

Performance, milk fatty acid composition and behaviour of high-yielding Holstein dairy cows given a limited grazing period

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1 **Performance, milk fatty acid composition and behaviour of high-yielding**
2 **Holstein dairy cows given a limited grazing period**

3

4 **Running title: Grazing high-yielding cows**

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20

21 **CONFLICT OF INTEREST STATEMENT**

22 The authors declare that there are no conflicts of interest regarding the publication of this article.

23

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27 **Abstract**

28 The effects of a limited grazing period on the performance, behaviour and milk composition of high-
29 yielding dairy cows was examined. A total of 56 Holstein cows yielding 44.7 ± 0.42 kg/d were
30 allocated to one of four treatments in one of two, 4-week periods. Treatments were: Control (C) -
31 cows housed and offered TMR *ad libitum*; Early Grazing (EG) - cows grazed for 6 hr after morning
32 milking then housed; Delayed Grazing (DG) - cows returned to housing for 1 hr after morning milking
33 followed by grazing for 6 hr, then housed; Restricted TMR (RT) - cows grazed for 6 hr after morning
34 milking then housed and fed TMR at 75% of *ad libitum*. Intake of TMR was highest in cows receiving
35 C, intermediate in EG and DG, and lowest in RT at 26.9, 23.6, 24.7 and 20.3 kg DM/d respectively.
36 Pasture intake was similar in cows receiving EG or DG, but was higher in RT at 2.4, 2.0 and 3.5 kg
37 DM/d respectively. Milk yield was similar between cows receiving C, EG or DG, but lowest in RT at
38 45.7, 44.2, 44.9 and 41.7 kg/cow respectively, whilst milk fat content of C18:3 n-3 was increased by
39 grazing. Cows in C spent more than 55 min/d longer lying and had three additional lying bouts/d,
40 whilst lying bouts were shorter than for cows receiving EG, RT or DG. It is concluded that high-
41 yielding cows can be grazed for 6 hr/d with little impact on performance, provided TMR is available
42 *ad-libitum* when housed.

43

44 **Keywords:** behaviour, fatty acid, grazing, intake, milk production

45

46

47 **1 | INTRODUCTION**

48 Grazing lactating dairy cows in many Western countries is decreasing as milk production per cow
49 increases, resulting in the greater use of summer housing and total mixed ration (TMR) feeding
50 (March et al., 2014; Wolf et al., 2015). Increasing the proportion of grazed pasture in the diet of dairy
51 cattle can however, offer economic, environmental, milk quality and animal welfare benefits
52 (Hennessy et al., 2015). For example, grazing dairy cows for part of the day can lower farm expenses
53 and increase profit in scenarios with high feed costs and low milk prices (Tozer et al., 2003), and
54 reduce the methane output of animals (Dall-Orsoletta et al., 2016; Mufungwe et al., 2014). Grazing
55 may also increase the concentration of fatty acids (FA) in milk that are beneficial to human health
56 (Barca et al., 2018; Mufungwe et al., 2014) and, because cows can exhibit motivation to be outside
57 at pasture (Motupalli et al., 2014), pasture access allows natural behaviour to be expressed which may
58 potentially improve animal welfare. Some farmers in European countries such as the United Kingdom
59 (UK), the Netherlands and Germany are currently incentivised by milk companies to graze milking
60 cows for a minimum of 6 hr per day, while in Scandinavia, legislation requires cows to have outdoor
61 access for at least 6 hr per day during the summer months. Providing pasture on its own however, is
62 insufficient to meet the nutrient requirements of high yielding dairy cows, limiting their daily milk
63 production to below 30 kg/cow (Kolver & Muller, 1998). Alternating grazing with TMR feeding
64 between the am and pm milking intervals has also been reported to lower milk yield compared with
65 housed and TMR fed cows (Bargo et al., 2002; Soriano et al., 2001), unless TMR was provided in the
66 field (Mufungwe et al., 2014). Recently it has been reported that limiting the time cows have at pasture
67 to 6 hr per day allows cows in mid to late lactation to graze pasture and consume sufficient TMR to
68 maintain milk yield in comparison with housed and TMR fed cows (Dall-Orsoletta et al., 2016).

69 Despite the potential benefits from grazing, little is known about the effects of the length or
70 timing of a grazing period on the performance and behaviour of high yielding cows. Behavioural
71 studies have revealed that cows with free access to pasture and housing, rapidly consume a meal of
72 TMR following milking before going out to pasture (Charlton et al., 2011; Motupalli et al., 2014), a

73 strategy that was able to maintain milk yield but limited pasture intake to less than 2 kg DM/cow per
74 d. The diurnal response of cows to consume feed around dawn/morning milking (Gregorini, 2011)
75 may also be used to increase the pasture intake of high yielding cows receiving limited access to
76 pasture and fed a TMR when housed. Additionally, restricting the access of cows to TMR prior to
77 grazing, or restricting the allowance of TMR when housed may promote grazing and pasture intake,
78 although few studies have been conducted in this area. The primary objective of this study was to
79 determine the effects of giving high yielding cows a 6 hr grazing period compared with continuously
80 housed cows on feed intake, milk yield, milk composition, and behaviour, and to determine the effects
81 of delaying the 6 hr grazing period or restricting TMR allowance with a 6 hr grazing period. It was
82 hypothesised that total feed intake and milk yield would be unaffected by a 6 hr grazing period, but
83 grazing behaviour, grass intake and milk composition would alter.

84

85 **2 | MATERIALS AND METHODS**

86 The study was conducted at Harper Adams University, Newport, Shropshire, UK (52°780'N,
87 2°434'W). Experimental work took place from the 12th May to 14th July 2015, with all procedures
88 involving animals, conducted in accordance with the UK Animals (Scientific Procedures) Act 1986
89 (amended 2013).

