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1 **Grass silage particle size when fed with or without maize silage alters**
2 **performance, reticular pH and metabolism of Holstein-Friesian dairy cows**

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12 Short title: Effect of forage particle size on cow performance

13 **Abstract**

14 The particle size (PS) of the forage has been proposed as a key factor to ensure a
15 healthy rumen function and maintain dairy cow performance, but little work has been
16 conducted on ryegrass silage (GS). To determine the effect of chop length of GS and
17 grass silage to maize silage (MS) ratio on the performance, reticular pH, metabolism
18 and eating behaviour of dairy cows, 16 multiparous Holstein-Friesian cows were used
19 in a 4×4 Latin square design with four periods each of 28-days duration. Ryegrass
20 was harvested and ensiled at two mean chop lengths (short and long) and included at
21 two ratios of GS:MS (100:0 or 40:60 DM basis). The forages were fed in mixed rations
22 to produce four isonitrogenous and isoenergetic diets: long chop GS (**LG**); short chop
23 GS (**SG**); long chop GS and MS (**LM**); short chop GS and MS (**SM**). The DM intake
24 (DMI) was 3.2 kg/day higher ($P < 0.001$) when cows were fed the MS than the GS
25 based diets. The short chop length GS also resulted in a 0.9 kg/d DM higher ($P < 0.05$)
26 DMI compared to the long chop length. When fed the GS:MS based diets cows
27 produced 2.4 kg/day more ($P < 0.001$) milk than when fed diets containing GS only.
28 There was an interaction ($P < 0.05$) between chop length and forage ratio for milk
29 yield, with a short chop length GS increasing yield in cows fed GS but not MS based
30 diets. An interaction for DM and organic matter digestibility was also observed ($P <$
31 0.05), where a short chop length GS increased digestibility in cows when fed the GS
32 based diets but had little effect when fed the MS based diet. When fed the MS based
33 diets cows spent longer at reticular pH levels below pH 6.2 and pH 6.5 ($P < 0.01$), but
34 chop length had little effect. Cows when fed the MS based diets had a higher ($P <$
35 0.05) milk fat concentration of C18:2n-6 and total polyunsaturated fatty acids (FA)
36 compared to when fed the GS only diets. In conclusion, GS chop length had little effect
37 on reticular pH but a longer chop length reduced DMI and milk yield, but had little effect

38 on milk fat yield. Including MS reduced reticular pH, but increased DMI and milk
39 performance irrespective of the GS chop length.

40

41 **Key words:** chop length, forage ratio, milk production, particle size distribution,
42 ryegrass silage

43

44 **Implications**

45 Too short a forage chop length may lead to digestive upsets in dairy cows, whereas a
46 long chop length may reduce intake and performance. Dairy cows were fed short or
47 long chop grass silage either alone or mixed with maize silage. When fed the short
48 compared to the long chop grass silage the cows produced more milk, but there was
49 no effect on reticular pH. Intake, milk production and milk protein content were all
50 higher when cows were fed diets that contained both grass and maize silage.

51 **Introduction**

52 The increased milk production of dairy cows in many Western countries such as the
53 United Kingdom (UK) has required an increase in the level of concentrate
54 supplementation and the production of high quality forages, with a trend towards lower
55 dietary fibre levels (March *et al.*, 2014). The consequences of these dietary changes
56 include an increased risk of metabolic disorders such as sub-acute ruminal acidosis
57 (SARA), displaced abomasum, milk fat depression, laminitis, reduced fibre digestion
58 and fat cow syndrome (Plaizier *et al.*, 2008). The particle size (PS) of the diet has been
59 proposed as a key factor, along with forage fibre and non-forage carbohydrate
60 concentration to ensure a healthy rumen function and maintain animal performance
61 (Zebeli *et al.*, 2012). Additionally, optimal rumen fermentation can lead to an increase
62 in the microbial protein and metabolisable protein supply to the small intestine and
63 therefore enhance milk protein yield (Sinclair *et al.*, 2014).

