre (Barry, 1998), and higher mineral and trace
92 element concentrations (Høgh-Jensen *et al.*, 2006; Marley *et al.*, 2013) than
93 perennial ryegrass, irrespective of the grazing season. Greater voluntary intake in
94 steers (Morel *et al.*, 2014) and sheep (Niderkorn *et al.*, 2014) has been found in
95 mixed swards including chicory compared with perennial ryegrass swards. Under
96 grazing management, the effect of including chicory in mixed swards on dairy cow
97 performance is unclear. Pasture DM intake and milk production were unaffected by
98 inclusion of chicory in the studies of Soder *et al.* (2006) and Muir *et al.* (2014), but
99 increases in milk production and pasture DM intake were found in the studies of Li
100 and Kemp (2005), and Chapman *et al.* (2008). In order to investigate the effect of

101 MSS on intake and milk production of grazing dairy cows, a two-year study was
102 conducted to test the hypothesis that increasing botanical complexity from 1 (grass)
103 to 5 sward species (grass-legume-forb) would affect pasture chemical composition
104 and nutritive value, milk production, milk solids production, pasture DM intake and
105 grazing behaviour of dairy cows. The duration of the study allowed for grazing in all
106 seasons, so as to investigate whether the effect of the sward's botanical complexity
107 on cow performance would be different depending on the season.

108

109 **Materials and methods**

110 *Location, treatments and experimental design*

111 The experiment was conducted over two years: September 2011 – August 2012
112 (year 1) and September 2012 – August 2013 (year 2) at the INRA experimental dairy
113 farm of Méjusseume (1.71°W, 48.11°N, Le Rheu, France). The soils are loamy with
114 a pH value of around 6.0, an organic matter content of 3%, and are particularly
115 sensitive to water stress during summer. The swards were sown in September 2010,
116 one year before starting the experiment. Four treatments were compared by seeding
117 pastures with a combination of 1 to 5 sward species as described in Table 1. The
118 sward species were: perennial ryegrass (L, *Lolium perenne* L.), white clover
119 (*Trifolium repens* L.) and red clover (*Trifolium pratense* L.), both referred to as T for
120 *Trifolium* since they are always sown together, chicory (C, *Cichorium intybus* L.), and
121 tall fescue (F, *Festuca arundinacea* Schreb.). The four treatments were applied by
122 combining 1 (L), 3 (LT), 4 (LTC) or 5 (LTCF) of these sward species, to increase
123 botanical complexity of pastures. Treatment L was considered as the control, and LT
124 as a commonly used grass-legume mixture in temperate pasture-based milk
125 production systems for its potential to supply consistent forage yields with low mineral

126 N-fertiliser due to symbiotic N₂ fixation by legumes. In LTC, chicory was added as a
127 deep-root forb well adapted to dry summers and considering its good nutritive value.
128 Finally, in LTCF, tall fescue was added, as a more drought-resistant grass than
129 perennial ryegrass, increasing tolerance to dry soil conditions. The experiment was a
130 randomized complete block design with four replicates of each treatment. The total
131 area (8.7 ha) was divided into four blocks (replicates) and each block was subdivided
132 into four paddocks (treatments) with random distribution of treatments within each
133 block. During the two-year study, this area was dedicated solely to grazing and no
134 silage or hay was harvested.

135

136 *Cows*

137 Treatments within each block were simultaneously grazed by four homogeneous
138 groups of 7 to 10 autumn-winter-calving Prim'Holstein dairy cows, using a rotational
139 grazing system. The same cows could not be used during the 2 years of the study,
140 and several reference periods were needed to allocate cows in the 4 groups. A total
141 of 6 pre-experimental periods were considered throughout the trial, during which all
142 cows were managed similarly as a single herd (Table 2). Cows were balanced at
143 each pre-experimental period according to lactation stage (181 ± 85.4 days in milk),
144 milk production (27.4 ± 7.52 kg/day), milk fat concentration (37.1 ± 3.79 g/kg), milk
145 protein concentration (31.2 ± 2.39 g/kg), body weight (BW) (627 ± 15.2 kg) and body
146 condition score (BCS) (2.16 ± 0.19). On average, cows were 100, 164 and 280 days
147 in milk and yielded 35.4, 26.4 and 20.3 kg/day of milk respectively in the pre-
148 experimental spring, summer and autumn periods considered.

149

150 *Grazing management and pastures*

151 Due to limited grazing area compared to herd size (high global stocking rate),
152 permanent grazing within the experimental area was not possible. Consequently,
153 grazing was organised by rotations and cows grazed non experimental pastures as a
154 single herd between 2 experimental rotations. In total, 13 grazing rotations (7 in year
155 1 and 6 in year 2) were carried out during the two-year study, with a yearly average
156 of 2 rotations in autumn, 2.5 rotations in spring and 2 rotations in summer. Within a
157 grazing rotation, the 4 blocks were grazed successively, always in the same order.
158 Within each block, the 4 herds grazed simultaneously in 1 of the 4 paddocks, 1 herd
159 being dedicated to 1 treatment for the entire rotation and until the next reference
160 period (Table 2). During the 8 rotations without intake measurement (see Animal
161 measurements section below), a rotational grazing system was used (2 to 6 days of
162 residence time per paddock according to season and pasture availability). During the
163 5 rotations with intake measurement (see Animal measurements section below), a
164 strip-grazing system was employed using temporary electric fences. Fresh pasture
165 was allocated once daily after the a.m. milking and the pasture access time was
166 approximately from 9.00 a.m. to 3.30 p.m. and from 5.00 p.m. to 6.30 a.m. Cows
167 were thus at grazing 20 hours daily and received no supplement. The total residence
168 time per paddock and therefore area on offer each day was calculated 1 or 2 days
169 before grazing from the pre-grazing pasture mass estimated as described in the
170 Sward measurements section, and considering the two following management rules:
171 1) same grazing calendar (i.e. same dates) between treatments to avoid time lag,
172 and 2) similar pasture allowance (19 kg DM/cow/day > 4 cm) between treatments, to
173 define a medium to high grazing pressure (Pérez-Prieto and Delagarde, 2013) for
174 controlling post-grazing sward height. To combine both rules, additional, mobile, non-
175 experimental dairy cows were needed to adjust grazing pressure within each block

176 and between treatments based on differences in pre-grazing pasture mass. Pasture
177 refusals were mowed once per paddock and per year in late spring to a 5-6 cm
178 stubble height, and the clipped residues were left in place. The nitrogen fertilisation
179 level was similar between treatments (75 kg N/ha/year) by implementing 3 equal
180 applications of 25 kg N/ha/rotation of ammonium nitrate in spring and early summer
181 after grazing. Water and mineral blocks were always available to each herd during
182 grazing. The walking distance from paddocks to the milking parlour averaged 610 m.

183

184 *Sward measurements*

185 Pre-grazing pasture mass, pre-grazing and post-grazing sward heights, sward bulk
186 density and pasture allowance were determined for each treatment in each block and
187 rotation. Pre- and post-grazing sward heights were measured with an electronic rising
188 plate meter (30 × 30 cm and 4.5 kg/m², AGRO-Systèmes, La Membrolle, France) on
189 the days before and after grazing by taking 60 and 50 measurements per treatment
190 at random, respectively, across 4 diagonals of each paddock, i.e. 100-120
191 measurements per ha. Pre-grazing sward height was adjusted by pasture daily
192 growth rate estimated by weekly measurement of sward height, and considering lag
193 time between measurement days and average grazing days within the paddock.
194 Adjusted pre-grazing pasture mass above 4 cm was calculated by multiplying the
195 adjusted pre-grazing sward height by the sward bulk density above 4 cm. To
196 determine sward bulk density, 4 strips of 8 m × 0.5 m per treatment were cut with a
197 motor scythe to a post-cutting sward height of 4 cm above ground level. The pasture
198 height on each strip was measured with a rising plate meter, before and after mowing
199 (15 measurements per strip), making it possible to estimate bulk density by dividing
200 pasture mass by cutting depth. The total quantity of pasture collected in each strip