90

91 **2.1 | Experimental design and routine**

92 **2.1.1 | Animals and treatments**

93 Fifty-six dairy cows with previous experience of grazing that were (mean \pm SE) 89 \pm 5.3 days post-
94 partum, yielding 44.7 \pm 0.42 kg milk/d, with a live weight (LW) of 644 \pm 7.7 kg and a body condition
95 score (BCS scale 1-5; Ferguson et al., 1994) of 2.78 \pm 0.029 were used. Twenty-eight cows were
96 allocated to one of four groups of seven cows for a period of 28 d duration. Another 28 cows were
97 allocated in the same way, in a consecutive period of 28 d. Period 1 occurred during May and June,
98 and period 2 during June and July 2015. At the start of each period, the cows were stratified according

99 to their milk yield, LW, feed intake and milk fat content measured in the week prior to allocation, and
100 randomly allocated to one of four treatments. The treatments were; Control (C) - cows were
101 continuously housed and offered a TMR *ad libitum*; Early grazing (EG) - cows grazed for 6 hr directly
102 post am milking and then housed and offered TMR *ad libitum*; Delayed Grazing (DG) - cows were
103 returned to housing for 1 hr post am milking before being grazed for 6 hr and then housed and offered
104 TMR *ad libitum*; Restricted TMR (RT) - cows were grazed for 6 hr directly post morning milking
105 then housed and offered TMR that was restricted to 75% of *ad libitum* intake. Cows remained on
106 treatment for four weeks with measurements undertaken during the final week.

107 Cows were milked twice daily at approximately 06:30 and 16:30 h. Immediately following
108 morning milking, treatments C and DG were allowed to return to the free-stall housing and feeding
109 area, while treatments EG and RT were separated as they left the milking parlour and moved to the
110 pasture. After 1 hr, cows receiving treatment DG were separated and moved to the pasture. After 6 hr
111 at pasture, cows were gathered and returned inside (approximately 12:30 and 13:30 h for treatments
112 EG/RT, and DG respectively). All cows had continuous access to water when indoors and at pasture.
113

114 **2.2 | Grazing and pasture allocation**

115 The grazing area consisted of a 3 ha paddock composed predominately of perennial ryegrass (*Lolium*
116 *perenne*), sown in spring 2011. The paddock had received 50 kg/ha N and 20 kg/ha S fertiliser, and
117 was grazed once that grazing season with dairy cows before the beginning of the study. The area was
118 subdivided with temporary electric fences to allow flexible rotational grazing. Cows were given a
119 fresh strip of grass each day in blocks of three days, and then that block was back fenced and allowed
120 to regrow. Each daily grass strip was further split into three parts of equal area and randomly allocated
121 to the treatment groups who grazed independently. Grazing was counter balanced so that no group
122 grazed the same area twice over a three-day block. Allocation of the grazing area was determined
123 from herbage mass (HM), estimated daily prior to grazing by walking the paddock in a 'W' pattern
124 and taking 30 random measurements using a rising plate meter (Jenquip, Fielding, New Zealand).

125 Daily fresh pasture allowance was initially set at 12 kg DM/d above a 4 cm base, but during the first
126 10 d of the study it became apparent that cows were consuming considerably less, and the daily
127 amount was reduced to 6 kg DM/cow/d above a 4 cm base (8 kg DM on day one and 5 kg DM in
128 each subsequent day of a 3 d block), plus the residual herbage from the previous days grazing during
129 days two and three of each block. Post-grazing herbage mass was also recorded daily for each group
130 using a rising plate meter. Target pre-grazing herbage mass was 2700-3000 kg DM/ha above ground
131 level, with a mean of 2842 ± 89.2 kg DM/ha during the collection period. A group of non-lactating
132 cows were used to graze residual herbage to 1500-1600 kg DM/ha above ground level, and
133 mechanical topping was used to maintain pasture quality when necessary.

134

135 **2.3 | Housing and TMR feeding**

136 When housed, the cows were located together in the same portion of a free-stall building containing
137 Super Comfort cubicles fitted with foam mattresses (IAE, Stoke-on-Trent, United Kingdom). Fresh
138 TMR was delivered at approximately 08:00 h daily using a forage mixer wagon (Hi-spec Engineering
139 Ltd, Bagenalstown, Ireland) calibrated to ± 0.1 kg, and was composed of maize silage, lucerne silage
140 and straight feeds, formulated according to Thomas (2004; Table 1). The TMR was accessed via 30
141 electronic roughage intake control (RIC) bins (Insentec, Marknesse, The Netherlands). Cows were
142 trained to use the bins at least one week prior to each study period. Cows receiving treatments C, EG
143 or DG received *ad-libitum* access to the TMR, with those receiving RT were restricted to 75% of the
144 DM intake of their corresponding pair in treatment C.

145

146 **2.4 | Experimental measurements**

147 **2.4.1 | Feed intake milk yield, composition and live weight**

148 Grass and TMR samples were collected during the final five days of each period. Samples of the
149 TMR were collected within 10 min of feeding, and grass 'pluck' samples were taken at approximately
150 07:30 and 11:30 h as described by Smit et al. (2005) to represent the herbage in the grazed horizon,

151 and stored at -20°C prior to subsequent analysis. Individual grass intake was estimated using the n-
152 alkane method as described by Mayes et al. (1986). For the final 12 d of each period, a daily dose of
153 C32 alkane (dotriacontane) was thoroughly incorporated into the TMR at 2.0 g/cow/d. Briefly; alkane
154 powder (56 g) was mixed with 1 kg of the straight feeds mix, and the straight feeds plus alkane mix
155 was then scattered across the TMR and mixed for 10 minutes. The daily TMR intake was then used
156 to calculate the quantity of n-alkane consumed by each cow. Faecal samples for each cow were
157 collected during the final 5 d of each period between 04:00 – 06:00 and 16:00 – 18:00 h from naturally
158 voided faecal deposits of certain origin at the time of deposition, and frozen at -20°C. Milk yield was
159 recorded at each milking for all cows during the collection period, with sub-samples collected on four
160 separate occasions (2 x am and 2 x pm) during week 4 of each period for subsequent analysis. Cow
161 LW was measured (Trutest, Auckland, New Zealand) and BCS (Ferguson et al., 1994) recorded at the
162 start and end of each four-week treatment period at approximately 16:30 h.

