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1 **Particle size distribution of forages and mixed rations, and their**  
2 **relationship with ration variability and performance of UK dairy herds**

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20 ABSTRACT

21 The particle size of the ration has been proposed as a key factor, along with its  
22 fibre and non-forage carbohydrate concentration, to ensure healthy rumen  
23 function and optimal performance of dairy cows. The current **particle size**  
24 distribution recommendations for forages and rations are primarily based on  
25 lucerne-haylage and maize silage (MS) and may not be suitable for the wetter  
26 grass silage (GS) based rations typically fed in Northern Europe. In order to  
27 characterize the **particle size** distribution of forages and rations in the UK, fifty  
28 commercial dairy herds feeding a range of GS and MS based rations were  
29 sampled during the winter of 2015/2016. The **particle size** distribution of the fresh  
30 forages and mixed rations (MR; total and partial mixed rations) were analysed  
31 using a modified Penn State Particle Separator with six screens of hole size 60,  
32 44, 26.9, 19, 8, and 4 mm. The fresh MR was collected at 5-equally-spaced  
33 locations along the length of the feed-face for each herd within 5-min of feeding  
34 to determine the consistency of ration mixing, and again from the same locations  
35 4h post-feeding. Grass silage was the main forage fed on 50 herds, with 80.3%  
36 of the dry matter (DM) being retained above the 19 mm sieve, which is  
37 considerably higher than the North-American recommendations for lucerne-  
38 haylage. The **particle size** distribution of MS followed the general  
39 recommendations for North American forages, however, the 8-19 mm fraction  
40 was higher and the <4 mm lower. The >60 mm fraction of the MR had the lowest  
41 (0.1% DM) DM retention, and the 8-19 mm fraction the highest (34.9% DM). The  
42 MR had a higher proportion of particles retained on the 26.9 mm sieve when GS  
43 was the sole forage. Fifty eight % of herds were considered to have either  
44 moderately or poorly mixed rations, whilst 66% had evidence of diet selection

45 (either preferential consumption or selective refusals). Particle size of the MR  
46 accounted for 33% of the variance in the milk fat content and 12% of milk yield.  
47 In conclusion, the **particle size** distribution of the GS and MR fed on UK dairy  
48 herds is different from the current recommendations, suggesting that the **particle**  
49 **size** of UK dairy rations is too long or new guidelines using additional sieves with  
50 larger pore sizes are required. There is also a high proportion of herds with poor  
51 mixing and/or evidence of diet selection.

52

53 *Key words:*

54 Dairy cows, ration variability, diet selection, particle size distribution

55

## 56 **1. Introduction**

57 Feeding dairy cows with a mixed ration (MR; either total or partial mixed ration)  
58 is an effective way to provide a homogeneous and balanced diet throughout the  
59 day (Coppock et al., 1981). The composition of MR can vary considerably but  
60 ryegrass (GS) and **maize silages (MS)** are the main forages used in the MR fed  
61 to dairy herds in Northern Europe (Johansen et al., 2018; March et al., 2014). In  
62 order to maintain animal performance and promote a healthy rumen function the  
63 inclusion of forages with an adequate particle size and dietary concentration of  
64 non-forage carbohydrate (fibre) in the MR are required (Zebeli et al., 2012). The  
65 physically effectiveness of a ration has been proposed as the product of the  
66 **particle size** multiplied by its neutral detergent fibre (NDF) content, defined as  
67 physically effective fibre (*peNDF*; Mertens, 1997). Achieving the correct **particle**  
68 **size** and *peNDF* in a ration can enhance rumen function leading to an increase  
69 in the production of rumen microbes, more efficient degradation of fibre and

70 increased milk fat content (De Brabander et al., 1999; Zebeli et al., 2012). A short  
71 forage **particle size** is associated with improved compaction in the bunker and  
72 can result in reduced aerobic spoilage at feed out (McDonald et al., 1991) and  
73 may increase **dry matter (DM)** intake, due to reduced rumen fill and increased  
74 fibre digestibility (Thomson et al., 2017). However, too short a forage particle  
75 length can increase the rate of volatile fatty acid production in the rumen, reduce  
76 rumination time, and decrease the production of saliva (Tafaj et al., 2007), with  
77 the consequence of inhibiting cellulolytic bacteria activity and increasing the risk  
78 of sub-acute ruminal acidosis (SARA; Tafaj et al., 2007). In a review of the  
79 literature, Zebeli et al. (2012) concluded that too **short** a **particle size** (and  
80 *peNDF*), increases the passage rate of digesta and rate of fibre degradation due  
81 to a higher surface area for microbial attachment. In contrast, too long a forage  
82 **particle size** may promote ration sorting and result in some cows receiving excess  
83 concentrates and others insufficient (Kononoff and Heinrichs, 2003).