201 was weighed and a fresh, representative 500-g subsample was oven-dried to
202 determine pasture DM concentration. Another fresh 500-g subsample was collected
203 at the same time, washed and oven-dried before being analysed for ash and crude
204 protein in blocks 1 to 4, for fibre in blocks 2 and 4, and for pepsin-cellulase
205 digestibility in blocks 2 and 4, but only during rotations with intake measurement.
206 Pasture botanical composition was determined for each treatment in 12 of the 13
207 grazing rotations carried out during the experiment (except in the grazing rotation 13
208 in late summer). A fresh pasture subsample of approximately 1,000 g was taken from
209 blocks 2 and 4 before grazing. Handfuls of pasture were randomly collected at each
210 of the 4 steps across 4 diagonals in each paddock to a cutting height of 3-4 cm
211 above ground level. A fresh representative 500-g pasture subsample was
212 immediately separated into 7 botanical items (*Lolium perenne* L., *Trifolium repens* L.,
213 *Trifolium pratense* L., *Cichorium intybus* L., *Festuca arundinacea* Schreb, unsown
214 species and senescent material). Each constituent was oven-dried for 48 h at 80°C to
215 determine botanical composition on a DM basis.

216

217 *Animal measurements*

218 Cows were milked twice daily at 7.30 a.m. and 4.00 p.m. Milk production per cow was
219 recorded at each milking throughout the experiment. Milk fat and milk protein
220 concentrations were measured using individual milk samples, collected during 6
221 consecutive milkings per week, by near infrared spectrophotometry using a
222 Milkoscan instrument (Foss Electric, DK-3400, Hillerød, Denmark). Production of 4%
223 fat-corrected milk (4% FCM) was calculated according to INRA (2007). The body
224 weight (BW) of each cow was recorded automatically once daily after morning
225 milking.

226 Individual pasture DM intake was determined during 5 rotations, namely rotations 2 in
227 autumn, 5 and 11 in spring and 6 and 12 in summer. Intake (I) was measured using
228 ytterbium as an external marker for estimating faecal output (FO) and using faecal
229 composition for estimating pasture digestibility (D), with the relation $I = FO / (1 - D)$
230 (Ribeiro-Filho *et al.*, 2005). Faecal DM output was calculated by dividing the daily
231 amount of dosed Yb by the faecal Yb concentration. The digestibility of selected
232 pastures was estimated using faecal nitrogen (N) and acid detergent fibre (ADF)
233 concentrations as faecal indices to estimate organic matter (OM) digestibility by
234 multiple regression (Ribeiro-Filho *et al.*, 2005).

235 At each intake measurement period, each experimental cow was dosed twice daily
236 before milking during at least 7 days and then sampling with a cellulose stopper (Carl
237 Roth, Germany) containing 0.8 g of ytterbium (Yb) marker. Faecal grab samples were
238 collected from each cow twice daily after milking during a total of 6 days, of which 3
239 days in block 2 and 3 days in block 4, and then stored at 4°C. The daily faecal
240 samples were composited by cow and rotation and then oven-dried before chemical
241 analyses. Net energy (in UFL, i.e. Unité Fourragère Lait) and protein (in PDIE, i.e.
242 protein truly digested in the intestine with no degradable-N deficiency) balance were
243 calculated according to INRA (2007) and expressed as a proportion of requirements.
244 Requirements were considered for maintenance (including additional 20% for grazing
245 activity), growth (for primiparous), 4%-fat corrected milk production and gestation
246 (INRA, 2007). Nutrients supplies were calculated from pasture DM intake and the
247 UFL and PDIE concentrations of pastures, taking into account digestive interactions
248 due to feeding level (INRA, 2007).

249 Grazing time was determined during rotations 11 (spring) and 12 (summer), using the
250 Kenz Lifecorder Plus device (Delagarde and Lamberton, 2015). In total, 24 dairy

251 cows (6 per treatment) were simultaneously equipped with collars containing the
252 Lifecorder Plus device. Cows were equipped for 48-h periods in both blocks 2 and 4,
253 so that each cow was recorded at least 2 days in each block, i.e. a total of 4 days per
254 rotation. Grazing bout and grazing time were defined as described by Delagarde and
255 Lambertson (2015). The first grazing bout was defined as such just after the a.m.
256 milking, when fresh pasture was allocated. The average duration for a grazing bout
257 was calculated by dividing the grazing time by the number of bouts. Pasture intake
258 rate was calculated as the ratio between pasture DM intake and grazing time.
259 Blood samples were taken from each cow via the caudal vein for urea nitrogen
260 analysis as an indicator of nitrogen supply. Blood sampling took place after morning
261 milking once in block 4 during grazing rotations 5 and 12 (spring) and 6 and 13
262 (summer). Plasma was separated by centrifugation, deproteinised by adding 2 ml of
263 HClO₄ per ml of plasma, and then frozen.

264

265 *Chemical analyses*

266 Oven-dried pasture (80°C during 48 h) and faeces (80°C during 72 h) samples were
267 ground through a 0.8-mm screen before chemical analyses. The nitrogen (N)
268 concentration was determined using the Dumas method (Association Française de
269 Normalisation, 1997) on a Leco instrument (Leco, St. Joseph, MI, USA). The
270 concentrations of neutral detergent fibre (NDF), acid detergent fibre (ADF) and acid
271 detergent lignin (ADL) were measured according to van Soest *et al.* (1991) using a
272 Fibersac extraction unit (Ankom Technology, Fairport, NY, USA). Pepsin-cellulase
273 digestibility (PCd) was determined following the Aufrère and Michalet-Doreau method
274 (1988). Ash concentration was determined by calcination at 550°C during 5 h in a
275 muffle furnace (Association Française de Normalisation, 1997). Ytterbium was

276 determined using atomic absorption spectrophotometry with a nitrous oxide/acetylene
277 flame (Varian spectra-20, Varian France, Les Ulis, France) after calcination and
278 digestion in nitric acid as reported by Siddons *et al.* (1985). A standard addition
279 method was applied for Yb determination (Pérez-Ramírez *et al.*, 2012). Uremia was
280 determined with a multi-parameter analyser (KONE Instruments Corporation, Espoo,
281 Finland) according to Talke and Schubert (1965).

282

283 *Statistical analyses*

284 Pasture data related to grazing management characteristics, pasture chemical
285 composition and nutritive value of the 4 grazing treatments considered were
286 evaluated using the following model of analysis of variance:

$$287 Y_{ijklm} = \mu + Yr_i + S_j + R_k (Yr_i \times S_j) + P_l + T_m + Yr_i \times S_j + T_m \times S_j + T_m \times Yr_i + T_m \times Yr_i \times S_j + e_{ijklm}$$

288 where Y_{ijklm} , μ , Yr_i , S_j , $R_k (Yr_i \times S_j)$, P_l , T_m , $Yr_i \times S_j$, $T_m \times S_j$, $T_m \times Yr_i$, $T_m \times Yr_i \times S_j$ and e_{ijklm}
289 represent the analysed variable; the overall mean; the fixed effect of the year ($i= 1-2$);
290 the fixed effect of the season ($j= 1-3$); the fixed effect of the grazing rotation within
291 year and season ($k= 1-13$); the fixed effect of the block ($l= 1-4$); the fixed effect of the
292 treatment ($m= 1-4$); the interaction between year and season; the interaction between
293 treatment and season; the interaction between treatment and year; the interaction
294 between treatment, year and season; and the residual error term; respectively. The
295 effect of year, season and year \times season were tested using rotation within each year
296 and season as the residual term.

297 Three orthogonal contrasts were applied on treatment means for testing: the effect of
298 introducing clover in perennial ryegrass swards (contrast T: L vs. LT); the effect of
299 MSS compared to single perennial ryegrass/clover mixtures (contrast M: LT vs.
300 LTC/LTCF); the effect of introducing tall fescue in MSS (contrast F: LTC vs. LTCF).