163

164 **2.4.2 | Visual and automatic behaviour recording**

165 On days 4 and 7 of week 3 of each experimental period, visually observed behaviour was recorded
166 for cows receiving treatments EG, DG and RT for the 6 hr whilst they were at pasture. Every 5 min
167 during each observational period the posture (lying, standing, walking) and jaw activity (grazing,
168 ruminating, drinking, idling) of each cow was recorded. Binoculars were used when necessary for
169 accurate identification of each cow and to maintain a distance from the cows to prevent disturbance.
170 Prior to the study each cow had an accelerometer (IceQube, IceRobotics Ltd, Edinburgh, UK)
171 attached to their hind left leg. The sensors recorded lying time (LT min/d), frequency of lying bouts
172 (LB/d), average lying bout duration (LBD; min/bout) and step count (steps/d). All data were stored
173 within the accelerometer device and subsequently uploaded each time the cows walked past a reader
174 (CowAlert system, IceRobotics Ltd, Edinburgh, UK), at the entrance to the milking parlour. Time
175 spent eating TMR was determined using the data recorded by the RIC feeders for each cow during
176 the final week of each period.

178 **2.5 | Chemical analysis**

179 Grass and TMR samples were bulked by period, and a sub-sample of grass was freeze dried (Edwards
180 Modulyo, Bolton, UK) and TMR oven dried to constant weight and analysed (AOAC, 2012) for ash
181 (942.05) and crude protein (CP; 990.03) content. Faecal samples were bulked within cow and a sub-
182 sample freeze dried for subsequent analysis. Fibre content of the TMR and grass was assessed
183 according to the method of Van Soest et al. (1991), and water soluble carbohydrate (WSC) according
184 to Thomas (1977). Grass metabolisable energy content (ME; MJ/kg DM) was predicted from the
185 concentration of modified acid detergent fibre (MADF; Givens et al., 1990). Milk samples were
186 analysed for fat, protein and lactose content using a Milkoscan Minor (FOSS, Warrington, UK)
187 calibrated according to AOAC (2012), and energy corrected milk yield (ECM) calculated using milk
188 yield, milk fat, protein and lactose content (Sjaunja et al., 1990). Fatty acid methyl esters (FAME) in
189 hexane were prepared from milk fat by the method of Feng et al., (2010) and from feeds by the method
190 of Jenkins (2010). Individual FAME were determined by GLC (Hewlett Packard 6890, Wokingham,
191 UK) fitted with a CP-Sil 88 column (100 m x 0.25 mm i.d. x 0.2 µm film). Hydrogen was used as the
192 carrier gas and a programmed temperature sequence was used; further details and conditions have
193 been described previously (Lock et al., 2006). Grass, TMR and faecal samples were analysed for n-
194 alkanes and grass intake for each cow calculated from the concentrations of the naturally occurring
195 odd-chain (C33) and the dosed even-chain (C32) n-alkane using the method described by Mayes et
196 al. (1986). Alkane analysis was conducted on a GLC (Phillips PU 4500; Phillips, Surrey, UK), fitted
197 with a 30 m x 0.32 mm 0.25µm fused silica capillary column (Restec Corporation, Bellefonte, USA)
198 using helium as the carrier gas. Oven temperature was programmed at 190°C for 3 min and then
199 increased by 6°C per min until 316°C.

200

201 **2.6 | Statistical analysis**

202 Data were evaluated by ANOVA in Genstat v.17 (VSN International 2015). Feed intake, milk

203 production, fatty acid, LW and BCS variables were tested for normality and fitted to the model:

$$204 \quad y_{ijk} = \mu + P_i + B_j + T_k + \epsilon_{ijk}$$

205 where y_{ijk} = dependent variable; μ = overall mean; P_i = random effect of period; B_j = random effect of
206 block; T_k = effect of treatment; ϵ_{ijk} = residual error. Measurements of feed intake, milk production,
207 LW and BCS taken prior to allocation were used as covariates in the model if appropriate. Behaviour
208 data were evaluated using the same model for the individual animals. Differences were considered
209 significant at $p < 0.05$ and a least significant difference test was conducted post hoc.

210

211 **3 | RESULTS**

212 **3.1 | Chemical analysis of the diets**

213 The DM content of the TMR was more than double that of the grazed grass (Table 1), but the ME and
214 CP content was similar in both feeds, with a mean value of 12.2 MJ/kg DM and 176 g/kg DM
215 respectively. The WSC and NDF concentration was 121 and 61 g/kg DM higher in the grazed grass
216 than the TMR respectively. Total FA content was 8 g/kg DM higher in the TMR than the grass. The
217 major FA in grass was C18:3 n-3, which contributed 41g/100g FA, whereas in the TMR C16:0,
218 C18:1n-9 and C18:2n-6 were the major FA, each contributing approximately 28 g/100g FA.

219

220 **3.2. | Intake and performance**

221 Intake of TMR was highest ($p < 0.05$) in cows offered C, and was approximately 3 kg DM/d higher
222 than in cows offered EG or DG, which did not differ ($p > 0.05$; Table 2). The lowest intake of TMR
223 was in cows receiving RT, which was 75% of the value of cows receiving the Control. In contrast,
224 grass intake was highest ($p < 0.05$) in cows receiving RT at 3.5 kg DM/d compared to the mean value
225 of 2.2 kg DM in those receiving EG or DG. In the 6 hr prior to grazing, cows receiving DG had the
226 highest ($p < 0.05$) TMR intake, at 7.6 kg DM, whilst those in C, EG and RT were similar at 4.5 kg
227 DM (Figure 1). In the 6 hr following grazing, cows receiving EG, DG and RT had a similar ($p > 0.05$)
228 intake, with a mean of 10.6 kg DM, whilst the TMR intake of those receiving C was the lowest ($p <$

229 0.05) at 7.4 kg DM. Total DM intake was similar in cows receiving C, EG or DG (mean value of 26.5
230 kg/d), and was approximately 2.7 kg DM/d higher than cows receiving RT ($p < 0.05$; Table 2). Milk
231 yield was similar in cows receiving C, EG or DG, with a mean value of 44.9 kg/d, but was lower (p
232 < 0.05) in cows fed RT compared to C or DG. However, ECM was similar ($p > 0.05$) between
233 treatments, with a mean value of 37.6 kg/d. There was no effect ($p > 0.05$) of treatment on milk fat or
234 protein content, whereas milk protein and lactose yield were higher ($p < 0.05$) in cows receiving C
235 compared to RT. Cows receiving C also had a higher LW and greater LW gain than those receiving
236 RT ($p < 0.05$), with EG and DG being intermediate. There was no effect ($p > 0.05$) of treatment on
237 BCS or BCS change.