64 A short forage PS when included in total mixed rations (TMR) based on lucerne and
65 maize silage has been shown to increase dry matter (DM) intake (DMI) and milk
66 protein yield (Tafaj *et al.*, 2007; Zebeli *et al.*, 2012), but may result in a reduction in
67 rumination, eating and total chewing time, as well as rumen pH (Tafaj *et al.*, 2007). In
68 contrast, a longer PS produced a higher milk fat content (Mertens, 1997), but can also
69 promote feed sorting, resulting in some cows receiving excess concentrates and
70 others insufficient (Kononoff and Heinrichs, 2003). However, the effects of PS in grass
71 silage (GS) based TMR on intake and milk production are inconsistent, mainly due to
72 differences in the PS and physically effective fibre (*peNDF*; particles long enough to
73 stimulate rumination, Mertens, 1997) measurement procedure.

74 In a recent study to determine the range of PS of grass and maize silages and TMR
75 fed to dairy cows on commercial farms in the UK (Tayyab *et al.*, 2017), it was reported

76 that the TMR fed on UK dairy herds had more longer (>19 mm) particles than
77 recommended for North American diets, and that the difference in PS distribution was
78 principally due to the inclusion of GS (Tayyab *et al.*, 2017). There is however, a lack
79 of information on the effects of PS of GS based diets on dairy cow performance.
80 Additionally, the greater inclusion of wheat and barley that are more commonly fed in
81 Europe (AHDB, 2017) and are rapidly degraded in the rumen (Offner *et al.*, 2003)
82 enhances the risk of SARA and increases the importance of PS and *peNDF*. The
83 hypothesis of the current study was that dairy cows fed diets with a short compared to
84 a long PS of GS when fed with or without maize silage (MS) would decrease rumen
85 pH and milk fat content, but increase intake and milk production. The objectives of the
86 study were to determine the effect of chop length of GS when fed at different ratios of
87 GS:MS on the intake, performance, reticular pH, diet digestibility, metabolism and
88 eating behaviour in Holstein-Friesian dairy cows.

89

90 **Materials and Methods**

91 *Animals, housing, forages, diets and experimental routine*

92 Sixteen early lactation (60 ± 10.6 days in milk) multiparous Holstein-Friesian dairy
93 cows producing 41.9 ± 3.86 kg (mean \pm SD) of milk per day and weighing 675 ± 60.9
94 kg at the beginning of the study were used in a 4×4 Latin square design with four
95 periods each of 28-days duration, with measurements undertaken during the final 12-
96 days of each period. At the start of the experiment cows were blocked according to
97 milk yield and randomly assigned to one of 4 dietary treatments. The cows were
98 housed in a building containing free stalls fitted with mattresses and had free access
99 to water.

100 A first cut perennial ryegrass (*Lolium perenne*) sward was mown at a leafy stage on
101 the 25th May 2016, wilted for 24 h and then alternate windrows harvested using a
102 precision chop self-propelled forage harvester (John Deere 7840i, Nottinghamshire
103 UK) at two different settings to provide a theoretical chop length of 10 mm (short chop)
104 or 44 mm (long chop). An additive (Axfast Gold, Biotal, Worcestershire, UK) was
105 applied at the rate of 2 litres per tonne to each GS which were ensiled in separate
106 roofed concrete clamps. Maize silage (*Zea mays*) was harvested on the 10th October
107 2016 using the same forage harvester as the GS to provide a theoretical chop length
108 of 15 mm. A silage additive (Maizecool Gold, Biotal, Worcestershire, UK) was applied
109 at 2 litres per tonne, and the MS ensiled in a concrete clamp.

110 The two GS (short or long) and two ratios of GS:MS (100:0 or 40:60 respectively, DM
111 basis) were used to formulate four diets (Table 1). The dietary treatments were: long
112 chop GS (**LG**); short chop GS (**SG**); long chop GS and MS (**LM**) and short chop GS
113 and MS (**SM**). All diets were fed as a TMR with a forage to concentrate ratio of 54:46
114 (DM basis) to provide a similar metabolisable energy and protein content (Thomas,
115 2004). Diet mixing and feeding protocol was adopted after Sinclair *et al.* (2015) using
116 16 Hokofarm roughage intake feeders (RIC feeders, Marknesse, Netherlands). Fresh
117 feed was offered daily at 1000 h at the rate of 1.05 of *ad-libitum* intake, with refusals
118 collected 3-times/week prior to feeding. Forages were sampled twice weekly; one
119 sample was oven dried at 105°C and the ratio of GS to MS adjusted to the desired
120 level, while the second sample was stored at -20°C for subsequent analysis. Samples
121 of all four TMR were collected daily during the final week of each period and stored at
122 -20°C for subsequent analysis.