84 The estimation of the **particle size** of forages and MR is problematic, and various  
85 methods have been proposed to characterise feed particle distribution using  
86 different sieving techniques, with no universally accepted standard. Maulfair and  
87 Heinrichs (2012) concluded that the Penn State Particle Separator was the most  
88 useful method and proposed dietary guidelines for use on-farm. These  
89 recommendations are primarily based on North American rations that consist of  
90 MS and lucerne haylage (Eastridge, 2006), and may therefore not be suitable for  
91 the typically wetter (e.g. less than 30% DM) MS and GS commonly fed in Northern  
92 Europe (Møller et al., 2000).

93 Heinrichs et al. (1999) reported that processing by the mixer wagon prior to  
94 feeding can also have a large effect on the consistency of the mix, and affect the

95 **particle size** and *peNDF* concentration of the ration subsequently consumed.  
96 Mixing protocols have been shown to affect feed intake and milk yield, particularly  
97 in rations containing longer chop lengths (Humphries et al., 2010; Maulfair and  
98 Henrichs 2010). Consideration should therefore also be given to the effect of  
99 **particle size** and consistency of mixing on the degree of diet selection by dairy  
100 cows.

101 The primary objective of the present study was to characterise the **particle size**  
102 distribution and *peNDF* content of GS, MS and MR fed on UK dairy herds using  
103 a modified Penn State Particle Separator, and to compare the observed **particle**  
104 **size** distribution with current guidelines. The secondary objective of the study was  
105 to evaluate the consistency of mixing of MR and extent of sorting of GS and  
106 GS/MS based MR, and to determine the relationship between **particle size** and  
107 cow performance on UK dairy herds.

108

## 109 **2. Material and methods**

### 110 *2.1. Herd characteristics*

111 Fifty commercial dairy herds located throughout the UK (32 in the Midlands of  
112 England, 9 in the South of England and 9 in Southwest Scotland) that were  
113 feeding GS and/or MS were visited between January and June, 2016. The herds  
114 were randomly selected from a database supplied by the Agricultural and  
115 Horticultural Development Board, the levy body covering England, Scotland and  
116 Wales, with the provision that they were using a MR (partial or total) feeding  
117 system and had a high yielding group that contained at least 50 cows. Herds were  
118 enrolled onto the study through an initial telephone contact and questionnaire  
119 survey to determine suitability and willingness to participate. On the day of the

120 visit a second questionnaire was completed to collect details of herd  
121 characteristics, performance levels and frequencies of fresh feed delivery, feed  
122 push up and orts removal. In addition, feeding space per cow, feed mixer make  
123 and model, forage harvester make and model, and mixing protocol were  
124 recorded. The ingredient composition of MR fed to the target group and the mean  
125 concentrate fed in the parlour was also recorded.

126 Out of the 50 herds, 50 fed GS, with 34 using MS in the MR. Other sources of  
127 forage being fed were; whole-crop wheat (19), wheat straw (15), fodder beet (5),  
128 grass haylage (2), whole-crop triticale (1), whole-crop barley (1), lucerne (1), pea  
129 silage (1) and oat silage (1). Forty-four of the herds had an all year around calving  
130 pattern, 4 were autumn block calving and 2 spring block calving. Holstein-Friesian  
131 was the major breed on 36 herds, with the predominant breed on the remaining  
132 herds being Ayrshire (2), Jersey (1), Brown Swiss (1), or (10) having a mixture of  
133 Holstein with other breeds (Brown Swiss, New Zealand Friesian, and Jersey) or  
134 crossbred. The main feeding system was total MR which was used on 28 herds,  
135 while the remaining 22 herds fed a partial MR with additional concentrate fed in  
136 the milking parlour. Twenty-four herds used a “tub” type mixer wagon, 18 a  
137 “barrel” type, 7 an “auger” design (vertical or horizontal) and one used a forage  
138 box.

