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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 physical 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) × [(38.3 × fat
201 (g/kg) + 24.2 × protein (g/kg) + 15.71 × 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 ($pef_{>8mm}$) or 4 mm ($pef_{>4mm}$, Lammers et al., 1996; Maulfair and Heinrichs, 2010).

206 The $peNDF_{>4mm}$ was calculated by multiplying the NDF content (% DM) of the MR
207 by the $pef_{>4mm}$, and $peNDF_{>8mm}$ by multiplying the NDF content (% DM) of the MR
208 by the $pef_{>8mm}$ (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 Xm
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). In a survey of Iranian herds, Esmaeili et al. (2016) reported a high
410 variability (CV >10%) in **particle size** distribution of MR with the highest variation
411 recorded for the >19 mm fraction, a finding in agreement with the current study.
412 There were 42% of herds that had a CV \leq 5% (indicating a well-mixed ration),
413 26% that had a CV of between 5-10% (moderately mixed), and 32% that had a
414 CV >10% (poorly mixed ration). There was no effect of mixer model on overall
415 ration variability across all herds. In contrast, Heinrichs et al. (1999) reported that
416 MR processing by the mixer wagon can have a significant effect on the ration

417 consistency, **particle size** and *peNDF* concentrations of the ration subsequently
418 consumed.

419

420 *4.4. Herd level diet selection*

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

438

439 *4.5. Associative effects of particle size and production*

440 Several authors have reported a relationship between *peNDF* and milk
441 performance (Tafaj et al., 2007; Zebeli et al., 2012). In the current study there

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

456

457 4.6. Comparison of fresh and dry separation

458 Compared with when measured fresh, the particle size distribution of dried
459 forages and MR differed, with the proportion of longer fractions decreasing while
460 short fractions increased after drying of samples (Kononoff et al., 2003). This
461 difference may be attributed to the wetter forages and rations used resulting in
462 adherence of short particles to larger particles, or the physical reduction in particle
463 size due to the shaking when undertaken dry. It is therefore recommended to
464 partially or completely dry the forages and MR before analyses in order to
465 overcome the moisture variation (Heinrichs, 2013). However, this may not be a
466 practical way of measuring particle size of wetter forages and MR on-farm.

467

468 **5. Conclusions**

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

481

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615 Figure captions

616

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

620

621 **Figure 2.** Relationship between mean particle size of MR (X_m , mm) and energy
622 corrected milk (ECM; Tyrrel and Reid, 1965) across 50 herds containing Holstein
623 Friesian (HF; ●=36), Ayrshire (▲=2), Jersey (+=1), Brown Swiss (■=1) and
624 Holstein crossbred (HFX; ◆=10).

Figure 1.

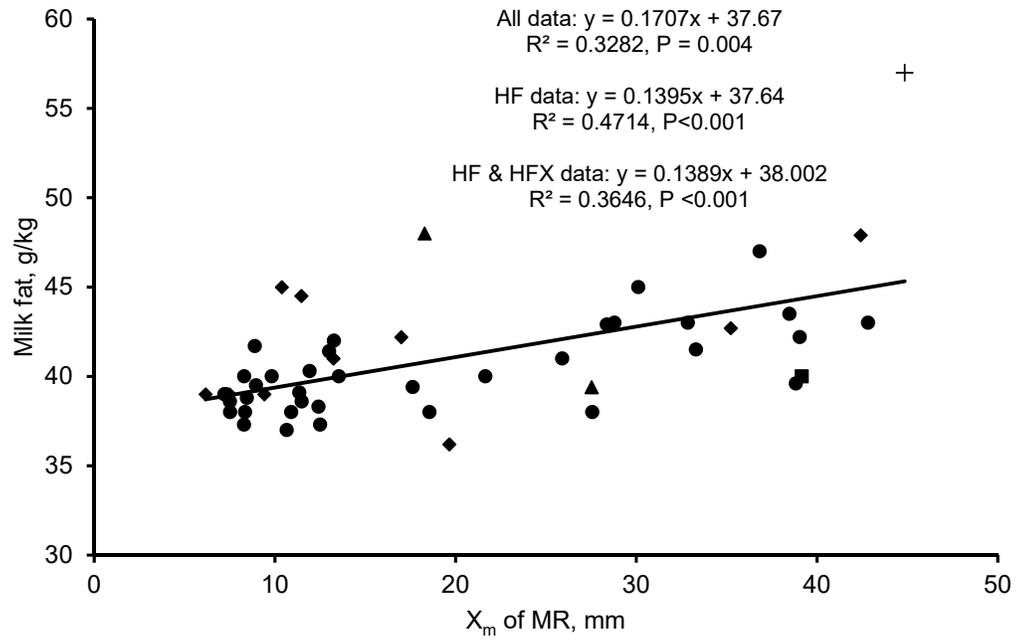


Figure 2.