301 Pasture data related to chemical composition of the 7 botanical items were analysed
302 using analysis of variance by the following model:

$$303 Y_{ijkno} = \mu + Y_{ri} + S_j + R_k (Y_{ri} \times S_j) + Sp_n + Sp_n \times S_j + Sp_n \times Y_{ri} + e_{ijkno}$$

304 where Y_{ijkno} , Sp_n , $Sp_n \times S_j$, $Sp_n \times Y_{ri}$ and e_{ijkno} represent the analysed variable; the fixed
305 effect of the botanical item ($n= 1-7$); the interaction between botanical item and
306 season; the interaction between botanical item and year; and the residual error term;
307 respectively.

308 Three orthogonal contrasts were applied on treatment means for testing: the effect of
309 chicory vs. legumes (C vs. L); the effect of chicory vs. grasses (C vs. G); the effect of
310 grasses vs. legumes (G vs. L).

311 Animal data related to milk production, milk solids production, milk composition and
312 body weight were analysed using covariance analysis by PROC Mixed SAS Institute
313 (1999) following the model below:

$$314 Y_{ijklmh} = \mu + Y_{ri} + S_j + R_k (Y_{ri} \times S_j) + Pr_l + T_m + Y_{ri} \times S_j + T_m \times S_j + C_h + b_1 X_{ijklm} + b_2 DIM_{ijklm} \\ 315 + e_{ijklmh}$$

316 where Y_{ijklmh} , Pr_l , C_h , $b_1 X_{ijklm}$, $b_2 DIM_{ijklm}$ and e_{ijklm} represent the analysed variable; the
317 fixed effect of the parity ($l= 1-2$); the random effect of the cow ($h= 1-40$); the pre-
318 experimental covariate for each experimental variable when available; the days in
319 milk at the start of the trial; and the residual error term; respectively. Covariates were
320 centered within parity and rotation. Days in milk was centered within rotation.

321 Intake, energy and protein balance, faecal output and uremia were analysed using
322 covariance analysis by PROC Mixed SAS Institute (1999), using the following model:

$$323 Y_{ijlmh} = \mu + Y_{ri} + S_j + Pr_l + T_m + T_m \times S_j + C_h + e_{ijlmh}$$

324 where Y_{ijlmh} and e_{ijlmh} represent the analysed variable and the residual error term;
325 respectively.

326 Grazing behaviour was evaluated using variance analysis by PROC Mixed SAS
327 Institute (1999), using the following model:

$$328 Y_{jlmh} = \mu + S_j + Pr_l + T_m + T_m \times S_j + C_h + e_{jlmh}$$

329 where Y_{jlmh} and e_{jlmh} represent the analysed variable and the residual error term;
330 respectively.

331 The three orthogonal contrasts (T, M and F) described above were applied to all
332 animal variables.

333

334 **Results**

335 Weather conditions were globally good for pasture growth in both years, with rainy
336 springs and medium temperatures in early summers, making it possible to maintain
337 pasture growth until late July. The average monthly temperatures in year 1 (12.0°C)
338 and year 2 (11.2°C) were in line with the last 30-year average (11.8°C).

339 Nevertheless, the cumulated rainfall was slightly lower in year 1 (627 mm) and then
340 marginally higher in year 2 (855 mm) compared to the last 30-year average (728 mm)
341 (Figure 1).

342

343 *Pasture botanical composition, pasture mass, height and allowance*

344 Perennial ryegrass proportion based on DM ranged from 70% in L to 34% in LTCF
345 swards (Table 3). Clover represented approximately 20% of DM in LT, LTC and
346 LTCF swards. The highest clover proportion was observed in autumn (25%) and the
347 lowest in spring (15%) (Table 4). Chicory represented 30% of DM in MSS, ranging
348 from 20% in spring to 46% in autumn. Tall fescue represented 10% of DM in LTCF
349 swards, independently of the season. Proportions of unsown species decreased with

350 increasing sward botanical complexity, and senescent material proportions were
351 similar between treatments.

352 Pre-grazing pasture mass averaged 2,350 kg DM/ha above 4 cm and was unaffected
353 by treatment (Table 3). Pre-grazing sward height averaged 14.3 cm and was greater
354 in MSS than in mixed swards (+1.8 cm, $P<0.05$), leading to lower sward bulk density
355 in MSS than in mixed swards. As planned, pasture DM allowance was similar
356 between treatments. Post-grazing sward height was lower in MSS than in mixed
357 swards (4.7 vs. 5.0 cm, $P<0.05$). There was no treatment \times season interaction for
358 pre-grazing pasture mass, pasture allowance, pre- or post-grazing sward height.

359

360 *Pasture chemical composition and nutritive value*

361 Pasture DM concentration was lower in mixed swards than in ryegrass swards (-12
362 g/kg, $P<0.01$), and in MSS than in mixed swards (-34 g/kg, $P<0.001$). Pasture OM
363 concentration was lower in MSS than in mixed swards (-28 g/kg DM, $P<0.001$), with a
364 greater effect in autumn than in spring and summer (interaction treatment \times season:
365 $P<0.001$). Pasture CP concentration was greater in LTCF than in LTC (+11 g/kg DM,
366 $P<0.05$). Pasture NDF concentration was lower in MSS than in mixed swards (-54
367 g/kg DM, $P<0.001$), and this occurred particularly during autumn (-85 g/kg DM,
368 interaction treatment \times season: $P<0.05$). Pasture ADL concentration was greater in
369 mixed swards than in ryegrass swards (+6 g/kg DM, $P<0.05$) and greater in MSS
370 than in mixed swards (+12 g/kg DM, $P<0.001$). Pasture pepsin-cellulase digestibility
371 was greater in MSS than in mixed swards (+33 g/kg DM, $P<0.01$), and this difference
372 occurred mainly in autumn (+88 g/kg DM, interaction treatment \times season: $P<0.07$).

373 Pasture ADF, net energy and metabolisable protein concentrations averaged 263 g,
374 0.88 UFL and 95 g PDIE/kg DM, respectively, and were unaffected by treatment.

375 The chemical composition differed between the 7 botanical items considered (Table
376 5). Perennial ryegrass and tall fescue showed similar chemical composition. White
377 clover and red clover showed also similar chemical composition, except for fibre
378 concentrations, greater in red clover than in white clover. On average, legumes were
379 characterised by lower DM and NDF concentrations, and by greater CP and ADL
380 concentrations than grasses. Chicory had a very specific chemical composition, with
381 lower DM and OM concentrations than grasses and legumes, low NDF concentration,
382 close to that of legumes, similar CP concentration to that of grasses, and similar ADF
383 and ADL concentrations as those in red clover.

384

385 *Milk production, milk composition and body weight*

386 Milk production averaged 17.3 kg/day and was greater by 1.1 kg/day in mixed swards
387 than in ryegrass swards ($P<0.01$), and greater by 0.8 kg/day in MSS as opposed to
388 mixed swards ($P<0.05$) (Table 6). On average, milk fat concentration was lower in
389 MSS than in mixed swards (-1.0 g/kg, $P<0.05$), but this mainly occurred in autumn (-
390 2.7 g/kg) and not in spring (+0.5 g/kg) (interaction treatment \times season, $P<0.001$,
391 Table 7). Milk protein concentration was unaffected by treatment. Fat-corrected milk
392 production, milk fat production, milk protein production and milk solids production
393 followed the same trends as milk production, and were greater in mixed swards than
394 in ryegrass swards ($P<0.01$), and greater in MSS than in mixed swards ($P<0.05$)
395 (Table 6). Milk production and milk solids production decreased from spring to
396 autumn due to the advancing lactation stage of the herd (Table 2). The treatment had
397 no effect on body weight (596 kg).