139 Total herd size ranged from 75 to 2220 animals, with a mean of 354 (Table 1).  
140 The number of lactating cows ranged from 67 to 1770 cows/herd, with a mean  
141 and median of 310 and 277, respectively. The annual milk yield ranged from 6000  
142 to 12500 kg/cow, with a mean of 9199 kg/cow (median = 9200). Annual energy  
143 corrected milk yield (corrected for milk fat and protein; Sjaunja et al., 1991)  
144 ranged from 7248 to 13209 kg/cow, with a mean of 10011 kg/cow. All herds



145 delivered fresh feed either once or twice daily, with a mean of 1.3 times/d. Of the  
146 50 herds, 20 were feeding the MR in a trough where there was no push up the  
147 feed. The average frequency of feed push up in the remaining 30 herds was 4.7  
148 times/d. The mean orts removal frequency was 4.4 times/wk, with a range from  
149 0.25 (monthly) to 7 (daily) times/wk. Feed space per cow ranged from 0.30 to  
150 0.76 m/cow, with a mean of 0.56 m/cow. Length of feed mixing was either  
151 manually recorded or provided by the farmer, and ranged from 5 to 60 min. The  
152 number of chews per bolus was manually counted for three full bouts for 10 cows  
153 randomly selected from the feeding group sampled (Kononoff et al., 2002).

154

## 155 2.2. Determination of *particle size* and *peNDF* distribution of forages and MR

156 Where more than one feeding group was present, data were collected from the  
157 high yielding group in each herd (n = 40). Where feed was delivered more than  
158 once (n = 15), the first (morning) feed was sampled. The feed face of the high  
159 yielding group of cows (or all cows if no subdivision was present) was divided into  
160 five equal sections to determine the consistency of mixing (Sova et al., 2014).  
161 Within each feed face section, a 30 cm × 30 cm quadrat was randomly placed  
162 over the MR within 5 mins of fresh feed-out, and all material removed and  
163 thoroughly mixed by hand (0hMR; Endres and Espejo, 2010). To determine the  
164 level of diet selection (feed sorting), the MR was sampled using the quadrat from  
165 the same locations along the feed fence again four hours post feeding (4hMR;  
166 Leonardi et al., 2005). Prior to fresh feed delivery, refusals, where available, were  
167 also sampled (n = 33).

168 The *particle size* distribution of the forage (GS and MS) and MR samples were  
169 analysed on both a fresh and dried basis. A modified Penn State Particle

170 Separator with four screens of 26.9, 19, 8, and 4 mm was used to determine the  
171 **particle size** of GS and GS/MS based MR, and three screens of 19, 8 and 4 mm  
172 for MS according to the manual shaking procedure described by Kononoff et al.  
173 (2003). Perennial ryegrass (*Lolium perenne*) and MS (*Zea mays* L.) were  
174 sampled from first, second or third cut GS and MS silage bunkers as described  
175 by Sinclair (2006) and the **particle size** measured using the modified Penn State  
176 Particle Separator described above. The **particle size** distribution (%) was  
177 calculated by dividing the weight of each fraction by the sum of all fractions and  
178 multiplying by 100.

179 The on-farm **particle size** distribution analysis using one additional Penn State  
180 Particle Separator sieve screen (26.9 mm) was found to be inadequate to  
181 accurately determine the geometric mean **particle size** ( $X_m$ ) of GS and GS based  
182 MR. Consequently, two larger sieve screens of size 44 and 60 mm were used to  
183 reanalyse **particle size** of 0hMR and GS using frozen and defrosted samples. The  
184 frozen samples were thawed at room temperature for 6h prior to analysis.

185

### 186 *2.3. Chemical analysis*

187 The DM content (AOAC, 2012; 988.05) of each fraction of 0hMR, 4hMR, refusals,  
188 GS and MS for each herd was determined by oven drying at 105°C to constant  
189 weight. Forage and MR samples were then milled in a hammer mill (Crompton  
190 Control Series 2000, Wakefield West Yorkshire UK) fitted with a 1 mm screen.  
191 The crude protein (988.05; Dumas method [ $N \times 6.25$ ]), ash (942.05; at 550°C for  
192 6 h) and ether extract (920.39) was analysed as described by AOAC (2012). The  
193 NDF (using sodium sulphite and heat stable amylase, and expressed residual of  
194 ash) and acid detergent fibre (ADF) content was analysed according to Van Soest

195 et al. (1991). The starch content of the 0hMR was analysed by Trouw Nutrition  
196 (Blenheim House, Blenheim Road, Ashbourne, Derbyshire, UK) using the  
197 procedure described by McCleary et al. (1997).