123 Cows were milked twice daily at 0700 and 1700 h with milk yield recorded at each
124 milking and samples taken during the final week of each period (two morning and two

125 evening milkings) for subsequent analysis. Body condition score (BCS, Ferguson *et*
126 *al.*, 1994) and live weight were recorded after the evening milking during the week
127 prior to commencing the study and then at the end of each period. Whole tract
128 apparent digestibility was estimated using acid insoluble ash as an internal marker
129 (Van Keulen and Young, 1977) with faecal samples collected at 1000 and 1600 h for
130 five consecutive days during the final week of each period, and stored at -20°C prior
131 to subsequent analysis.

132 *Reticular pH and blood collection*

133 To determine reticular pH, pH boluses (eCow® Devon Ltd, Exeter Devon, UK) were
134 administered orally to all cows one week prior to data collection. Boluses were
135 calibrated prior to administration by immersing in warm water (39°C) for 30 min
136 according to the manufactures instructions. Data were recorded every 15 min, and
137 downloaded at the end of each period. A second set of pH boluses were administered
138 to all cows during the first week of the 3rd period to monitor reticular pH during periods
139 3 and 4. Blood samples were collected from 12 cows (3 per treatment) by jugular
140 venepuncture over 2-days during the collection week at 0900, 1100, 1230 and 1400
141 h, centrifuged at 3 000 g for 15 min, the plasma extracted and stored at -20°C prior to
142 subsequent analysis.

143 *Particle size distribution and eating behaviour*

144 The PS distribution of the fresh TMR was measured by collecting samples 5 min post-
145 feeding on days 20 to 25 of each period and using a modified Penn State Particle
146 Separator (PSPS) with 5 sieve screens of size 44, 26.9, 19, 8, and 4 mm (Tayyab *et*
147 *al.*, 2017). A manual shaking procedure was adopted (Kononoff *et al.*, 2003), and each
148 diet was separated into six fractions; >44, 26.9-44, 19-26.9, 8-19, 4-8 and <4 mm. Jaw
149 movement (eating, ruminating and idling) was visually recorded for 48 h commencing

150 at 0530 h on day-18 of each period by instantaneous scan monitoring of all cows at 5
151 min intervals (Martin and Bateson, 2007). All observers were trained for 1 h before the
152 start of the study with a 96% similarity index achieved. Observations were conducted
153 using 2 observers for a duration of 4 h to minimise fatigue and enhance accuracy
154 (Martin and Bateson, 2007).

155 *Chemical analysis*

156 Forage and TMR samples were analysed according to AOAC (2012) for DM (934.01),
157 CP (988.05; intra-assay CV of 2.3%) and ash (942.05), while NDF (using heat-stable
158 α -amylase; Sigma, Gillingham, UK), ADF and ADL were analysed according to Van
159 Soest *et al.* (1991) and expressed exclusive of residual ash (intra-assay CV of 1.4 and
160 1.3% for NDF and ADF respectively). Starch concentration was analysed using the
161 procedure described by McCleary *et al.* (1997). Milk samples were analysed using a
162 Milkoscan Minor analyser (Foss, Denmark). Plasma samples were analysed for
163 glucose, β -hydroxybutyrate (3-OHB) and urea (Randox Laboratories, County Antrim,
164 UK; kit catalogue no. GL1611, RB1008 and UR221 with an intra-assay CV of 0.6, 4.5
165 and 2.3%, respectively) using a Cobas Miras Plus autoanalyser (ABX Diagnostics,
166 Bedfordshire, UK). Faecal samples were pooled for each cow within each period, dried
167 and analysed for acid insoluble ash (Van Keulen and Young, 1977), nitrogen, NDF
168 and ADF. Forage pH was determined using a pH meter (HI 2210, Hanna Instruments,
169 Bedfordshire UK) after suspending 50 g forage in 100 ml distilled water for 30 min.
170 Milk and feed fatty acids (FA) analysis are provided in the Supplementary Material S1.