398

399 *Faecal output, digestibility, intake, energy and protein balance, and uremia*

400 Faecal OM output was on average unaffected by treatment (Table 6). In summer,
401 however, faecal output was greater in mixed swards than in ryegrass swards, and
402 greater in MSS than in mixed swards (interaction treatment x season, $P<0.001$, Table
403 7). Pasture OM digestibility averaged 792 g/kg and was greater in MSS than in mixed
404 swards, regardless of season (+10.0 g/kg, $P<0.001$). Pasture DM intake averaged
405 15.6 kg/day and was 1.6 kg greater in MSS than in mixed swards ($P<0.01$, Table 6).
406 This positive effect of MSS compared to mixed swards on pasture DM intake was at
407 its lowest in autumn (+0.7 kg DM/day) and its greatest in summer (+2.3 kg DM/day)
408 (interaction treatment x season: $P<0.05$, Table 7). Pasture OM intake and pasture
409 digestible OM intake followed the same trends as pasture DM intake, with greater
410 values in MSS than in mixed swards, and greater positive effect of MSS in summer
411 than in autumn and spring (Tables 6 and 7). Pasture intake (in DM, OM, or digestible
412 OM) was only greater in mixed swards than in ryegrass swards in summer (no
413 average effect, but interaction treatment x season: $P<0.05$, Table 7). Considering
414 only the 5 rotations with pasture DM intake measurement, milk production, fat-
415 corrected milk production and milk solids production were greater in MSS than in
416 mixed swards ($P<0.05$), and tended to be greater in LTCF than in LTC ($P<0.10$)
417 (Table 6), with no interaction based on the season (Table 7). On average, there were
418 no difference between treatments on UFL and PDIE balances, which averaged 107%
419 and 122% of requirements, respectively. Grazing MSS in summer enabled an
420 increase in energy balance compared to mixed swards, which did not occur in
421 autumn nor in spring (interaction treatment x season, $P<0.001$, Table 7). The
422 treatment had no effect on blood urea nitrogen concentration (238 mg/l).

423

424 *Feeding behaviour*

425 Grazing time averaged 532 min/day and was greater by 57 min/day in MSS than in
426 mixed swards ($P<0.01$, Table 6). This difference tended to be greater in summer as
427 opposed to spring (+74 vs. 41 min/day, respectively; interaction treatment \times season:
428 $P=0.07$). The average duration of a grazing bout tended to be greater in MSS than in
429 mixed swards (+16 min/bout, $P=0.06$), and particularly in summer (interaction
430 treatment \times season: $P<0.05$). No treatment effect was observed on the first grazing
431 bout duration (157 min), nor the number of grazing bouts (5.9 bouts/day), nor pasture
432 intake rate (31.7 g DM/min).

433

434 **Discussion**

435 The aim of this study was to determine how pastures sown with increasing botanical
436 complexity from 1 (grass) to 5 sward species (grass-legume-forb) affect pasture
437 chemical composition and nutritive value, milk production, milk solids production,
438 pasture DM intake and the grazing behaviour of dairy cows in different seasons. This
439 was successfully achieved, as botanical composition clearly differed between
440 treatments, even if clover and fescue proportions were lower, and chicory proportions
441 were greater than expected. Although some significant interactions between season
442 and treatment occurred for pasture botanical and chemical composition, the overall
443 effects of sward type on animal performance did not strongly differ between seasons.
444 This could be related to the weather conditions, globally favorable for pasture growth
445 and quality in both years, including early summer in the ryegrass swards. Finally, pre-
446 grazing pasture mass and pre-grazing pasture allowance were similar between
447 treatments, enabling us to compare sward types under similar grazing conditions and
448 management.

449

450 *Effect of introducing clover in ryegrass swards*

451 Including clovers in perennial ryegrass swards had no effect on pasture nutritive
452 value and pasture DM intake, yet presented a small positive effect on daily milk
453 production (+1.1 kg/day) and milk solids production. These results may be regarded
454 as consistent with the literature given the small difference in clover content between
455 ryegrass and mixed swards observed in our experiment (6 vs. 22%), along with the
456 high quality of the ryegrass pastures.

457 Clovers are recognized as highly digestible and protein-rich forage species (Peyraud,
458 1993; Ribeiro-Filho *et al.*, 2003; INRA, 2007). Similarly, voluntary intake of legumes,
459 and particularly clovers, is known to be 10% to 20% greater than that of grasses
460 (Ribeiro-Filho *et al.*, 2003; INRA, 2007), due to their lower fibre concentration, lower
461 resistance to chewing and faster rate of particle breakdown (Dewhurst *et al.*, 2009).
462 For these reasons, inclusion of white clover in a grass-based diet generally enhances
463 diet quality, daily pasture DM intake and milk production of dairy cows, either at
464 grazing (Harris *et al.*, 1997; Phillips and James, 1998; Ribeiro-Filho *et al.*, 2003) or
465 indoors (Harris *et al.*, 1998). In our study, the increase in milk production of 1.1
466 kg/day in mixed compared to ryegrass swards is within the range of 1-3 kg/day, as
467 reported by Ribeiro-Filho *et al.* (2003), in several short-term experiments at the same
468 daily pasture allowance. The amplitude of the positive effect of clover may depend on
469 the ratio between grass and clover quality, and on the proportion of clover in the
470 swards (Harris *et al.*, 1997; Harris *et al.*, 1998). The greatest milk production
471 response to clover inclusion, almost 3 kg/day, was observed with low grass quality,
472 and clover content of more than 50% (Harris *et al.*, 1997), where clover largely
473 increases diet quality, which is not the case in this experiment.

474 No differences concerning grazing behaviour were found between ryegrass and
475 mixed swards, corroborating previous results of Phillips and James (1998), Ribeiro-
476 Filho *et al.* (2003) and Ribeiro-Filho *et al.* (2012). The latter found that the daily
477 patterns of grazing and ruminating activities were similar between cows grazing on
478 grass-legume swards or pure grass swards, and that the average daily pasture intake
479 rate was only slightly affected by the inclusion of 40% of clover. Including clover in
480 ryegrass swards had no clear effect on milk composition, similarly to the results of
481 Harris *et al.* (1997) and Ribeiro-Filho *et al.* (2003).

482

483 *Effect of multi-species swards compared to single ryegrass/clover mixtures*

484 In our study, MSS were characterised by a high proportion of chicory (30%) that
485 replaced perennial ryegrass, with no changes in the clover proportion compared to
486 the single ryegrass/clover mixture. The high quality of MSS observed must thus be
487 related to the high proportion of chicory and to the specific chemical composition of
488 this species. When analysed separately, chicory was mainly characterised by low DM
489 and NDF concentrations, and high ash concentration, as previously found in many
490 studies (Barry, 1998; Sanderson, 2010; Muir *et al.*, 2014). Due to the high mineral
491 concentration of chicory, as in other forbs, MSS containing chicory may be regarded
492 as an interesting option for enhancing macro- and micro-minerals supply (Barry,
493 1998; Marley *et al.*, 2013). Low NDF concentration, related to high OM digestibility,
494 may also be regarded as a nutritional advantage, leading to high energy
495 concentration and nutritive value of pastures (INRA, 2007; Muir *et al.*, 2014). Low
496 NDF concentration is also cited as one of the main factors explaining high voluntary
497 intake, such as in clovers, through faster ruminal particle breakdown and passage
498 rates (INRA, 2007; Dewhurst *et al.*, 2009). Conversely, the low DM concentration of

499 chicory may be regarded as a potential factor limiting its intake (Tinworth *et al.*, 1999)
500 and thus the intake of MSS containing chicory, given that internal water is known to
501 limit pasture DM intake rate and daily DM intake in dairy cows fed on fresh grass
502 (Cabrera-Estrada *et al.*, 2004). In our experiment, the positive effect of the presence
503 of chicory on pasture DM intake suggests that the negative effect of chicory's low DM
504 concentration is largely compensated by the positive effect of the low NDF
505 concentration or any other component on daily intake. This is in line with several
506 previous studies, where intake and/or milk production have been found to increase
507 when chicory was included in mixed pastures and fed to dairy cows, either at grazing
508 (Chapman *et al.*, 2008; Totty *et al.*, 2013) or indoors (Barry, 1998; Minnee *et al.*,
509 2012). The increase in milk production after inclusion of chicory in the diet generally
510 ranges from 1 to 2 kg/day, but an increase in milk production as high as 6 kg/day has
511 been observed for cows grazing clover-chicory mixtures in summer when compared
512 to low quality grass-based pastures (Chapman *et al.*, 2008).