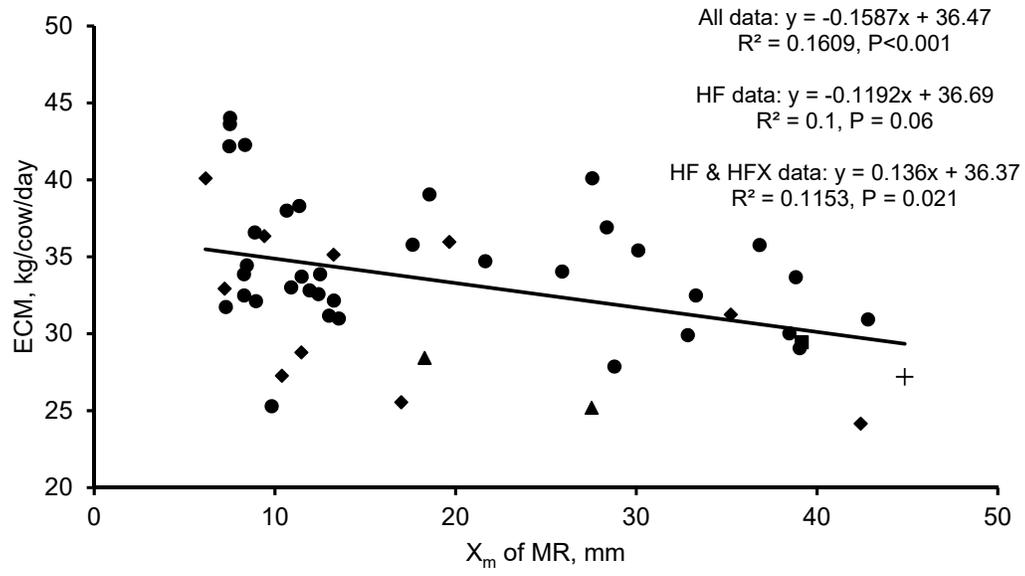


Table 1. Herd and feeding characteristics on 50 UK dairy herds.

| | Mean | SD | Min | Max | Median |
|--|-------|--------|------|-------|--------|
| Herd size (n) | 354 | 343.9 | 75 | 2220 | 277 |
| Cows in milk (n) | 310 | 282.3 | 67 | 1770 | 240 |
| Milk yield (kg/cow/year) | 9199 | 1583.2 | 6000 | 12500 | 9200 |
| Milk fat (g/kg) | 41.0 | 0.36 | 36.2 | 57.0 | 40.0 |
| Milk protein (g/kg) | 32.9 | 0.21 | 29.3 | 41.0 | 32.8 |
| ECM yield (kg/cow/year) ¹ | 10011 | 1434.9 | 7248 | 13209 | 9891 |
| Frequency of fresh feed delivery (n/d) | 1.3 | 0.46 | 1 | 2 | 1.0 |
| Frequency of feed push up (n/d) ² | 4.7 | 3.19 | 1 | 16 | 4.0 |
| Frequency of refusals removal (n/wk) | 4.4 | 2.75 | 0.25 | 7 | 5.5 |
| Feed space per cow (m/cow) | 0.56 | 0.098 | 0.30 | 0.76 | 0.61 |
| Length of feed mixing (min/mix) | 19 | 10.2 | 5 | 60 | 15 |
| No. of chews/bolus | 66 | 9.81 | 44 | 105 | 66 |

¹Energy corrected milk.

²Herds feeding into a trough (n = 20) have been excluded.