198

#### 199 2.4. Calculations and statistical analysis

200 Energy corrected milk yield (kg) was calculated as: milk yield (kg)  $\times$  [(38.3  $\times$  fat  
201 (g/kg) + 24.2  $\times$  protein (g/kg) + 15.71  $\times$  lactose (g/kg) + 20.7)/3,140], as described  
202 by Sjaunja et al. (1991). The geometric mean **particle size** ( $X_m$ ) was calculated  
203 using the method described by ANSI (1992). The physical effectiveness factor  
204 (*pef*) was determined as the DM proportion of particles longer than 8 mm  
205 (*pef*<sub>>8mm</sub>) or 4 mm (*pef*<sub>>4mm</sub>, Lammers et al., 1996; Maulfair and Heinrichs, 2010).  
206 The *peNDF*<sub>>4mm</sub> was calculated by multiplying the NDF content (% DM) of the MR  
207 by the *pef*<sub>>4mm</sub>, and *peNDF*<sub>>8mm</sub> by multiplying the NDF content (% DM) of the MR  
208 by the *pef*<sub>>8mm</sub> (Lammers et al., 1996; Mertens, 1997).

209 The consistency of ration mixing of each herd was calculated using the co-  
210 efficient of variation (CV%) of each **particle size** fraction of the 0hMR (Buckmaster  
211 et al., 2014; Oelberg and Stone, 2014; Sova et al., 2014), with a CV of >5%  
212 considered significant (Silva-del-Rio and Castillo, 2012). The CV of each fraction  
213 was weighted for the respective percentage **particle size** distribution and then the  
214 corrected CV summed. Herd-level diet selection was calculated for each fraction  
215 by dividing the proportion (DM basis) at 0hMR by the corresponding proportion  
216 at 4hMR and refusals, and presented as a percentage. A sorting value of 100%  
217 indicated no sorting, <100% indicated preferential consumption, and >100%  
218 indicated selective refusal.

219 All data were summarised by herd and tested for normality using the general  
220 descriptive statistics component of GenStat 17.1 ® (VSN International Ltd.,  
221 Oxford, UK). Associations between measures of productivity (energy corrected  
222 milk yield, milk fat g/kg, milk protein g/kg), feeding management and ration  
223 characteristics were analysed using a standard linear model (i.e. ANOVA) with  
224 forage source and shaking technique as fixed effects and herds and location as  
225 random effects. A linear regression model was used to determine the association  
226 between  $X_m$  and energy corrected milk yield and milk fat using GenStat 17.1 ®  
227 (VSN International Ltd., Oxford, UK). For multiple comparisons, all fractions of  
228 the mixed ration were analysed by general ANOVA followed by a Tukey test, with  
229 the significant level set at  $P < 0.05$ .

230

### 231 **3. Results**

#### 232 *3.1. Forage proximate and physical characteristics*

233 The mean DM of the GS was 23 g/kg lower ( $P = 0.022$ ) and the CP 54 g/kg DM  
234 higher than the MS (Table 2). The NDF and ADF content were also 65 and 64  
235 g/kg DM higher in the GS than the MS ( $P < 0.001$ ). The highest % DM retention  
236 of GS was the 26.9-44 mm fraction (51.6%,  $P < 0.001$ ), with the majority of the  
237 DM (80.3%) being longer than 19 mm. In contrast, the highest retention of DM for  
238 MS was between 8-19 mm (73.2%,  $P < 0.001$ ). The  $X_m$ ,  $peNDF_{>4mm}$  and  
239  $peNDF_{>8mm}$  content was higher ( $P < 0.001$ ) in GS than MS (mean values of 42.6  
240 and 10.5 mm, 48 and 40%, and 47 and 34% for  $X_m$ ,  $peNDF_{>4mm}$  and  $peNDF_{>8mm}$   
241 for GS and MS respectively).

242

243 3.2. Mixed ration proximate and physical characteristics

244 The mean forage to concentrate ratio across the 50 herds was 77:23 on a fresh  
245 weight basis, and 57:43 on a DM basis, with a GS to MS ratio on the 34 herds  
246 that fed both forages of 50:50 (fresh weight basis) or 48:52 (DM basis; Table 3).  
247 The DM concentration of the MR ranged from 213 to 544 g/kg, with a mean value  
248 of 373 g/kg across the 50 herds, whilst the mean CP ranged from 116 to 205 g/kg  
249 DM, with a mean value of 160 g/kg DM. The mean and median NDF  
250 concentration of the MR was 391 and 381 g/kg DM respectively. For the MR, the  
251 lowest proportion of DM was retained on the 60 mm fraction ( $P < 0.001$ ), with the  
252 8-19 mm fraction having the highest proportion ( $P < 0.001$ ), and there was no  
253 difference ( $P > 0.05$ ) between the 44-60 and 19-26.9 mm fractions. The  
254  $peNDF_{>4mm}$  concentration of the MR ranged from 22 to 47% with a mean of 33%,  
255 and the mean  $peNDF_{>8mm}$  was 73%. The mean  $X_m$  of the MR was 19.5 mm,  
256 ranging from 6.2 to 44.9 mm. The starch concentration of MR ranged from 63 to  
257 237 g/kg DM with a mean value of 138 g/kg DM. The mean DM of the 0h, 4h and  
258 refusals did not differ ( $P = 0.10$ ) between sampling times, and the DM  
259 concentration of the various fractions of MR did not change over time ( $P > 0.05$ ;  
260 data not shown).