171 *Calculations and statistical analysis*

172 Calculations for forage PS are presented in the Supplementary Material S2. All data
173 were tested for normality using the general descriptive statistics and analysed as a
174 Latin Square design with a 2 x 2 factorial treatment structure using GenStat 17.1 (VSN

175 International Ltd., Oxford, UK), with main effects of chop length (C), forage ratio (F)
176 and their interaction (C × F). The model used was: $Y = \mu + C_i + F_j + C \times F_{ij} + P_j + A_k +$
177 ϵ_{ijk} , where Y is the observation, μ the overall mean, C_i is the chop length effect, F_j is
178 the forage ratio effect, $C \times F_{ij}$ is the interaction between chop length and forage ratio, P_j
179 the fixed period effect, A_k the random effect of animal and ϵ_{ijk} the residual error. Blood
180 plasma, rumen pH and sorting activity data were analysed as repeated measures
181 ANOVA. Results were reported as treatment means with SED, with the level of
182 significance set at $P < 0.05$ and a tendency stated at $P < 0.1$.

183

184 **Results**

185 Preliminary results of this study have previously been presented (Tayyab et al., 2018).

186 *Forage and feed composition*

187 The nutrient composition of the long and short chop GS were similar with a mean DM,
188 CP and NDF concentration of 201 g/kg, 121 and 487 g/kg DM respectively, whilst the
189 GS had a lower DM concentration, but a higher NDF and CP concentration than the
190 MS (Table 2). The mean particle size (X_m) of the long chop GS was 13.3 mm more
191 than the short GS, with the MS having the shortest X_m . The MS based diets (LM and
192 SM) had a higher DM compared to the GS based diets (LG and SG), but all four diets
193 had a similar CP content, with a mean value of 174 g/kg DM. The GS based diets had
194 a higher ash, NDF and ADF content compared to the MS based diets. The mean X_m
195 of the GS based diets was 10.5 mm greater than the MS based diets, and was 9 mm
196 less for the short chop than the long chop GS based diets. The $peNDF_{>4mm}$ was also
197 higher for the GS than the MS based diets.

198 *Production performance*

199 Average DMI was 3.2 kg/day lower ($P < 0.001$) in cows when fed the GS than the MS
200 based diets (Table 3). The short chop length diets resulted in a 0.9 kg DM/day higher
201 ($P = 0.035$) intake in cows compared to the long chop length diet. Cows fed the GS
202 based diets produced 2.4 kg/day less ($P < 0.001$) milk than when fed diets containing
203 grass and maize silages (Table 3). There was an interaction ($P = 0.011$) between chop
204 length and forage ratio on milk yield, with a short chop length increasing yield in cows
205 when fed GS but not MS based diets. There was a tendency ($P = 0.09$) for a higher
206 milk fat content in cows when fed the long chop length diets. Live weight change was
207 0.85 kg/day higher ($P < 0.001$) in cows when fed the MS compared to the GS based
208 diets, and there was a tendency ($P = 0.065$) for a lower live weight gain in cows when
209 fed long chop compared to the short chop length diets.

210 *Whole tract digestibility*

211 There was an interaction for DM ($P = 0.019$) and OM ($P = 0.022$) digestibility, where
212 the short chop length increased digestibility in cows when fed the GS but not the MS
213 based diets (Table 4). There was also an interaction ($P = 0.003$) for N digestibility,
214 where a short chop length increased N digestibility when cows were fed the GS based
215 diets, and decreased digestibility when fed the MS based diet. Digestibility of NDF was
216 0.228 kg/kg higher ($P < 0.001$) in cows when fed the GS compared to the MS based
217 diets, and there was an interaction ($P = 0.014$) between chop length and forage ratio
218 on ADF digestibility, where a shorter chop length GS increased digestibility for the GS
219 based diet, and decreased digestibility for the MS based diet.