513 Advantages of MSS on a per cow basis were also clear for milk production and milk
514 solids production, and were due to the cumulative effect of greater pasture quality
515 and greater pasture intake. An additional reason may be that a mixture of several
516 forages has positive associative effects on daily intake (Harris *et al.*, 2004), probably
517 through an increased motivation to eat while no digestive interactions have been
518 detected when mixing several forages (Niderkorn *et al.*, 2014). This may be also
519 related to the high ingestibility of chicory due to its low NDF concentration and rapid
520 ruminal particle breakdown (Niderkorn *et al.*, 2014), as it occurs with legumes
521 compared to grasses. Pasture digestibility was only slightly affected by pasture type,
522 suggesting that the low NDF concentration in chicory is partly compensated by its
523 high lignin concentration and potentially by its lower fibre digestibility.

524 Some studies reported no positive effects of MSS or chicory on daily pasture DM
525 intake or milk production in grazing dairy cows, but these studies were generally
526 carried out at high concentrate supplementation levels, namely 9 kg/day in Soder *et al.*
527 *(2006)* and 6 kg/day in Muir *et al.* *(2014)*. In our study, the lower milk fat
528 concentration found in MSS compared to mixed swards during autumn may be
529 related to the greater milk production and to the highest chicory proportion in the
530 swards, leading to the lowest diet fibre concentration and probably greatest ruminal
531 degradation rate and associated changes in VFA profile.

532 Greater daily pasture DM intake in MSS was reached through more time spent
533 grazing while no change occurred in pasture DM intake rate between MSS and mixed
534 swards. This suggests no fundamental changes in the short-term feeding behaviour
535 of cows when grazing chicory-based pastures, although large structural differences
536 exist between grasses, clovers and chicory. Similarly, no effect has been reported on
537 daily pasture intake rate of grazing dairy cows by including 30 to 40% of chicory in an
538 orchardgrass and white clover pasture (Soder *et al.*, 2007) or in a perennial ryegrass
539 sward (Gregorini *et al.*, 2013).

540

541 *Effect of introducing tall fescue in multi-species swards*

542 In our study, the fact that introducing tall fescue had no overall effect on pasture DM
543 intake nor on milk production may be related to the low tall fescue proportion in
544 swards (10%); as fescue partly replaced perennial ryegrass, while clover and chicory
545 proportions were unaffected. Grazing pure tall fescue generally had no effect, or else
546 decreased milk production in dairy cows by 1 or 2 kg/day when compared to grazing
547 pure perennial ryegrass (Lowe *et al.*, 1999), due to greater fibre concentration and
548 lower digestibility, pasture intake being only slightly affected (INRA, 2007). There is

549 evidence that replacing 10% of DM diet from ryegrass to fescue would only have
550 small effects on cow nutrition, which is in line with the results of Chapman *et al.*
551 (2008) in mixed swards. Greater effects would be expected with older swards, or
552 greater development and proportion of tall fescue in the swards, as tall fescue is well
553 known for its relatively low rate of establishment compared to other grass species.

554

555 **Conclusion**

556 The comparison of perennial ryegrass monoculture, grass-legume mixed swards, and
557 multispecies swards containing grasses, legumes and chicory, only grazed by
558 lactating dairy cows during 2 years, has shown that increasing sward botanical
559 complexity from 1 to 5 species has a positive effect on a per cow performance basis
560 under similar grazing management. All sown species were of good quality, but
561 inclusion of both clovers and of chicory made it possible to enhance sward quality,
562 milk production and milk solids production on a per cow basis. The advantages of
563 multispecies grazing over mixed swards on milk production and milk solids
564 production are due to the cumulative effect of enhanced sward quality and increased
565 pasture DM intake, in all seasons, with relation to the very specific chemical
566 composition of chicory. How increasing sward botanical complexity may increase the
567 grazing system's resilience to climate events such as drought, and may affect
568 pasture utilisation and milk production on a per hectare basis still remains to be
569 investigated.

570

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579

580 **References**

581 Association Française de Normalisation 1997. Aliments des animaux - Dosage de
582 l'azote - Méthode par combustion (DUMAS) - NF V18 - 120 : Dosage des cendres
583 brutes - NF V18 - 101. AFNOR Editions, Saint-Denis La Plaine, France.

584 Aufrère J and Michalet-Doreau B 1988. Comparison of methods for predicting
585 digestibility of feeds. *Animal Feed Science and Technology* 20, 203-218.

586 Barry TN 1998. The feeding value of chicory (*Cichorium intybus*) for ruminant
587 livestock. *Journal of Agricultural Science (Cambridge)* 131, 251-257.

588 Cabrera-Estrada JI, Delagarde R, Faverdin P and Peyraud JL 2004. Dry matter
589 intake and eating rate of grass by dairy cows is restricted by internal, but not external
590 water. *Animal Feed Science and Technology* 114, 59-74.

591 Chapman DF, Tharmaraj J and Nie ZN 2008. Milk-production potential of different
592 sward types in a temperate southern Australian environment. *Grass and Forage
593 Science* 63, 221-233.

594 Deak A, Hall MH and Sanderson MA 2009. Grazing schedule effect on forage
595 production and nutritive value of diverse forage mixtures. *Agronomy Journal* 101,
596 408-414.

597 Delagarde R and Lamberton P 2015. Daily grazing time of dairy cows is recorded
598 accurately using the Lifecorder Plus device. *Applied Animal Behaviour Science* 165,
599 25-32.

600 Dewhurst RJ, Delaby L, Moloney A, Boland T and Lewis E 2009. Nutritive value of
601 forage legumes used for grazing and silage. *Irish Journal of Agricultural and Food*
602 *Research* 48, 167-187.

603 Gregorini P, Minnee EMK, Griffiths W and Lee JM 2013. Dairy cows increase
604 ingestive mastication and reduce ruminative chewing when grazing chicory and
605 plantain. *Journal of Dairy Science* 96, 7798-7805.

606 Harris SL, Clark DA, Auldish MJ, Waugh CD and Laboyrie PG 1997. Optimum white
607 clover content for dairy pastures. *Proceedings of the New Zealand Grassland*
608 *Association* 59, 29-33.

609 Harris SL, Auldish MJ, Clark DA and Jansen EBL 1998. Effect of white clover content
610 in the diet on herbage intake, milk production and milk composition of New Zealand
611 dairy cows housed indoors. *Journal of Dairy Research* 65, 389-400.

612 Høgh-Jensen H, Nielsen B and Milan-Thamsborg S 2006. Productivity and quality,
613 competition and facilitation of chicory in ryegrass/legume-based pastures under
614 various nitrogen supply levels. *European Journal of Agronomy* 24, 247-256.

615 Hoskin S, Stafford K and Barry T 1995. Digestion, rumen fermentation and chewing
616 behavior of red deer fed fresh chicory and perennial ryegrass. *Journal of Agricultural*
617 *Science* 124, 289-295.

618 Huyghe C, De Vlieghe A, van Gils B and Peeters A 2014. Grassland and herbivore
619 production in Europe and effect of common policies. Ed Quae, 287 pp.

620 Institut National de la Recherche Agronomique 2007. Alimentation des bovins, ovins
621 et caprins. Besoins des animaux, valeur des aliments, Tables INRA 2007. Editions
622 QUAE, Versailles, France.

623 Li G and Kemp PD 2005. Forage chicory (*Cichorium intybus L.*): A review of its
624 agronomy and animal production. *Advances in Agronomy* 88, 187-222.

625 Lowe KF, Bowdler TM, Casey ND and Moss RJ 1999. Performance of temperate
626 perennial pastures in the Australian subtropics. 2. Milk production. *Australian Journal*
627 *of Experimental Agriculture* 39, 677-683.