261 Herds that fed GS as the main forage had a higher ( $P < 0.01$ ) proportion of the  
262 DM retained on the 26.9-44 mm fraction of the 0hMR compared to those that  
263 used a mixture of GS and MS (Table 4). In contrast, herds that used a mixture of  
264 both forages had a higher ( $P < 0.01$ ) proportion of the DM retained on the 8-19  
265 mm fraction. The type of mixer wagon (barrel, tub or auger) had no effect ( $P >$   
266  $0.05$ ) on the particle size distribution of any fraction of the 0hMR (data not shown).  
267 When the partial or total MR were considered separately, the proportion of longer

268 fractions (26.9-44 and 44-60 mm) was higher ( $P < 0.05$ ) when in the partial MR,  
269 while the shorter fractions (8-19, 4-8 and <4 mm) were highest ( $P < 0.05$ ) when  
270 fed as a total MR (Supplementary Table S1).

271

### 272 *3.3. Variability in mixed ration mixing*

273 The coefficient of variation of mixing of MR was highest for the 19-26.9 and >26.9  
274 mm fractions at 15 and 13.7% respectively, while the minimum CV of 6.4% was  
275 for the 8-19 mm fraction (Table 5). The type of wagon mixer, forage source, total  
276 MR or partial MR, and  $X_m$  had no effect ( $P > 0.05$ ) on ration variability across all  
277 five fractions (data not shown).

278

### 279 *3.4. Particle size distribution of mixed rations post-feeding and diet selection*

280 Diet selection calculated between 0-4h, 4-24h and 0-24h, demonstrated that  
281 there was selective refusal of the >26.9 and 19-26.9 mm fractions and a  
282 preferential consumption of the 8-19, 4-8 and <4 mm fractions between 0-24h  
283 period (Table 6), although there was considerable variation between herds.  
284 Sorting activity calculated between 0 and 4h showed preferential consumption ( $P$   
285 < 0.001) for the 4-8 and 8-19 mm fraction of the MR while the >26.9, 19-26.9 and  
286 <4 mm fractions were selectively refused. The inclusion of whole-crop wheat ( $n$   
287 = 19) and straw ( $n = 15$ ), the mixer wagon type or  $X_m$  had no effect ( $P > 0.05$ ) on  
288 the level of feed sorting (data not shown).

289

### 290 *3.5. Association between particle size and production*

291 There was a positive relationship ( $R^2 = 0.33$ ;  $P = 0.004$ ) between  $X_m$  and mean  
292 milk fat content (g/kg) across all herds (Figure 1). The relationship was improved

293 when Holstein-Friesian and Holstein-Friesian crosses were analysed separately  
294 ( $R^2 = 0.36$ ;  $P < 0.001$ ), with the  $R^2$  being highest when Holstein-Friesian herds  
295 were analysed alone, with almost 50% of the variation in milk fat content between  
296 herds being accounted for by  $X_m$  ( $R^2 = 0.47$ ;  $P < 0.001$ ). In contrast, there was a  
297 negative relationship between  $X_m$  and energy corrected milk across the 50 dairy  
298 herds, accounting for 16% of the variation ( $P < 0.001$ ).

299

### 300 3.6. Fresh vs dried particle size distribution

301 When dried prior to separation there was a difference in particle size distribution,  
302 with less long material and more short material than when measured fresh and  
303 then dried (Table 7 and Supplementary Table S2). For GS the >26.9 mm fraction  
304 decreased ( $P < 0.001$ ), while the 8-19, 4-8 and the <4 mm fractions increased ( $P$   
305  $< 0.001$ ) when analysed in a dried form. Similarly, the 4-8 and <4 mm fractions of  
306 the MS increased ( $P < 0.001$ ) when analysed in a dried compared to a wet form.  
307 For the MR, the proportion of the >26.9 mm decreased ( $P < 0.001$ ), while the  
308 proportion of the 4-8 and the <4 mm fractions increased ( $P < 0.01$ ) when analysed  
309 in a dried form compared to fresh and then dried.