220 *Reticular pH and eating behaviour*

221 Reticular pH was highest prior to the morning feeding in all treatments and then
222 declined with time ($P < 0.001$; Figure 1). There was a time x forage ratio interaction on
223 reticular pH, which was lower in cows fed MS for most of the day except around fresh

224 feed delivery, but there was no effect of GS chop length. When cows were fed the GS
225 based diets the mean minimum reticular pH was 0.1 higher ($P = 0.001$) than when fed
226 the MS based diet (Table 5). Cows fed the MS based diets also spent a longer time at
227 reticular pH levels below pH 6.2 and 6.5 ($P = 0.003$) compared to the GS based diets.
228 Cows spent 1.1 h/day longer eating ($P < 0.001$) when offered the GS compared to the
229 MS based diets and 0.9 h/day longer ($P = 0.003$) eating the long chop compared to
230 the short chop GS (Table 5). Similarly, eating time (ET) was 4.7 min/kg DM higher
231 when cows were fed the GS compared to the MS based diets ($P < 0.001$), and 2.4
232 min/kg DMI higher ($P < 0.05$) when fed the longer compared to the shorter GS. There
233 was an interaction ($P < 0.05$) for rumination time (RT; h/day), with the shorter chop
234 length increasing the RT in cows when fed the GS but not the MS based diets, whereas
235 when expressed on a min/kg DMI, a shorter chop length increased RT on the GS and
236 decreased RT on the MS based diets. The PS distribution of fractions 8-19 and 4-8
237 mm decreased ($P < 0.05$) with time post-feeding, and the DM proportion of the 26.9-
238 44 mm fraction was higher ($P < 0.001$) for diets that contained long chop GS or when
239 mixed with MS (Supplementary Table S1).

240 *Milk fatty acids and blood metabolites*

241 Cows fed the short chop length diets had a 0.04 g/100g higher milk fat C18:3n-3
242 concentration ($P < 0.001$), whereas, those receiving the long chop length diets had a
243 0.05 g/100g higher concentration of *cis*-9, *trans*-11 conjugated linoleic acid (CLA; $P =$
244 0.032; Supplementary Table S2). For cows fed the GS based diets, milk
245 concentrations of C16:0, C16:1n-7, C18:1c9 and C18:3n-3 were higher ($P < 0.05$),
246 compared to when the MS based diets were fed. In contrast, milk from cows fed the
247 MS based diets had a higher ($P < 0.01$) concentration of C10:0, C12:0, C14:1, C18:0,

248 C18:1 *trans*-8, C18:1 *trans*-9, C18:1 *trans*-12, C18:2n-6 and total polyunsaturated FA (P
249 = 0.015) compared to when fed the GS based diets.

250 Plasma glucose concentration decreased ($P < 0.001$) post feeding (Figure 2a) and
251 was 0.17 mmol/l higher ($P = 0.008$) in cows when fed the MS compared to the GS
252 based diets. Plasma 3-OHB concentrations increased ($P < 0.001$) with time post-
253 feeding, but there was no effect of chop length or forage ratio (Figure 2b). Similarly,
254 plasma urea concentration increased ($P = 0.004$) post-feeding to a maximum at 1230
255 h, with cows fed the MS based diets having a 0.86 mmol/l higher ($P < 0.001$)
256 concentration than when fed the GS based diet (Figure 2c).

257

258 **Discussion**

259 *Nutrient composition and particle length*

260 The current study was conducted to determine the effect of chop length of GS when
261 fed alone or mixed with MS on cow performance, rumen pH, eating behaviour and
262 blood metabolites. The PS of the long chop length GS and MS used in the current
263 study were similar to the mean values fed on UK dairy farms reported by Tayyab *et al.*
264 (2017; 43 and 11 mm respectively), whereas the short chop length GS was within the
265 shortest 5% of the GS surveyed. The DM of the GS was lower than typically reported
266 for 1st cut ryegrass silages (Sinclair *et al.*, 2015), although the chemical composition
267 of both chop length GS was similar, a finding in agreement with previous studies that
268 have altered forage chop length prior to ensiling (Kononoff and Heinrichs, 2003; Yang
269 and Beauchemin, 2007). The lactic acid content was however, higher and the acetic
270 acid content lower in the short chop compared to the long chop length GS, a finding in
271 agreement with others who have reported that a shorter chop length can enhance

272 consolidation in the clamp and improve the fermentation profile (McDonald *et al.*,
273 1991).