238

239 **3.3 | Milk fatty acid profile**

240 Milk fat content of C10:0 and C14:0 were lowest ($p < 0.05$) in cows receiving RT and highest in EG
241 (Table 3). The odd-chain FA C15:0 was lowest in milk from cows fed RT and highest in C ($p < 0.05$),
242 whereas C17:0 was lowest in cows receiving C and highest in those offered DG ($p < 0.05$). There was
243 no effect ($p > 0.05$) of treatment on the trans FA content of milk, except C18:1 t12, which was lower
244 in C than any of the treatments that received access to pasture. Milk content of C18:1 c9 was highest
245 ($p < 0.05$) in cows offered RT and lowest in cows offered EG. There was an effect of the inclusion of
246 access to pasture on the milk fat content of C18:3 n-3, which was approximately one-third higher in
247 cows receiving any of the grazing treatments (EG, DG or RT) compared to those receiving C ($p <$
248 0.001). Cows receiving RT had a lower content of saturated and a higher content of MUFA compared
249 to those offered EG ($p < 0.05$). Similarly, milk FA of $<C16$ was lowest ($p < 0.05$) in cows receiving
250 RT compared to EG, whereas milk FA $>C16$ was highest ($p < 0.05$) in cows receiving RT compared
251 to C.

252

253 **3.4 | Behavioural measurements**

254 When visually observed, cows that had access to pasture spent on average 42.9% of their time at

255 pasture grazing, 32.7% ruminating, 1.4% drinking and 22.9% idle, which was not affected by
256 treatment ($p > 0.05$; Table 4). There was also no difference ($p > 0.05$) in jaw activity (grazing,
257 ruminating, drinking and idling) between cows receiving EG, DG or RT. Whilst at pasture, cows spent
258 on average 42.2% of their time lying, and 55.7% standing, which was not affected by treatment ($p >$
259 0.05). In contrast, there was a difference ($p < 0.01$) in the proportion of time spent walking at pasture;
260 cows receiving EG spent a greater proportion of their time walking than either DG or RT, which did
261 not differ.

262 When activity was recorded over 24 h/d (during pasture access and indoors) using
263 accelerometers, there was a difference ($p < 0.001$) between cows receiving C compared to those
264 receiving EG, RT or DG in all behavioural activities. Cows receiving C spent at least 55 min/d
265 longer lying and had three additional LB/d, whilst lying bouts were shorter than for cows receiving
266 EG, RT or DG ($p < 0.001$; Table 4). Cows in C also took less steps/d compared to EG, RT or DG (p
267 < 0.001). There were no differences ($p > 0.05$) in behavioural activity, measured using the
268 accelerometers, between cows receiving treatments EG, RT or DG. When recorded by the RIC
269 system, time spent feeding on TMR was highest ($p < 0.05$) in C (191 ± 11.1 mins/d), lowest in RT
270 (124 ± 10.8 mins/d) and intermediate in EG and RT (both 152 ± 10.8 mins/d).

271

272 **4 | Discussion**

273 **4.1. | Intake and performance**

274 The current study was conducted over the summer months when the reproductive growth of pasture
275 can reduce its feed quality compared to earlier in the grazing season (Givens et al., 1993). Summer
276 grazing is, however, common practice on many dairy systems, with approximately 92% of dairy farms
277 in Britain grazing for at least part of the day during summer (March et al., 2014). Typical values for
278 pasture ME on commercial farms in the UK are less than 12 MJ ME/kg DM over the summer
279 (Wilkinson et al., 2014). In comparison, the grass used in the current study, at 12.5 and 12.0 MJ
280 ME/kg DM in the first and second periods respectively, was above average, and similar to the TMR.

281 Similarly, NDF and ADF content of the pasture was low (Wilkinson et al., 2014), indicative of a leafy
282 well-managed pasture (Dale et al., 2018), although the pasture contained a moderate concentration of
283 CP, but CP was similar to the TMR and sufficient for animal performance (Sinclair et al., 2014). The
284 cows in the current study grazed laxly, with post-grazing HM of approximately 2400 kg DM/ha above
285 ground level, and the high-quality pasture was maintained using hard grazing with dry cows following
286 the experimental cows, then mechanical topping if still required for an even sward. Commercially,
287 following grazing with dry cows may be insufficient to maintain pasture quality, as dry cows may be
288 too few in number on a commercial farm to keep up with a grazing rotation and greater use of
289 mechanical topping may be required with an associated increase in pasture wastage.