310

## 311 4. Discussion

### 312 4.1. Herd characteristics and proximate analysis

313 The mean annual milk yield and herd size recorded in the current study were  
314 higher than the values reported for the UK (yield of 8180 kg and 143 cows/ herd,  
315 respectively; AHDB, 2016). This difference may be due in part to the selection  
316 criteria for the current study, with all herds recruited feeding MR and using GS,  
317 MS or a mixture as the main forage source. As a consequence, spring calving,

318 grazed grass based herds that have a lower mean milk yield (AHDB, 2016; Garcia  
319 and Holmes, 1999) were not used, although the trend in the UK is for more  
320 continuous housing, indoor feeding rather than grazing (March et al., 2014).

321 The MS being fed in the current study had a lower DM content at 300 g/kg  
322 compared to the 395 g/kg reported by Lammers et al. (1996) in the northeast of  
323 the United States of America (USA). The nutrient composition of the GS used in  
324 the current study was, however, typical of European ryegrass silage (Møller et  
325 al., 2000), with a mean CP of 136 g/kg DM and NDF of 492 g/kg DM. The mean  
326 forage to concentrate ratio of the MR in the current study (57:43 DM basis) was  
327 higher than that reported for 50 herds in Minnesota (52:48, Endres and Espejo,  
328 2010). A higher forage to concentrate ratio is more likely to maintain an efficient  
329 rumen function and should minimise the risk of SARA (Zebeli et al., 2012).

330 However, twenty four out of the 50 herds fed a lower proportion of forage in the  
331 MR than the minimum of 56% proposed by Zebeli et al. (2012), and may  
332 subsequently have been at risk of SARA.

333 The average DM of the MR in the current study of 373 g/kg was lower than that  
334 reported by Eastridge (2006) and Sova et al. (2013) for typical North American  
335 rations. In similar cross-sectional studies, Sova et al. (2013) reported a mean total  
336 MR DM of 477 g/kg in 22 Canadian herds, while Endres and Espejo (2010)  
337 reported a mean of 523 g/kg DM in the total MR of 50 herds in Minnesota, USA.

338 Rations with a high DM content may increase DM intake, but may also encourage  
339 cows to sort (Leonardi et al., 2005). The CP content of the MR in the current study  
340 was also lower compared to that of 50 herds in the USA (175 g/kg DM; Endres  
341 and Espejo, 2010) or 22 herds in Canada (165 g/kg DM; Sova et al., 2013). This  
342 difference may be due to the greater use of concentrates and lower use of forages



343 in North American rations as reflected in the lower forage to concentrate ratio  
344 (Endres and Espejo, 2010). The average NDF content of the MR in the current  
345 study was approximately 90 g/kg DM higher than that reported in the USA (298  
346 g/kg DM; Endres and Espejo, 2010) or Canadian rations (313 g/kg DM; Sova et  
347 al., 2013). This was probably due to the greater use of forage in the current study,  
348 especially GS, which has a higher NDF concentration than MS or lucerne haylage  
349 (Hoffman et al., 1993), but may also be affected by maturity at harvesting which  
350 increases NDF concentration (Dawson et al., 2002). The higher concentration of  
351 NDF in the MR along with a sufficient **particle size** are associated with a more  
352 efficient rumen function for fibre degrading microbiota by resisting a depression  
353 in rumen pH (Zebeli et al., 2012). Similarly, the ADF content was approximately  
354 50 g/kg DM higher in the current study compared to that fed in the USA (198 g/kg  
355 DM; Endres and Espejo, 2010) or Canadian rations (205 g/kg DM; Sova et al.,  
356 2013), but was typical of Northern European rations (Johansen et al., 2018).

357

#### 358 *4.2. Ration physical characteristics*

359 The **particle size** distribution of MS followed the general guidelines suggested by  
360 Heinrichs (2013) of 3-8% above 19 mm, 45 to 65% between 8-19 mm, 20 to 30%  
361 between 4 and 8 mm, and <10% below 4 mm although the 8-19 mm fraction of  
362 MS in the current study was higher than that reported by Maulfair et al. (2010).  
363 This difference may be due to the higher moisture content of MS used in the UK  
364 that promotes the adherence of shorter particles, but may also reduce sorting  
365 (Leonardi et al., 2005). Overall, the **particle size** distribution of MS in the UK was  
366 similar to the current guidelines for MS based on North America rations, and  
367 consequently, there is little requirement for additional research or separate

368 recommendations for UK and northern European MS. Out of the 50 herds used  
369 in the current study, the minimum % DM of GS retained on the >19 mm sieve  
370 was 49%, considerably higher than the 10-20% guidelines for lucerne haylage in  
371 the USA (Heinrichs, 2013). Feeding a longer **particle size** may result in a higher  
372 rumen pH and avoid SARA, but is also associated with a reduction in feed intake  
373 due to a greater rumen fill (Tafaj et al., 2007; Zebeli et al., 2012).