628 Lüscher A, Mueller-Harvey I, Soussana JF, Rees RM and Peyraud JL 2014. Potential
629 of legume-based grassland-livestock systems in Europe: a review. *Grass and Forage*
630 *Science* 69, 206-228.

631 Marley CL, Fychan R, Scott MB, Davies JW and Sanderson R 2013. Yield, nitrogen
632 and mineral content of chicory compared with perennial ryegrass, red clover and
633 white clover over two harvest years. In *The role of grasslands in a green future,*
634 *threats and perspectives in less favoured areas* (eds À Helgadóttir and A Hopkins),
635 pp. 249-251. European Grassland Federation, Akureyri, Iceland.

636 Minnee EMK, Clark CEF; McAllister TB, Hutchinson KJ and Lee JM 2012. Chicory
637 and plantain as feeds for dairy cows in late lactation. In *Proceedings of the 5th*
638 *Australasian Dairy Science Symposium, 13-15 November 2012, Melbourne,*
639 *Australia*, pp. 426-428.

640 Morel I, Schmid E, Soney C, Aragon A and Dufey PA 2014. Influence of ryegrass
641 alone or blended with clover and chicory on feed intake and growth performance of
642 steers. *Grassland Science in Europe* 19, 731-733.

643 Muir SK, Ward GN and Jacobs JL 2014. Milk production and composition of mid-
644 lactation cows consuming perennial ryegrass- and chicory-based diets. *Journal of*
645 *Dairy Science* 97, 1005-1015.

646 Niderkorn V, Martin C and Baumont R 2014. Associative effects between forage
647 species on intake and digestive efficiency in sheep. In *The future of European*
648 *grasslands* (eds A Hopkins, RP Collins, MD Fraser, VR King, DC Lloyd, JM Moorby
649 and PRH Robson), pp.734-736. European Grassland Federation, Aberystwyth, UK.

650 Pérez-Prieto LA and Delagarde R 2013. Meta-analysis of the effect of pasture
651 allowance on pasture intake, milk production, and grazing behaviour of dairy cows
652 grazing temperate grasslands. *Journal of Dairy Science* 96, 6671-6689.

653 Pérez-Ramírez E, Peyraud JL and Delagarde R 2012. N-alkanes vs. ytterbium/faecal
654 index as two methods for estimating herbage intake of dairy cows fed on diets
655 differing in the herbage: maize silage ratio and feeding level. *Animal* 6(2), 232-244.

656 Peyraud JL 1993. Comparaison de la digestion du trèfle blanc et des graminées
657 prairiales chez la vache laitière. *Fourrages* 135, 465-473.

658 Phillips CJC and James NL 1998. The effects of including white clover in perennial
659 ryegrass swards and the height of mixed swards on the milk production, sward
660 selection and ingestive behaviour of dairy cows. *Animal Science* 67, 195-202.

661 Ribeiro-Filho HMN, Delagarde R and Peyraud JL 2003. Inclusion of white clover in
662 strip-grazed perennial ryegrass pastures: herbage intake and milk yield of dairy cows
663 at different ages of pasture regrowth. *Animal Science* 77, 499-510.

664 Ribeiro-Filho HMN, Delagarde R and Peyraud JL 2005. Herbage intake and milk
665 yield of dairy cows grazing perennial ryegrass swards or white clover/perennial
666 ryegrass swards at low- and medium-herbage allowances. *Animal Feed Science and*
667 *Technology* 119, 13-27.

668 Ribeiro-Filho HMN, Peyraud JL and Delagarde R 2012. Foraging behavior and
669 ruminal fermentation of dairy cows grazing ryegrass pasture alone or with white
670 clover. *Pesquisa Agropecuaria Brasileira* 47(3), 458-465.

671 Sanderson MA 2010. Nutritive value and herbage accumulation rates of pastures
672 sown to grass, legume and chicory mixtures. *Agronomy Journal* 102(2), 728-733.

673 Sanderson MA, Labreveux M, Hall MH and Elwinger GF 2003. Forage yield and
674 persistence of chicory and English plantain. *Crop Science* 43, 995-1000.

675 Sanderson MA, Soder KJ, Muller LD, Klement KD, Skinner RH and Goslee SC 2005.
676 Forage mixture productivity and botanical composition in pastures grazed by dairy
677 cattle. *Agronomy Journal* 97, 1465-1471.

678 Sanderson MA, Soder KJ, Brzezinski N, Taube F, Klement K, Muller LD and
679 Wachendorf M 2006. Sward structure of simple and complex mixtures of temperate
680 forages. *Agronomy Journal* 98, 238-244.

681 Siddons RC, Paradine J, Beever DE and Cornell PR 1985. Ytterbium acetate as a
682 particulate-phase digesta-flow marker. *British Journal of Nutrition* 75, 2235-2247.

683 Skinner RH 2008. Yield, root growth, and soil water content in drought-stressed
684 pasture mixtures containing chicory. *Crop Science* 48, 380-388.

685 Soder KJ, Sanderson MA, Stack JL and Muller LD 2006. Intake and performance of
686 lactating cows grazing diverse forage mixtures. *Journal of Dairy Science* 89, 2158-
687 2167.

688 Statistical Analysis System Institute Inc. 1999. User's Guide, Version 8, Cary, NC:
689 SAS Institute Inc., 3884 pp.

690 Talke H and Schubert GE 1965. Enzymatic urea determination in the blood and
691 serum in the Warburg optical test. *Klin Wochenschr* 43, 174-175.

692 Tinworth TRH, Barry TN, Wilson PR 1999. The effect of wilting chicory on its
693 voluntary feed intake and digestion by red deer. *Journal of Agricultural Science* 133,
694 217-221.

695 Totty VK, Greenwood SL, Bryant RH, Edwards GR 2013. Nitrogen partitioning and
696 milk production of dairy cows grazing simple and diverse pastures. *Journal of Dairy*
697 *Science* 96, 141-149.

698 van Soest PJ, Roberson JB and Lewis BA 1991. Carbohydrate methodology,
699 metabolism, and nutritional implications in dairy cattle. *Journal of Dairy Science* 75,
700 2215-2225.

701 **Table 1.** *Sowing rate (kg/ha) of each species in the four types of sward treatments*

Treatment	Number of species	<i>Lolium perenne</i> L. cv. Aberstar	<i>Trifolium repens</i> L. cv. Alice	<i>Trifolium pratense</i> L. cv. Segur	<i>Cichorium intybus</i> L. cv. Puna 2	<i>Festuca arundinacea</i> Schreb. cv. Callina
L ¹	1	35	-	-	-	-
LT	3	24	3	3	-	-
LTC	4	22	3	3	1.5	-
LTCF	5	11	3	3	1.5	11

702 ¹L: *Lolium*; T: *Trifolium*; C: *Cichorium*; F: *Festuca*

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Table 2. *Pre-experimental characteristics of Holstein-Friesian cows grazing multi-species swards during the thirteen experimental rotations*

Reference periods	1 ¹	2	3	4	5	6
Pre-experimental period						
start date	29/08/11	06/02/12	06/04/12	27/08/12	25/02/13	23/04/13
end date	04/09/11	19/02/12	15/04/12	16/09/12	17/03/12	10/05/13
Number of cows	36	28	40	32	36	40
primiparous	12	9	17	16	12	12
multiparous	24	19	23	16	24	28
Lactation stage (DIM)	267	68	142	292	132	185
Milk production (kg/day)	24.6	38.1	26.8	16.0	32.7	26.0
Milk fat concentration (g/kg)	42.2	31.9	37.3	39.9	33.8	37.5
Milk protein concentration (g/kg)	33.8	29.5	29.9	34.6	29.3	29.8
Body weight (kg)	644	631	615	613	646	615
Body condition score (scale 0-5)	2.39	2.31	2.16	2.23	2.02	1.86
Experimental rotations	1-2	3	4 ² -7	8-9	10	11-13

710 ¹ Reference periods considered for determination of pre-experimental dairy cow
711 characteristics.

712 ² The grazing rotation 4 was interrupted from 24/04/12 to 03/05/12 due to heavy rain that did
713 not allow grazing in any of the paddocks.