290 The major limiting factor for production in pasture-based systems for dairy cows is often not
291 grass quality, but DM intake (Kolver, 2003). The current study aimed to maintain overall DM intake,
292 with TMR offered *ad libitum* while housed, except for cows in RT that were restricted to 75% of *ad-*
293 *libitum* intake, and the provision of sufficient grass HM to facilitate a high rate of intake while at
294 pasture. A feeding bout was expected immediately following morning milking, therefore moving the
295 cows receiving EG to pasture at this point was hypothesised to promote a greater desire to consume
296 grass, and therefore increase grass intake. Delaying access to pasture for cows in DG following
297 milking was hypothesised to reduce grass intake as a result of increased TMR intake prior to grazing.
298 The DMI results for the 6-hr period prior to grazing indicate that cows in DG did consume the greatest
299 quantity of TMR during this period, however, grass intake of cows in EG or DG was similar (mean
300 of 2.2 kg DM/cow/d, or 8.2% of total DMI). Approximately 40% of the time at pasture for cows in
301 EG or DG was spent grazing, and therefore the rate of intake at pasture was low in comparison with
302 predominately pasture-fed dairy cows. For example, in cows with unrestricted access to pasture the
303 mean rate of intake of grass ranged from 16.2 to 44.8 g of DM/min (Pérez-Prieto & Delagarde, 2013),
304 and at these values, the 6 kg DM/d offered in the current study could have been consumed between
305 2.2 and 6.2 hr at pasture. Low intakes of grass in TMR-fed dairy cows offered pasture has also been
306 reported in previous work, with Motupalli et al. (2014) reporting a grass intake of only 0.8 to 1.6 kg

307 DM/cow/d when cows had free access to pasture and TMR. Similarly, in a study comparing
308 continuous housing with daytime or night time grazing, either with or without TMR access at pasture,
309 Mufungwe et al. (2014) reported grass intakes of 1.1 kg DM/cow during the day, or 0.7 kg DM/cow
310 at night, when cows had access to TMR while at pasture. When TMR was not provided in the field,
311 Mufungwe et al. (2014) reported that grass intake increased to 2.6 kg DM/cow, but total DMI was
312 decreased. The continuously housed cows in the current study were able to consume more feed (8.5
313 kg DM), during the 6 hr following the morning milking, which corresponds to the grazing period of
314 the other treatments. Despite this, cows in both EG and DG achieved a similar total DMI to C cows,
315 which was a consequence of the grazing groups increasing the duration and intensity of TMR feeding
316 at other times of the day.

317 It was hypothesised that the intake of grass would be highest in cows receiving RT, as these
318 animals would be hungrier and have a greater drive to consume grass, which would compensate for
319 the restriction of the TMR. Although the intake of grass for RT cows was higher than DG or EG, it
320 was still only 3.5 kg DM/cow, approximately 14.7% of total DMI for these cows, and the time spent
321 grazing (47.7% of time at pasture) was not different to cows in either of the other two grazing groups.
322 Previous research has reported a longer grazing time when TMR was more severely restricted. For
323 example, Fajardo et al. (2015), reported that when cows were offered 50% of the TMR of
324 continuously housed cows and had 6 hr/d of grazing, they spent 64% of their time at pasture grazing,
325 whilst cows with 9 hr/d of pasture access, grazed for 52% of the time, with a similar grazing duration
326 for both groups. In combination, these results suggest that when TMR is the major component of a
327 cows DM intake, their preference may be to wait for the TMR if it is not immediately available, even
328 if this results in a reduced total DMI. Alternatively, evidence suggests that grazing is a partially
329 learned behaviour (Charlton & Rutter, 2017), and thus limited early grazing exposure or limited
330 incentive to graze may limit the intake of grass, although all the cows in the current study had
331 extensive access to pasture prior to the study.

332 Milk yield reflected the pattern of DM intake, with a similar yield in cows receiving C, EG or

333 DG (mean of 44.9 kg/d), and was 3.2 kg/d lower in cows receiving RT. In contrast, previous research
334 has reported that cows fed TMR alone had a higher milk yield than those receiving a combination of
335 grazing and TMR (Bargo et al., 2002; Mufungwe et al., 2014; Soriano et al., 2001). However, these
336 studies had a longer grazing period (7-12 hr/d), suggesting that the 6 hr/d used in the current study
337 was within the ability of cows yielding 45 kg/d to compensate for a period without access to TMR.
338 Although DMI from grass was low, this small amount may have had a positive impact on the overall
339 nutrient digestibility of the diet, and it is also possible that there was increased mobilisation of adipose
340 tissue in cows that grazed grass which may have supported the high level of milk production, as LW
341 gain was lower in cows receiving EG or RT compared to C. Chapinal et al. (2010) reported that early
342 lactation cows could be grazed for approximately 10.5 hr overnight from 20:00 h without a reduction
343 in TMR intake or milk production. However, both the TMR intake and milk yield were lower in the
344 study of Chapinal et al. (2010) at 15.5 kg DM/d and 38.3 kg milk/d, and the cows may therefore have
345 been able to consume sufficient TMR before overnight grazing with less reliance on the pasture. Other
346 studies have observed negative production responses in early lactation when pasture has been a major
347 component of the diet. For example, Fajardo et al. (2015), restricted the TMR of cows to 50% with
348 either a 6 or 9 hr of grazing per day for 13 weeks from calving, and reported a milk yield of 33.8 kg/d,
349 which was approximately 91% of the continuously housed cows. In the current study, with a 75%
350 TMR allowance, the cows receiving RT did not fully compensate for their reduced DM intake when
351 at pasture, despite sufficient time and HM allowance, with the consequence that their yield was
352 reduced.

353 No difference was observed in milk fat or protein concentration in the current study, although
354 milk fat content was nominally higher in RT and subsequently ECM was similar in all treatments.
355 Lower milk yield of cows receiving RT compared to C resulted in a lower daily yield of protein, but
356 not fat. Milk fat concentration has previously been reported to increase (Fajardo et al., 2015), decrease
357 (Dall-Orsoletta et al., 2016; Morales-Almaráz et al., 2010), or remain unaffected (Soriano et al., 2001;
358 Vibart et al., 2008) when grazing was included in the diet of TMR-fed cows. These differences may

359 be a function of NDF intake, as pasture is typically higher in NDF than the TMR, which may be
360 expected to increase milk fat concentration (Sutton, 1986). In the current study, the NDF content of
361 the grass was approximately 20% higher than the TMR, but the intake of the grass was low and the
362 subsequent effects on total NDF intake small. Additionally, other factors of the TMR, such as particle
363 length and physically effective NDF content, may also have a large influence on milk fat content
364 (Tayyab et al., 2018, 2019).