Table 2. Mean chemical (g/kg DM \pm SD) and physical characteristics (%DM \pm SD) of grass (n = 50) and maize silage (n = 34) on 50 dairy herds.

| Chemical composition | Grass silage | | | Maize silage | | | SED | P value |
|--|-------------------------------|------|------|------------------------------|------|------|------|---------|
| | Mean | Min | Max | Mean | Min | Max | | |
| Dry matter (g/kg) | 273 \pm 46.1 | 205 | 390 | 300 \pm 55.8 | 219 | 420 | 11.2 | 0.022 |
| Organic matter | 899 \pm 20.0 | 854 | 945 | 961 \pm 7.1 | 942 | 974 | 3.6 | < 0.001 |
| Ash | 101 \pm 20.0 | 55 | 146 | 39 \pm 7.1 | 26 | 58 | 3.6 | < 0.001 |
| Crude protein | 136 \pm 26.0 | 81 | 184 | 82 \pm 9.3 | 56 | 98 | 4.7 | < 0.001 |
| Neutral detergent fibre | 492 \pm 75.0 | 362 | 702 | 427 \pm 74.1 | 276 | 559 | 16.8 | < 0.001 |
| Acid detergent fibre | 331 \pm 41.9 | 242 | 459 | 267 \pm 44.8 | 176 | 347 | 9.7 | < 0.001 |
| Physical composition | | | | | | | | |
| Fractions (mm) ¹ | | | | | | | | |
| >60 | 2.1 \pm 5.12 ^a | 0 | 31.8 | - | - | - | ND | ND |
| 44-60 | 23.1 \pm 13.38 ^c | 0 | 53.5 | - | - | - | ND | ND |
| 26.9-44 | 51.6 \pm 14.01 ^d | 5.9 | 77.2 | - | - | - | ND | ND |
| 19-26.9 | 3.5 \pm 3.29 ^a | 0.7 | 20.5 | 6.9 \pm 4.55 ^a | 2.0 | 22.8 | 0.75 | < 0.001 |
| 8-19 | 15.8 \pm 10.01 ^b | 0.8 | 39.8 | 73.2 \pm 8.75 ^d | 37.7 | 84.1 | 2.02 | < 0.001 |
| 4-8 | 2.4 \pm 1.44 ^a | 0.6 | 6.9 | 13.1 \pm 5.02 ^c | 7.7 | 33.1 | 0.77 | < 0.001 |
| <4 | 1.6 \pm 1.35 ^a | 0.1 | 6.0 | 6.8 \pm 4.14 ^a | 1.4 | 18.8 | 0.64 | < 0.001 |
| <i>pef</i> _{>4mm} (%) ² | 98 \pm 1.5 | 93 | 100 | 93 \pm 4.1 | 81 | 99 | 0.6 | < 0.001 |
| peNDF _{>4mm} (%) | 48 \pm 7.0 | 36 | 66 | 40 \pm 7.7 | 24 | 54 | 1.7 | < 0.001 |
| <i>pef</i> _{>8mm} (%) ³ | 96 \pm 3.1 | 86 | 100 | 80 \pm 8.0 | 48 | 90 | 1.3 | < 0.001 |
| peNDF _{>8mm} (%) | 47 \pm 6.7 | 35 | 62 | 34 \pm 7.7 | 19 | 48 | 1.6 | < 0.001 |
| <i>X</i> _m ⁴ | 42.6 \pm 5.63 | 17.5 | 53.9 | 10.6 \pm 1.21 | 7.4 | 13.6 | 0.98 | < 0.001 |

DM = dry matter, SD = standard deviation, SED = standard error of difference

¹Grass silage was separated into 7 fractions; >60, 44-60, 26.9-44, 19-26.9, 8-19, 4-8 and <4 mm. Maize silage was separated into 4 fractions; >19, 8-19, 4-8 and <4 mm.

^{a,b,c,d} Within each forage, different superscripts between fractions indicate a significant ($P < 0.05$) difference.

²Physical effective factor; % proportion of particles >4 mm.

³Physical effective factor; % proportion of particles >8 mm.

⁴Geometric mean particle size.