274 *Animal performance*

275 The increase in DMI when cows were fed the MS compared to the GS based diets is
276 in agreement with previous studies that have investigated the effect of including MS
277 (Hart *et al.*, 2015; O'Mara *et al.*, 1998). Mulligan *et al.* (2002) reported an increase in
278 intake of 3.5 kg/d DM when GS was replaced by MS in the diet of late lactation dairy
279 cows, whereas a linear increase in DMI was observed when MS replaced GS in the
280 diet of mid-lactation dairy cows (Kliem *et al.*, 2008). However, a higher acetate content
281 of the long chop GS coupled with its low DM content may have resulted in a lower
282 quality and subsequently decreased DMI and production (McDonald *et al.*, 1991).
283 Feeding cows with diets containing a short chop length GS increased DMI in the
284 current study, possibly due to less time required for chewing prior to swallowing, a
285 finding in accordance with other studies that have investigated the effect of chop length
286 (MS or alfalfa) on DMI in dairy cows (Nasrollahi *et al.*, 2015). The increase in the DMI
287 of cows fed the short chop length diets in the current study could be attributed to a
288 reduced rumen fill and lower rumen retention time, both of which are associated with
289 an increased intake (Zebeli *et al.*, 2007).

290 The current finding of a higher milk yield in cows when MS replaced GS is in
291 agreement with O'Mara *et al.* (1998) and Hart *et al.* (2015), and is most likely to be the
292 result of the higher DMI in cows fed the MS based diets. There was an interaction
293 between chop length and forage ratio on milk yield in the current study, with a short
294 chop length GS increasing yield in cows when GS was the sole forage, but not when
295 GS was fed along with MS. This difference may be explained by the mean PS of the
296 diets, with the LG diet having a substantially longer PS than any of the other 3 diets.

297 Longer particles in LG may have passed out of the rumen at a slower rate, resulting in
298 a lower DMI and subsequent milk production (Kononoff and Heinrichs, 2003; Zebeli *et*
299 *al.*, 2012). Milk fat production was not affected by chop length in the current study,
300 possible due to a sufficient dietary $peNDF_{>4mm}$ content of all four diets (minimum of
301 26%), as it has been suggested that milk fat content is only influenced by chop length
302 when dietary $peNDF$ levels are lower than the recommended level of 18-22% DM
303 (Zebeli *et al.*, 2012). Cows receiving the MS based diets in the present study gained
304 live weight whereas when they received the GS based diets they lost weight, which
305 may be attributed to differences in DM and ME intake as a consequence of feeding
306 mixed forage diets as suggested by O'Mara *et al.* (1998). In contrast, chop length did
307 not significantly alter body weight or body weight change, a finding in agreement with
308 that reported by Kononoff and Heinrichs (2003), and reflects that in the current study
309 DMI was less affected by chop length than the GS:MS ratio.

310 The digestibility co-efficients of the dietary components in the current study were
311 similar to previous studies that have evaluated GS and MS in the diet of dairy cows
312 (Sinclair *et al.*, 2015). In a review of the literature Khan *et al.* (2015) concluded that
313 increasing stage of maturity was one of the major factors influencing fibre digestibility
314 in MS, and the comparatively high DM of the MS used in the current study (350 g/kg
315 DM) may have resulted in a more resistant fibre structure, reducing the digestibility of
316 the fibre in the MS compared to the GS diets. Alternatively, the decreased rumen pH
317 due to the higher concentration of non-structural carbohydrates in the MS diets may
318 have had a negative impact on the fibre degrading microbiota, decreasing diet
319 digestibility (Nasrollahi *et al.*, 2015; Tafaj *et al.*, 2007).