365

366 **4.2. | Milk fatty acid profile**

367 Increasing forage intake has the potential to improve the FA profile of milk by increasing
368 unsaturated and decreasing saturated FA, with increases in beneficial long chain and reductions in
369 medium chain fatty acids (Dewhurst et al., 2006; Elgersma, 2015). For example, pasture-fed vs. maize
370 silage-based TMR-fed cows generally produce milk fat with increased concentrations of 18:3 n-3,
371 C18:2 c9,t-11, C18:1 t11, C18:1 n-9, and C18:0, with decreasing concentrations of C12:0, C14:0 and
372 C16:0 (Dewhurst et al., 2006). Other studies that have also incorporated a period of grazing with
373 TMR feeding have also reported increases in the C18:3 n-3 concentration of milk fat (Barca et al.,
374 2018; Morales-Almaráz et al., 2010; Vibart et al., 2008). In the current study, there was an increase
375 of C18:3 n-3 by approximately 29% in the milk fat of cows with a 6-hr grazing period compared to
376 TMR-only cows, although the amount was still comparatively small at less than 0.5% of the total fat
377 content. The major source of C18:3 n-3 was from the grass, which contained 41g/100g FA compared
378 with only 2.8 g/100g FA in the TMR. Despite the low intake of grass of cows receiving EG, DG or
379 RT, the intake of C18:3 n-3 was calculated to be increased by approximately 43, 36 and 64 g/cow/d,
380 respectively, compared to C. Approximately 90% of C18:3 n-3 would be expected to be
381 biohydrogenated in the rumen (Sinclair et al., 2005), but would still result in a net absorption of
382 between 4 and 6 g/d, and may explain the increase of this FA in milk of the grass-fed cows.

383 The majority of C18:3 n-3 is biohydrogenated in the rumen to c18:1t-11 and then C18:0, with
384 the mammary gland desaturating the majority of C18:t-11 to C18:2 c9,t11, or C18:0 to C18:1 c9

385 (Elgersma, 2015). An increase in these intermediaries may also have been expected with the inclusion
386 of grass in the diet, but no differences were observed in the milk from the cows receiving the 6 hr
387 grazing compared to continuously housed cows, although C18:1 t-12 was higher and C18:2 t-10,c12
388 tended to be higher in cows in grazing treatments compared to C. The FA C18:2 t-10,c12 is a potent
389 inhibitor of milk fat synthesis (Lock et al., 2006), and although not statistically significant, milk fat
390 content was numerically lowest in C and was relatively low overall, with a mean of 32.1 g/kg across
391 all treatments. Factors other than the proportion of grass in the diet can also affect the concentration
392 of these FA in milk fatty acids, with for example basal forage and concentrate composition and ratio,
393 pasture composition and vegetative state, rumen pH and animal factors playing an important role
394 (Dewhurst et al., 2006).

395 Overall, none of the grazing treatments substantially affected total SAT, MUFA or PUFA content
396 of the milk compared with continuously housed cows, although restricting TMR allowance did result
397 in a lower SAT, higher MUFA, less *de novo* synthesised FA and more preformed FA in milk than
398 unrestricted, early-grazed cows. Some of the differences in the milk FA composition between grazing
399 treatments may be explained by the higher grass intake in RT compared to EG, and potentially less
400 selective grazing or grazing to a lower sward horizon altering the FA profile, as grazing intensity has
401 been shown to alter the chemical composition of grass (Dale et al., 2018).

402

403 **4.3. | Behaviour**

404 Lying is a high-priority activity for dairy cows, and is essential for health, welfare and
405 productivity (Charlton & Rutter, 2017). When soil conditions are dry, and temperature and humidity
406 temperate, cows have been reported to show a preference for lying outside (Krohn et al., 1992;
407 Legrand et al., 2009). The cows that received 6 hr/d of grazing in the current study spent
408 approximately 42% of their time at pasture lying down, compared with 38% of their time indoors
409 lying. Continuously housed cows spent just over one hr/d more lying than those grazing, although the
410 lying bouts were shorter in the continuously housed cows. This reduced lying time of the grazed cows

411 is likely to be due to the additional time required for grazing. Previous studies have found that
412 continuously housed cows will spend an average of 3 to 6 hr/d feeding (Charlton & Rutter, 2017).
413 Although cows in C had a high DMI, which they were able to achieve in a relatively short feeding
414 time of 191 min, cows in EG and DG achieved a similar DMI but in 298 minutes of feeding plus
415 grazing. Interestingly, cows in RT spent a similar total time feeding and grazing (296 min), whilst
416 total lying time was also similar between the grazing groups (566 min). There may therefore have
417 been a conflict between lying and feeding/grazing, with the grazed cows in the current study perhaps
418 reaching a minimum acceptable lying time, or maximum feeding time with the conditions presented
419 to them. Alternatively, social interactions may have moderated the grazing behaviour of cows
420 receiving RT, although each treatment grazed in an independent area. Variation in grazing behaviour
421 has been observed to be related to milk yield, which influences appetite (Rind and Phillips, 1999) and
422 the similarity in grazing time between groups may be due to the lower milk yield of RT cows rather
423 than social influence from the adjacent groups.

424

425 **5 | CONCLUSIONS**

426 For cows with a milk yield of approximately 45 kg/d, providing access to pasture for 6 hr per day did
427 not have a major effect on intake or milk performance, with grass only contributing approximately
428 8% of DMI. In contrast, having access to pasture for 6 hr/d and restricting TMR intake to 75% of *ad*
429 *libitum* resulted in the highest grass intake, but this was not sufficient to compensate for the lower
430 TMR intake, and milk performance was reduced. Providing access to pasture can increase the milk
431 content of C18:3 n-3 but values were still low, at under 0.5% of the total fat. Having access to pasture
432 also resulted in cows spending more time feeding and spending less time lying, but when they did lie,
433 they did so for a longer period of time.