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719 **Figure 1.** *Monthly mean temperature and cumulated rainfall of the experimental*
720 *years (year 1, September 2011 – August 2012 and year 2, September 2012 – August*
721 *2013) and of the last 30-year average*

722 *See specific file*

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725 **Table 3.** Mean pasture botanical composition (12 rotations) and pasture
 726 characteristics (13 rotations) of multi-species swards rotationally grazed by dairy
 727 cows (2 years)

	Sward treatments ¹				SD _t ²	Contrasts ³		
	L	LT	LTC	LTCF		T	M	F
Botanical composition of pasture offered (proportion of DM)								
<i>Lolium perenne</i> L.	0.70	0.59	0.44	0.34				
<i>Trifolium repens</i> L.	0.06	0.13	0.10	0.10				
<i>Trifolium pratense</i> L.	0	0.09	0.10	0.07				
<i>Cichorium intybus</i> L.	0	0	0.29	0.30				
<i>Festuca arundinacea</i> Schreb.	0	0	0	0.10				
Unsovn species	0.18	0.13	0.05	0.04				
Senescent material	0.09	0.08	0.06	0.06				
Pasture mass (kg DM/ha, > 4 cm)	2218	2436	2388	2354	971.1	0.259	0.700	0.853
Pre-grazing sward height (cm)	12.6	13.7	15.8	15.2	4.23	0.204	0.013	0.509
Sward bulk density (kg DM/ha/cm, > 4 cm)	267	270	219	229	37.5	0.646	0.001	0.195
Pasture allowance (kg DM/day, > 4 cm)	18.7	19.1	19.3	19.4	2.13	0.384	0.418	0.881
Post-grazing sward height (cm)	4.8	5.0	4.7	4.7	0.78	0.125	0.030	0.798
Chemical composition and nutritive value of pasture offered (> 4 cm)								
DM (g/kg)	185	173	138	141	20.7	0.004	0.001	0.395
OM (g/kg DM)	897	895	866	869	9.4	0.230	0.001	0.228
CP (g/kg DM)	187	190	189	200	23.3	0.504	0.247	0.019
NDF (g/kg DM)	535	530	469	484	29.6	0.563	0.001	0.081
ADF (g/kg DM)	257	263	263	268	15.7	0.161	0.523	0.244
ADL (g/kg DM)	32	38	51	49	9.1	0.012	0.001	0.435
PCd (g/kg DM)	752	734	776	758	24.9	0.159	0.005	0.168
UFL (/kg DM)	0.88	0.86	0.88	0.86	0.024	0.135	0.580	0.153
PDIE (g/kg DM)	96	94	95	94	2.7	0.168	0.696	0.337

728 DM = Dry Matter; OM = Organic Matter; CP = Crude Protein; NDF = Neutral Detergent Fibre;

729 ADF = Acid Detergent Fibre; ADL = Acid Detergent Lignin; PCd = Pepsin-cellulase

730 Digestibility; UFL = Unité Fourragère Lait (Feed unit for milk production; 1UFL = 7.115 MJ

731 NE); PDIE = Protein truly digestible in the intestine, with energy-limiting microbial synthesis in
 732 the rumen.

733 ¹ See Table 1.

734 ² Standard deviation of the model for the effect of the treatment.

735 ³ Orthogonal contrasts: T (L vs. LT), M (LT vs. LTC/LTCF) and F (LTC vs. LTCF).

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737**Table 4.** Seasonal pasture botanical composition (12 rotations) and pasture characteristics (13 rotations) of multi-species swards rotationally grazed by dairy cows (2 years)

Grazing season	Autumn				Spring				Summer				p-value		
	L	LT	LTC	LTCF	L	LT	LTC	LTCF	L	LT	LTC	LTCF	SD _s ²	s	t × s
Sward treatments ¹															
Botanical composition of pasture offered (proportion of DM)															
<i>Lolium perenne</i> L.	0.62	0.49	0.24	0.18	0.77	0.71	0.59	0.47	0.65	0.50	0.38	0.33			
<i>Trifolium repens</i> L.	0	0.19	0.10	0.09	0.05	0.09	0.08	0.07	0.13	0.17	0.12	0.15			
<i>Trifolium pratense</i> L.	0	0.14	0.12	0.10	0	0.06	0.09	0.06	0	0.10	0.09	0.06			
<i>Cichorium intybus</i> L.	0	0	0.46	0.46	0	0	0.19	0.22	0	0	0.29	0.26			
<i>Festuca arundinacea</i> Schreb.	0	0	0	0.08	0	0	0	0.07	0	0	0	0.11			
Unsovn species	0.27	0.14	0.06	0.03	0.15	0.11	0.04	0.04	0.16	0.17	0.07	0.04			
Senescent material	0.16	0.14	0.11	0.09	0.06	0.05	0.03	0.05	0.06	0.06	0.05	0.05			
Pasture mass (kg DM/ha) ³	1138	1357	1581	1474	3452	3722	3482	3549	2064	2228	2101	2038	4791.7	0.001	0.946
Pre-grazing sward height (cm)	8.3	8.9	11.3	10.7	17.3	18.8	20.2	20.0	12.3	13.3	15.8	14.9	82.22	0.001	0.998
Sward bulk density (kg DM/ha/cm) ³	268	296	240	253	275	268	234	243	257	246	184	191	220.9	0.001	0.054
Pasture allowance (kg DM/day) ³	16.5	17.6	18.2	17.8	20.7	20.9	20.3	21.1	18.9	18.8	19.5	19.3	6.88	0.001	0.539
Post-grazing sward height (cm)	3.9	3.9	3.7	3.6	5.4	5.8	5.2	5.3	4.9	5.3	5.1	5.3	8.93	0.001	0.464
Chemical composition and nutritive value of pasture offered ³															
DM (g/kg)	182	176	131	133	176	167	140	139	197	175	142	152	133.9	0.003	0.206
OM (g/kg DM)	889	888	849	850	905	902	879	882	897	895	871	874	54.1	0.001	0.001
CP (g/kg DM)	210	206	211	230	164	169	178	183	185	194	178	187	84.6	0.001	0.077
NDF (g/kg DM)	539	527	439	445	525	528	472	498	541	536	497	508	72.5	0.001	0.011
ADF (g/kg DM)	249	256	259	259	255	262	252	263	267	272	278	282	53.8	0.001	0.695
ADL (g/kg DM)	31	38	55	54	32	37	47	44	32	40	50	48	28.9	0.111	0.611
PCd (g/kg DM)	788	763	861	840	756	748	771	741	711	690	697	694	57.1	0.001	0.066
UFL (/kg DM)	0.92	0.89	0.95	0.93	0.88	0.87	0.87	0.84	0.84	0.82	0.80	0.80	0.165	0.001	0.213
PDIE (g/kg DM)	101	98	104	103	95	94	94	90	92	91	88	89	17.4	0.001	0.075

738 ¹ See Tables 1 and 3.739 ² Standard deviation of the model for the effect of the season.740 ³ Above 4 cm

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744 **Table 5.** Chemical composition (g/kg DM: 12 rotations) of the botanical items in multi-species swards rotationally grazed by dairy cows

	<i>Lolium</i>	<i>Trifolium</i>	<i>Trifolium</i>	<i>Cichorium</i>	<i>Festuca</i>	Unso	Senescent	SD ¹	p-value		Contrasts ²		
	<i>perenne</i>	<i>repens</i>	<i>pratense</i>	<i>intybus</i>	<i>arundinacea</i>	n	material		sp	sp x s	C vs. L	C vs. G	G vs. L
	L.	L.	L.	L.	Schreb.								
DM (g/kg)	196	156	177	102	202	148	271	26.6	0.001	0.820	0.001	0.001	0.002
OM	892	891	896	833	889	875	865	11.5	0.001	0.021	0.001	0.001	0.428
CP	196	253	239	193	204	210	114	18.5	0.001	0.004	0.001	0.386	0.001
NDF	520	345	389	350	559	375	619	30.8	0.001	0.501	0.190	0.001	0.001
ADF	229	208	244	244	249	232	342	22.9	0.001	0.451	0.064	0.644	0.089
ADL	26	56	91	94	21	77	58	10.1	0.001	0.187	0.001	0.001	0.001

745 ¹ Standard deviation of the model for the effect of the year and the season.746 ² Orthogonal contrasts: chicory vs. legumes (C vs. L), chicory vs. grasses (C vs. G) and grasses vs. legumes (G vs. L).