Table 3. Mean chemical composition and physical characteristics of mixed rations (MR) on 50 herds.

| | Fresh basis | DM basis | | |
|--|------------------|----------|------|--------|
| Forage (kg/cow/d) | 40.5 | 12.2 | | |
| Concentrate (kg/cow/d) ¹ | 11.7 | 9.5 | | |
| Forage to concentrate ratio (F:C) ¹ | 77:23 | 57:43 | | |
| Grass to maize silage ratio (GS:MS) ² | 50:50 | 48:52 | | |
| Composition (g/kg DM \pm SD) ¹ | Mean | Min | Max | Median |
| Dry matter (g/kg) | 373 \pm 78.6 | 213 | 544 | 380 |
| Organic matter | 920 \pm 11.5 | 883 | 944 | 922 |
| Ash | 80 \pm 11.5 | 56 | 117 | 78 |
| Crude protein | 160 \pm 18.9 | 116 | 205 | 162 |
| Ether extract | 28 \pm 8.2 | 11 | 40 | 30 |
| Starch | 138 \pm 44.1 | 63 | 237 | 139 |
| Neutral detergent fibre | 391 \pm 59.3 | 290 | 507 | 381 |
| Acid detergent fibre | 249 \pm 42.6 | 173 | 329 | 245 |
| Physical composition (%DM \pm SD) | | | | |
| Fractions (mm) ³ | | | | |
| >60 | 0.1 \pm 0.29 | 0 | 1.4 | 0 |
| 44-60 | 7.3 \pm 9.27 | 0 | 32.8 | 2.4 |
| 26.9-44 | 26.0 \pm 15.10 | 1.6 | 75.9 | 24.7 |
| 19-26.9 | 4.4 \pm 3.38 | 0.9 | 21.8 | 3.7 |
| 8-19 | 34.9 \pm 13.31 | 3.5 | 67.8 | 34.9 |
| 4-8 | 11.8 \pm 5.58 | 0.9 | 29.6 | 10.9 |
| <4 | 15.5 \pm 9.72 | 0.4 | 37.4 | 14.9 |
| <i>pef</i> _{>4mm} (%) ⁴ | 85 \pm 9.6 | 63 | 100 | 85 |
| <i>peNDF</i> _{>4mm} (%) | 33 \pm 6.8 | 22 | 47 | 33 |
| <i>pef</i> _{>8mm} (%) ⁵ | 73 \pm 12.9 | 44 | 99 | 70 |
| <i>peNDF</i> _{>8mm} (%) | 29 \pm 7.3 | 16 | 43 | 28 |
| <i>X_m</i> ⁶ | 19.5 \pm 12.09 | 6.2 | 44.9 | 13.3 |

¹Includes the concentrates offered in the parlour.

²Ratio of GS to MS in 34 herds, where both silages were fed.

³Rations were separated into 7 fractions; >60, 44-60, 26.9-44, 19-26.9, 8-19, 4-8 and <4 mm; SED = 2.72 and *P* < 0.001.

⁴Physical effective factor; % proportion of particles >4 mm.

⁵Physical effective factor; % proportion of particles >8 mm.

⁶Geometric mean particle size.

Table 4. Particle size distribution of mixed rations (0hMR) at feed out containing grass silage (16) and mixtures of grass and maize silage (34) on 50 herds.

| Fractions ¹ (mm) | Particle size distribution (%DM) | | SED | <i>P</i> value |
|-----------------------------|----------------------------------|-------|------|----------------|
| | GS | GS+MS | | |
| >60 | 0.1 | 0.1 | 0.08 | 0.55 |
| 44-60 | 10.6 | 5.7 | 2.75 | 0.08 |
| 26.9-44 | 34.6 | 22.0 | 4.25 | < 0.01 |
| 19-26.9 | 3.5 | 4.8 | 1.01 | 0.22 |
| 8-19 | 26.4 | 39.0 | 3.65 | < 0.01 |
| 4-8 | 10.2 | 12.6 | 1.67 | 0.15 |
| <4 | 14.6 | 15.9 | 2.97 | 0.68 |
| X_m ² | 23.1 | 17.8 | 3.63 | 0.15 |

GS = grass silage, GS+MS= mixture of grass and maize silage, SED = standard error of difference

¹Rations were separated into seven fractions; >60, 44-60, 26.9-44, 19-26.9, 8-19, 4-8 and <4 mm.

²Geomatic mean particle size.