374 The mean **particle size** distribution of the 0hMR in the current study differed from  
375 the guidelines based on North American rations (Heinrichs, 2013), with the long  
376 (>19 mm) **particle size** distribution being 38%, approximately 50% higher than  
377 that reported by Sova et al. (2013), DeVries et al. (2011) or Hosseinkhani et al.  
378 (2008), and approximately 4 times higher than that reported by Heinrichs (2013),  
379 Endres and Espejjo (2010), Miller-Cushon and DeVries (2009), or Heinrichs and  
380 Kononoff, (1996) (Supplementary Table S3). The difference in **particle size**  
381 distribution of MR in the current study reflected the high inclusion of GS that  
382 contained a very long **particle size** (>19 mm = 80% DM,  $X_m = 42.6$  mm). The use  
383 of other forages (e.g. whole-crop wheat, wheat straw, fodder **beet**) in the MR in  
384 the current study did not significantly affect the **particle size** distribution of the MR,  
385 and supports that the high proportion of GS in the ration was the major factor  
386 causing the differences. The higher proportion of the 26.9-44 and 8-19 mm  
387 particle fractions in the MR may also be explained by the high moisture content,  
388 as 4-8 and <4 mm particles may have adhered to longer particles (Leonardi et  
389 al., 2005). However, the considerably longer **particle size** of GS than lucerne  
390 haylage based MR suggests that either the **particle size** of UK dairy rations is too  
391 long or the need for more specific **particle size** measurement methods and  
392 distribution recommendations when wetter GS is the major forage in the MR.

393 When GS was the sole forage in the MR, rations had a higher proportion of the  
394 26.9-44 and 44-60 mm fractions which may promote ration sorting (DeVries et  
395 al., 2007), although in the current study there was no relationship between  $X_m$   
396 and degree of sorting after 4 or 24 h. The additional 26.9, 44 and 60 mm pore  
397 size sieves used in the Penn State Particle Separator in the current study allowed  
398 a more even distribution of **particle size** for GS and MR samples than the  
399 traditional Penn State Particle Separator. However, as a very small proportion of  
400 particles was retained on the 19-26.9 mm screen, a screen larger than 26.9 mm  
401 may be more appropriate.

402

#### 403 *4.3. Variability in ration mixing*

404 Feeding MR is an effective method to provide all the required nutrients to dairy  
405 cows, and a properly mixed ration ensures a uniform delivery of all feed ingredient  
406 to the animal (Coppock et al., 1981). Mixer wagons and mixing protocols can  
407 however, influence **particle size** distribution and result in differences in feed intake  
408 and milk yield, particularly for rations with longer chop lengths (Humphries et al.,  
409 2010). Heinrichs et al. (1999) also reported that processing by the mixer wagon  
410 prior to feed-out can have a large effect on the **particle size** and *peNDF*  
411 subsequently fed and the consistency of the mix. In a survey of Iranian herds,  
412 Esmaeili et al. (2016) reported a high variability (CV >10%) in **particle size**  
413 distribution of MR with the highest variation recorded for the >19 mm fraction, a  
414 finding in agreement with the current study. There were 42% of herds that had a  
415 CV  $\leq$ 5% (indicating a well-mixed ration), 26% that had a CV of between 5-10%  
416 (moderately mixed), and 32% that had a CV >10% (poorly mixed ration). There  
417 was no effect of mixer model on overall ration variability across all herds. In

418 contrast, Heinrichs et al. (1999) reported that MR processing by the mixer wagon  
419 can have a significant effect on the ration consistency, **particle size** and *peNDF*  
420 concentrations of the ration subsequently consumed.