434

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TABLE 1 Ingredient (kg/kg DM) and chemical composition of a TMR and pasture offered to dairy cows that were continuously housed or receiving TMR and grazed pasture

	TMR	Pasture
Ingredient		
Maize silage	0.416	
Lucerne silage	0.117	
Sweetstarch ^a	0.129	
Rapeseed meal	0.074	
Wheat distillers dark grains	0.074	
Pot ale syrup ^b	0.061	
Soya hulls	0.035	
Hipro soyabean meal	0.031	
Palm kernel meal	0.021	
Rumen protected fat ^c	0.018	
Molasses	0.006	
Minerals ^d	0.006	
Limestone flour	0.005	
Feed grade urea	0.003	
Buffer ^e	0.002	
Yeast ^f	0.002	
Chemical composition		
DM (g/kg)	482	206
ME (MJ/kg DM)	12.2	12.3
OM (g/kg DM)	930	895
Ash (g/kg of DM)	70	105
CP (g/kg of DM)	176	175
WSC (g/kg DM)	81	202
NDF (g/kg of DM)	329	390
ADF (g/kg of DM)	196	210
Hemicellulose (g/kg DM)	133	180
Fatty acids (g/kg DM)	52.6	44.6
Fatty acids (g/100 g FA)		
C16:0	29.9	11.7
C18:0	4.2	0.3
C18:1 c9	28.9	1.5
C18:2 c9 c12 (n-6)	26.7	10.3
C18:3 c9 c12 c15 (n-3)	2.8	41.0

^aKW Alternative Feeds, Andover, UK; g/kg 360 cake products, 140 breakfast cereals, 140 cocoa hulls, 140 wheat feed, 70 sugar confectionery, 140 flour.

^bSpey syrup, KW Alternative Feeds, Andover, UK.

^cMegalac, Volac International Limited, Royston, UK.

^dContained the following macro minerals (g/kg) 210 Ca, 100 Mg, 50 Na, 30 P and trace minerals (mg/kg) 6000 Zn, 5000 Mn, 2500 Cu, 400 I, 70 Co, 40 Se.

^eAcid Buff, AB Vista, Wiltshire, UK.

^fVistacell Ultra, AB Vista, Co. Antrim, Ireland.

TABLE 2 Performance of dairy cows that were continuously housed or received 6 hr of access to pasture.

	C ¹	EG	DG	RT	SED	<i>p</i> -value
TMR intake (kg DM/d)	26.9 ^c	23.6 ^b	24.7 ^b	20.3 ^a	0.698	<0.001
Pasture intake (kg DM/d)	---	2.35 ^a	1.98 ^a	3.48 ^b	0.449	0.006
Total intake (kg DM/d)	26.9 ^b	26.0 ^b	26.7 ^b	23.8 ^a	0.524	<0.001
Milk yield (kg/d)	45.7 ^b	44.2 ^{ab}	44.9 ^b	41.7 ^a	0.993	<0.001
ECM ² (kg/d)	38.2	37.8	37.8	36.5	1.32	0.588
Milk fat (g/kg)	30.6	32.7	31.3	33.7	0.18	0.293
Milk protein (g/kg)	29.7	29.1	28.9	29.4	0.56	0.492
Milk lactose (g/kg)	44.2	43.8	44.5	44.2	0.57	0.686
Milk fat (kg/d)	1.44	1.44	1.36	1.39	0.088	0.769
Milk protein (kg/d)	1.36 ^b	1.29 ^{ab}	1.29 ^{ab}	1.23 ^a	0.032	0.002
Milk lactose (kg/d)	2.01 ^b	1.93 ^{ab}	2.00 ^b	1.84 ^a	0.051	0.005
Live weight (kg)	668 ^b	653 ^a	656 ^{ab}	647 ^a	4.9	<0.001
Live weight change (kg/d)	0.86 ^b	0.31 ^a	0.41 ^{ab}	0.09 ^a	0.177	<0.001
Body condition	2.87	2.83	2.76	2.76	0.058	0.186
Body condition change, units 28 d	0.086	0.047	-0.021	-0.021	0.0549	0.185

¹C = cows that were continuously housed and offered a TMR *ad libitum*; EG = cows that were grazed for 6 hr directly post am milking and then housed and offered a TMR *ad libitum*; DG = cows that were returned to housing for 1 hr post am milking before being grazed for 6 hr and then housed and offered a TMR *ad libitum*; RT = cows that were grazed for 6 hr directly after morning milking then housed and offered a TMR restricted to 75% of *ad libitum* intake.

²Energy corrected milk yield.

TABLE 3. Milk fatty acid composition of dairy cows that were continuously housed or received 6 hr of access to pasture.

	C ¹	EG	DG	RT	SED	<i>p</i> -value
Fatty acid (g/100g)						
C4:0	5.45	5.34	5.31	5.27	0.202	0.830
C6:0	2.62	2.69	2.41	2.44	0.131	0.115
C8:0	1.33 ^b	1.41 ^{ab}	1.20 ^a	1.22 ^{ab}	0.079	0.040
C10:0	2.69 ^{ab}	2.91 ^b	2.43 ^a	2.42 ^a	0.177	0.025
C12:0	3.11 ^{bc}	3.25 ^c	2.80 ^{ab}	2.68 ^a	0.173	0.006
C14:0	9.63 ^{ab}	9.88 ^b	9.33 ^{ab}	8.80 ^a	0.341	0.018
C14:1 c9	1.06	0.88	0.94	0.91	0.097	0.321
C15:0	0.89 ^b	0.84 ^{ab}	0.91 ^b	0.75 ^a	0.051	0.015
C16:0	28.2 ^b	27.6 ^{ab}	26.1 ^a	26.8 ^{ab}	0.56	0.004
C16:1 c7	1.66	1.48	1.66	1.82	0.163	0.253
C17:0	0.46 ^a	0.48 ^{ab}	0.52 ^b	0.50 ^{ab}	0.019	0.009
C18:0	7.16	7.55	7.40	7.41	0.446	0.851
C18:1 t9	0.53	0.51	0.55	0.57	0.040	0.562
C18:1 t11	0.65	0.68	0.64	0.74	0.086	0.638
C18:1 t12	0.96 ^a	1.24 ^b	1.22 ^b	1.24 ^b	0.086	0.004
C18:1cis 9	21.5 ^{ab}	21.1 ^a	23.1 ^{ab}	23.3 ^b	0.93	0.044
C18:1 c9, c12 (n-6)	2.61	2.45	2.53	2.58	0.107	0.457
C18:2 c9 t11	0.69	0.78	0.78	0.82	0.052	0.123
C18:2 t10 c12	0.12	0.07	0.07	0.05	0.027	0.084
C18:3 c9 c12 c15 (n-3)	0.31 ^a	0.40 ^b	0.40 ^b	0.41 ^b	0.022	<0.001
C22:0	0.09	0.05	0.04	0.06	0.019	0.057
C20:5 c7 c8 c11 c14 c17 (n-3)	0.02	0.03	0.03	0.02	0.008	0.506
Other	7.04	7.07	8.24	7.57	0.687	0.274
Summation by degree of saturation						
SAT	61.6 ^{ab}	62.0 ^b	58.5 ^a	58.4 ^a	1.29	0.008
MUFA	26.9 ^{ab}	26.5 ^a	28.6 ^{ab}	29.3 ^b	0.99	0.016
PUFA	4.53	4.51	4.68	4.74	0.161	0.397
Summation by length						
<C16	26.8 ^{ab}	27.2 ^b	25.3 ^{ab}	24.5 ^a	0.09	0.020
C16:0 and C16:1	30.2 ^b	29.5 ^{ab}	28.2 ^a	29.1 ^{ab}	0.58	0.012
>C16	36.0 ^a	36.2 ^{ab}	38.2 ^{ab}	38.8 ^b	1.12	0.032