Table 5. Within farm standard deviation (SD) and coefficient of variation (CV) of particle fractions of mixed ration at 5 points along feed face on 50 dairy herds.

| Fractions ¹ (mm) | Mean ² | Standard deviation ³ | | | CV (%) ⁴ | | |
|--------------------------------|-------------------|---------------------------------|-----|------|---------------------|-----|------|
| | | Mean ± SD | Min | Max | Mean ± SD | Min | Max |
| >26.9 | 33.4 | 2.9 ± 2.28 | 0.1 | 10.8 | 13.7 ± 13.25 | 0.1 | 10.8 |
| 19-26.9 | 4.4 | 0.7 ± 1.16 | 0.1 | 7.7 | 15.0 ± 12.56 | 0.1 | 7.7 |
| 8-19 | 34.9 | 2.1 ± 1.60 | 0.0 | 7.9 | 7.3 ± 8.09 | 0.0 | 7.9 |
| 4-8 | 11.8 | 0.7 ± 0.53 | 0.1 | 2.9 | 6.4 ± 4.59 | 0.1 | 2.9 |
| <4 | 15.5 | 1.1 ± 1.26 | 0.1 | 5.9 | 8.0 ± 7.43 | 0.1 | 5.9 |

¹Ration was separated into five fractions; >26.9, 19-26.9, 8-19, 4-8 and <4 mm.

²Average particle size distribution of MR on 50 herds.

³SD of each fraction at 5 sampling points at each farm.

⁴CV = (SD of each fraction at 5 sampling points at each farm/ average value of each fraction) × 100.

Table 6. Group level sorting¹ (% ± SD) on 50 dairy herds.

| Fractions ² (mm) | Sorting | | |
|--------------------------------|------------|--------------------|--------------------|
| | 0-4h | 4-24h ³ | 0-24h ³ |
| >26.9 | 115 ± 59.5 | 158 ± 98.8 | 165 ± 113.0 |
| 19-26.9 | 101 ± 10.6 | 117 ± 47.8 | 106 ± 9.0 |
| 8-19 | 99 ± 28.0 | 92 ± 39.1 | 89 ± 32.4 |
| 4-8 | 99 ± 25.7 | 85 ± 32.5 | 83 ± 36.5 |
| <4 | 103 ± 52.8 | 96 ± 143.7 | 93 ± 83.6 |

¹Sorting was calculated for each fraction by dividing the proportion (DM basis) at 0hMR by the corresponding proportion at 4hMR and RefMR, and presented as a percentage. A sorting value of 100% indicated no sorting, <100% indicated preferential consumption, and >100% indicated selective refusal.

²Rations were separated into 5 fractions; >26.9, 19-26.9, 8-19, 4-8 and <4 mm.

³24h sorting activity was calculated across 33 herds, where refusals were available.

Table 7. Comparative particle size distribution of mixed rations (n = 50), grass silage (n = 50) and maize silage (n = 34) analysed by fresh and dry shaking on 50 dairy herds.

| Sample | Fractions ¹ (mm) | % Dry matter | | SED | P value |
|--------------|--------------------------------|--------------|------|------|---------|
| | | Fresh | Dry | | |
| Grass silage | >26.9 | 78.7 | 45.7 | 3.15 | < 0.001 |
| | 19-26.9 | 2.7 | 2.9 | 0.38 | 0.75 |
| | 8-19 | 14.3 | 34.4 | 2.16 | < 0.001 |
| | 4-8 | 2.6 | 10.1 | 0.63 | < 0.001 |
| | <4 | 1.7 | 6.9 | 0.57 | < 0.001 |
| Maize silage | >19 | 6.9 | 4.3 | 0.87 | 0.004 |
| | 8-19 | 73.2 | 52.6 | 2.37 | < 0.001 |
| | 4-8 | 13.1 | 28.8 | 1.53 | < 0.001 |
| | <4 | 6.8 | 14.3 | 1.28 | < 0.001 |
| Mixed ration | >26.9 | 32.8 | 16.2 | 4.08 | < 0.001 |
| | 19-26.9 | 4.4 | 3.5 | 0.54 | 0.10 |
| | 8-19 | 35.6 | 38.0 | 2.58 | 0.35 |
| | 4-8 | 12.2 | 21.2 | 1.25 | < 0.001 |
| | <4 | 15.0 | 21.1 | 1.88 | 0.002 |

¹MR and GS were separated into 5 fractions; >26.9, 19-26.9, 8-19, 4-8 and <4 mm. MS was separated into 4 fractions; >19, 8-19, 4-8 and <4 mm.