421

#### 422 *4.4. Herd level diet selection*

423 Herd level diet selection was calculated as the proportional change in each  
424 fraction of the MR over time post-feeding. Feed sorting activity is usually  
425 associated with the preferential consumption of fine starch or protein rich particles  
426 in the ration (DeVries et al., 2007). However, in the current study, there were  
427 selective refusals for the >19 mm fraction and preferential consumption for the  
428 <8 mm fraction. To more easily determine the variability of diet selection across  
429 herds, the long fractions (>60, 44-60, 26.9-44 and 19-26.9 mm) were summed  
430 (>19 mm), and the short (4-8 and <4 mm) fractions summed (<8 mm), while  
431 assuming that a sorting value of 100% ± 5 indicated no sorting, >105% indicated  
432 selective refusal and a sorting value of <95% indicates preferential consumption.  
433 Of the 50 herds, 82% had either selective refusal or did not show preferential  
434 consumption for the >19 mm fraction which may be associated with the inclusion  
435 of long particles of GS. There was no sorting activity observed for the <8 mm  
436 fraction in 46% of the herds. As discussed previously, this may have been due to  
437 the comparatively high moisture content of the MR in the current study that  
438 caused the cohesion of smaller particles to larger particles making it more difficult  
439 to sort (Beauchemin, 1991; Fish and DeVries, 2012; Leonardi et al., 2005).

440

441 4.5. Associative effects of particle size and production

442 Several authors have reported a relationship between *peNDF* and milk  
443 performance (Tafaj et al., 2007; Zebeli et al., 2012). In the current study there  
444 was also a positive relationship between *peNDF*<sub>>4mm</sub> or *peNDF*<sub>>8mm</sub> and milk fat  
445 content ( $R^2 = 0.14$  and  $R^2 = 0.16$ ;  $P < 0.01$ , respectively), but these were not as  
446 strong as with  $X_m$ , although due to the nature of the data caution should be  
447 exercised when interpreting the results. The positive relationship between  $X_m$  and  
448 milk fat content, and the negative relationship with milk yield is in agreement with  
449 De Brabander et al. (1999). A long fibrous particle size is associated with an  
450 increase of acetic acid production in the rumen that can subsequently lead to a  
451 higher milk fat content (Merten, 1997). Alternatively, a higher fibre ration may  
452 increase rumen pH and reduce the ruminal production of *trans*-10, *cis*-12  
453 conjugated linoleic acid that has been associated with milk fat reduction  
454 (Harvatiné and Bauman, 2011). Contrary to our findings, Tafaj et al. (2007)  
455 reported no correlation between particle size and milk yield or milk components  
456 and suggested that any effect of particle size on milk yield mainly depends on its  
457 influence on DM intake, which was not measured in the current study.

458

459 4.6. Comparison of fresh and dry separation

460 Compared with when measured fresh, the particle size distribution of dried  
461 forages and MR differed, with the proportion of longer fractions decreasing while  
462 short fractions increased after drying of samples (Kononoff et al., 2003). This  
463 difference may be attributed to the wetter forages and rations used resulting in  
464 adherence of short particles to larger particles, or the physical reduction in particle  
465 size due to the shaking when undertaken dry. It is therefore recommended to

466 partially or completely dry the forages and MR before analyses in order to  
467 overcome the moisture variation (Heinrichs, 2013). However, this may not be a  
468 practical way of measuring **particle size** of wetter forages and MR on-farm.

469

## 470 **5. Conclusions**

471 The **particle size** distribution of GS and MR based on GS in UK dairy herds was  
472 found to be considerably higher than current guidelines that are based on North  
473 American forages and rations. This suggests that the **particle size** of UK dairy  
474 rations is either too long, or that new guidelines or methods of **particle size**  
475 evaluation for GS and GS/MS based MR in Northern Europe are required. The  
476 poor consistency of mixing and high degree of selection recorded on the majority  
477 of herds is of concern, and further research into reasons for this variation and its  
478 impact on cow performance is required. Finally, the high use of concentrates by  
479 50% of the herds in the current study is a potential threat to SARA and reiterates  
480 the need for more appropriate means of **particle size** characterisation and  
481 guidelines for wetter, GS based dairy rations, with further controlled studies  
482 required to determine the optimal **particle size** distribution of these rations.

483

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619 Figure captions

620

621 **Figure 1.** Relationship between mean particle size of MR ( $X_m$ , mm) and milk fat  
622 (g/kg/herd) across 50 herds containing Holstein Friesian (HF; ●=36), Ayrshire  
623 (▲=2), Jersey (+=1), Brown Swiss (■=1) and Holstein crossbred (HFX; ◆=10).

624

625 **Figure 2.** Relationship between mean particle size of MR ( $X_m$ , mm) and energy  
626 corrected milk (ECM; Sjaunja et al., 1991) across 50 herds containing Holstein  
627 Friesian (HF; ●=36), Ayrshire (▲=2), Jersey (+=1), Brown Swiss (■=1) and  
628 Holstein crossbred (HFX; ◆=10).