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750**Table 6.** Milk production, milk composition and body weight (13 rotations), pasture intake, nutrient balance and uremia (5 rotations), and grazing behaviour (2 rotations) of dairy cows rotationally grazing on multi-species swards

	Sward treatments ¹				RSD _t ²	Contrasts		
	L	LT	LTC	LTCF		T	M	F
Milk production (kg/day)	16.1	17.2	17.8	18.2	1.94	0.005	0.022	0.357
4% FCM production (kg/day)	15.9	17.0	17.5	17.9	1.81	0.003	0.026	0.377
Milk fat concentration (g/kg)	41.0	41.0	40.3	39.8	2.75	0.989	0.049	0.430
Milk protein concentration (g/kg)	32.8	32.6	32.4	32.0	1.57	0.481	0.152	0.237
Milk solids production (kg/cow)	1.14	1.22	1.25	1.28	0.129	0.003	0.039	0.439
Milk fat production (g/day)	633	673	696	708	73.6	0.006	0.034	0.474
Milk protein production (g/day)	511	542	559	568	59.8	0.006	0.049	0.474
Body weight (kg)	594	599	593	598	14.5	0.083	0.210	0.221
Faecal output OM (kg/day)	2.73	2.84	2.92	2.94	0.308	0.298	0.370	0.854
Pasture OM digestibility (g/kg)	788	786	798	794	0.3	0.518	0.001	0.123
Pasture OM intake (kg/day)	13.0	13.4	14.5	14.3	1.65	0.424	0.054	0.774
Pasture DM intake (kg/day)	14.4	15.0	16.6	16.5	1.95	0.388	0.008	0.835
Pasture digestible OM intake (kg)	10.2	10.6	11.6	11.4	1.37	0.463	0.034	0.695
Milk production (kg/day)	15.3	15.8	17.2	18.5	1.95	0.481	0.012	0.064
4% FCM production (kg/day)	15.4	16.0	17.0	18.1	1.76	0.384	0.007	0.080
Milk solids production (kg/cow)	1.12	1.15	1.21	1.29	0.128	0.445	0.012	0.084
UFL balance (% of requirements)	1.05	1.06	1.09	1.06	0.104	0.720	0.580	0.352
PDIE balance (% of requirements)	1.23	1.19	1.25	1.20	0.117	0.295	0.322	0.138
Blood urea nitrogen concentration (mg/l)	245	224	233	251	20.3	0.198	0.209	0.298
Grazing time (min/day)	503	503	570	550	30.7	0.978	0.002	0.306
First grazing bout duration (min)	146	161	164	155	17.5	0.271	0.931	0.508
Number of grazing bouts (/day)	5.7	6.1	5.5	6.2	0.80	0.436	0.595	0.124
Mean grazing bout duration (min)	97	90	114	98	16.3	0.432	0.056	0.113
Pasture intake rate (g DM/min)	31.9	32.1	31.1	31.5	2.38	0.903	0.619	0.856

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¹ See Tables 1 and 3. ² Residual standard deviation of the model for the effect of the treatment.

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753**Table 7.** Seasonal variation of milk production, milk composition and body weight (13 rotations), pasture intake, nutrient balance and uremia (5 rotations), and grazing behaviour (2 rotations) of cows rotationally grazing on multi-species swards

Grazing season	Autumn				Spring				Summer				p-value		
	L	LT	LTC	LTCF	L	LT	LTC	LTCF	L	LT	LTC	LTCF	RSD _s ²	s	t × s
Sward treatments ¹															
Milk production (kg/day)	10.5	11.9	12.0	13.2	22.5	23.0	24.0	23.7	15.3	16.5	17.3	17.6	0.27	0.001	0.405
4% FCM production (kg/day)	11.8	13.3	13.1	13.9	21.3	21.8	22.9	22.7	14.7	15.9	16.5	17.1	0.25	0.001	0.431
Milk fat concentration (g/kg)	49.1	48.1	46.9	43.9	36.6	36.5	36.7	37.3	37.4	38.5	37.2	38.1	0.41	0.001	0.001
Milk protein concentration (g/kg)	38.0	37.0	37.3	35.9	29.4	29.7	29.7	29.6	31.0	31.0	30.1	30.4	0.21	0.001	0.010
Milk solids production (kg/cow)	0.90	1.00	1.00	1.04	1.48	1.52	1.61	1.58	1.04	1.13	1.16	1.20	0.018	0.001	0.464
Milk fat production (g/day)	506	564	557	574	821	835	889	881	572	621	642	667	10.5	0.001	0.335
Milk protein production (g/day)	396	438	439	470	663	684	717	699	473	505	520	534	8.1	0.001	0.492
Body weight (kg)	598	607	599	603	589	599	593	599	594	592	588	591	2.5	0.001	0.116
Faecal output OM (kg/day)	2.71	2.77	2.50	2.70	2.80	2.71	2.90	2.80	2.69	3.05	3.35	3.31	0.064	0.001	0.001
Pasture OM digestibility (g/kg)	779	773	790	788	797	800	809	805	789	786	796	789	2.0	0.001	0.307
Pasture OM intake (kg/day)	12.2	12.3	11.9	12.8	13.9	13.7	15.1	14.4	12.8	14.3	16.4	15.7	0.34	0.001	0.010
Pasture DM intake (kg/day)	13.4	13.6	13.7	14.9	15.5	15.2	17.4	16.5	14.4	16.1	18.8	18.0	0.39	0.001	0.019
Pasture digestible OM intake (kg)	9.5	9.5	9.4	10.1	11.1	10.9	12.2	11.6	10.2	11.3	13.1	12.4	0.28	0.001	0.028
Milk production (kg/day)	10.1	9.7	11.3	14.4	19.8	20.5	21.9	22.2	16.0	17.2	18.4	18.8	0.41	0.001	0.578
4% FCM production (kg/day)	12.1	12.3	12.7	12.5	19.0	19.3	20.8	20.9	15.3	16.3	17.5	17.9	0.38	0.001	0.659
Milk solids production (kg/cow)	0.94	0.96	0.97	1.18	1.33	1.34	1.45	1.45	1.08	1.14	1.21	1.24	0.027	0.001	0.603
UFL balance (% requirements)	1.10	1.06	1.02	1.00	1.01	0.98	1.04	0.98	1.03	1.13	1.21	1.19	0.018	0.001	0.001
PDIE balance (% requirements)	1.32	1.20	1.21	1.40	1.17	1.09	1.16	1.09	1.20	1.29	1.37	1.36	0.064	0.001	0.001
Blood urea nitrogen (mg/l)	-	-	-	-	225	203	206	236	266	244	261	265	9.2	0.005	0.882
Grazing time (min/day)	-	-	-	-	513	512	557	548	492	494	583	552	8.3	0.738	0.064
First grazing bout duration (min)	-	-	-	-	142	154	148	143	150	167	180	167	5.5	0.001	0.149
Number of grazing bouts (/day)	-	-	-	-	5.8	6.3	6.0	6.8	5.7	5.9	5.1	5.6	0.19	0.001	0.212
Mean grazing bout duration (min)	-	-	-	-	102	89	104	90	93	91	124	107	4.1	0.041	0.023
Pasture intake rate (g DM/min)	-	-	-	-	30.4	30.1	29.9	30.3	33.4	34.1	32.3	32.6	0.81	0.001	0.681

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¹ See Tables 1, 3 and 6. ² Residual standard deviation of the model for the effect of the season.

Figure 1 Roca-Fernandez et al, Animal, 2015