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TABLE 4 Behaviour of dairy cows that were continuously housed or received 6 hr of access to pasture.

	C ¹	EG	DG	RT	<i>p</i> -value
Behaviour at pasture, % of time visually observed					
Grazing	---	40.9 ± 4.3	40.2 ± 3.4	47.7 ± 3.2	0.292
Ruminating	---	33.7 ± 2.3	32.7 ± 1.8	31.7 ± 2.3	0.792
Drinking	---	1.5 ± 0.2	1.7 ± 0.2	1.1 ± 0.2	0.108
Idling	---	23.8 ± 3.4	25.4 ± 2.7	19.6 ± 2.2	0.318
Lying	---	41.5 ± 3.5	45.8 ± 3.8	39.2 ± 4.0	0.462
Standing	---	55.2 ± 3.5	52.5 ± 3.6	59.3 ± 3.9	0.433
Walking	---	3.4 ± 0.4	1.7 ± 0.3	1.5 ± 0.4	0.003
Behavioural measurements (grazing and housed) measured automatically					
Lying time (min/d)	630 ± 13.0 ^b	563 ± 15.4 ^a	574 ± 11.7 ^a	560 ± 10.7 ^a	<0.001
Lying bouts (per d)	12.8 ± 0.35 ^b	9.6 ± 0.31 ^a	9.6 ± 0.30 ^a	9.9 ± 0.32 ^a	<0.001
Lying bout duration (min/bout)	53.5 ± 1.23 ^b	61.4 ± 1.66 ^a	64.9 ± 2.19 ^a	60.1 ± 1.54 ^a	<0.001
Steps (per d)	1137 ± 65.3 ^b	1592 ± 48.8 ^a	1592 ± 34.6 ^a	1563 ± 28.7 ^a	<0.001

¹C = cows that were continuously housed and offered a TMR *ad libitum*; EG = cows that were grazed for 6 hr directly post am milking and then housed and offered a TMR *ad libitum*; DG = cows that were returned to housing for 1 hr post am milking before being grazed for 6 hr and then housed and offered a TMR *ad libitum*; RT = cows that were grazed for 6 hr directly after morning milking then housed and offered a TMR restricted to 75% of *ad libitum* intake.

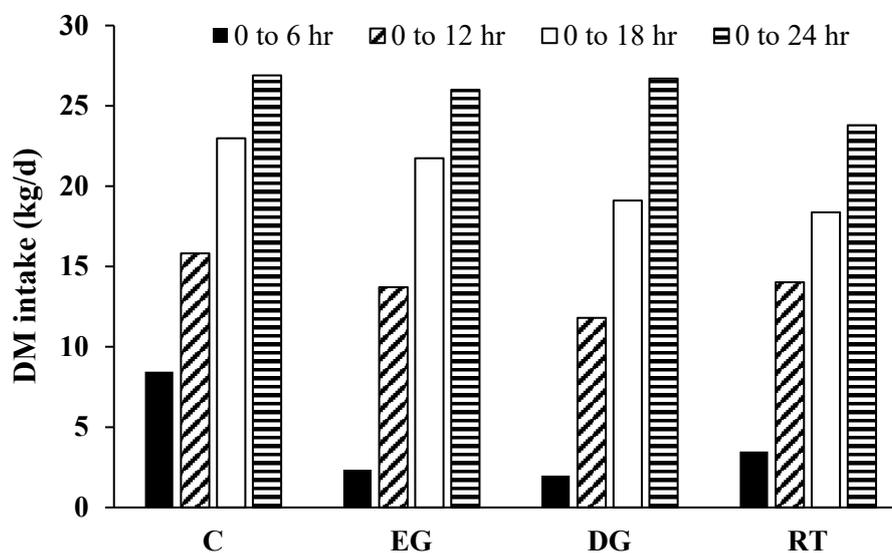


FIGURE 1 Cumulative DM intake in cows that were continuously housed and offered a TMR *ad libitum* (C); grazed for 6 hr directly post am milking and then housed and offered a TMR *ad libitum* (EG); returned to housing for 1 hr post am milking before being grazed for 6 hr and then housed and offered a TMR *ad libitum* (DG); grazed for 6 hr directly after morning milking then housed and offered a TMR restricted to 75% of *ad libitum* intake (RT). Hours 0 to 6 relate to the grazing period. For cows that were continuously housed the 6 hr blocks relate to the same period as EG and RT. Treatment $p < 0.001$; SED 0.86, Time $p < 0.001$; SED 0.39, Treatment x Time $p < 0.001$; SED 1.09.