320 *Reticular pH and eating behaviour*

321 Similar to previous studies (Yang and Beauchemin, 2007), the highest reticular pH
322 was recorded prior to feeding, with a nadir reached at approximately 9 h after fresh
323 feed delivery. Cows fed the MS compared to the GS based diets had a lower mean
324 and minimum reticular pH, which may be associated with the higher concentration of
325 starch and lower concentration of $peNDF_{>8mm}$ in the MS diets (130 vs 199 g starch/kg
326 DM and 27.1 vs 19.1% $peNDF_{>8mm}$, for the GS and MS based diets respectively). In
327 contrast, chop length had no effect on reticular pH, a finding in agreement with Tafaj
328 *et al.* (2007). In contrast, Yang and Beauchemin (2007) reported an increase in mean
329 rumen pH when a longer chop length forage was fed, although the results were based
330 on lucerne silage rather than the ryegrass silage used in the current study.

331 Chop length did influence eating time in the current study, with cows spending more
332 time eating the long than the short chop diets, a finding in agreement with Kammes
333 and Allen (2012) who reported a tendency for a longer daily eating time when cows
334 were offered a long versus short chop length orchard grass. Kammes and Allen (2012)
335 reported no effect of chop length on ruminating time, but in the current study the effect
336 of chop length was unclear, with a decrease in ruminating time per kg DMI in cows
337 when fed GS, and increase when fed the MS based diets, although there was a clear
338 effect of forage source, with cows fed the MS diets (which had the shortest PS),
339 spending significantly less time ruminating.

340 *Metabolism and milk fatty acids*

341 The higher plasma glucose concentration in cows fed the MS diets in the current study
342 may be due to the higher dietary content of sugar and starch (Oba and Allen, 2003),
343 whereas the lower plasma urea concentration in cows fed the GS based diets may
344 reflect a lower content of rumen degradable N as a greater proportion of dietary N was
345 from rumen-protected protein sources in these diets, although all diets were

346 formulated to have a similar excess of rumen degradable nitrogen. Alternatively, the
347 GS based diets may have resulted in a more suitable rumen microbial environment for
348 the capture of degraded N, as demonstrated by the higher reticular pH.
349 Overall, the inclusion of MS in the diet altered the FA profiles of the milk more than the
350 GS chop length. Chilliard *et al.* (2000) reviewed the literature on diet and milk FA profile
351 and concluded that cows fed MS based diets had a higher concentration of C10:0,
352 C12:0 and C18:2n-6, due to the higher concentrations in MS compared to GS, a finding
353 in agreement with the current results. Hart *et al.* (2015) also reported a 0.99 g/100g
354 higher milk fat content of C16:0 in cows when fed a 70:30 (DM basis) grass to MS
355 based diets compared to those receiving a 30:70 GS:MS diets, a finding in agreement
356 with the current findings. Soita *et al.* (2005) reported no effect of chop length on milk
357 FA, but in the current study a shorter GS chop length increased the milk fat proportion
358 of C18:3n-3, which may be related to a lower rate and extent of biohydrogenation in
359 the rumen, possibly due to a shorter rumen retention time.

360

361 **Conclusions**

362 The short chop length grass silage used in the current study was within the shortest
363 5% of that fed in the UK but had no effect on reticular pH compared to an average
364 chop length grass silage, but increased intake and milk performance when fed as the
365 sole forage. Milk performance can also benefit from replacing a proportion of grass
366 silage with maize silage in a TMR when fed to high producing dairy cows, irrespective
367 of the chop length of the grass silage, but with a reduction in reticular pH and fibre
368 digestion. The effects of a shorter chop length grass silage when fed at a high
369 concentrate to forage ratio, or with a greater dietary content of rapidly fermentable
370 starch, requires further investigation.

371

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376

377 **Declaration of interest**

378 None.

379

380 **Ethics statement**

381 All the procedures involving animals were conducted in accordance with the UK
382 Animals Scientific Procedures Act (1986; amended 2012) and received local ethical
383 approval.

384

385 **Software and data repository resources**

386 None.

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Table 1 Dietary inclusion (kg/kg DM) and predicted nutrient composition for diets fed to cows that contained long chop grass silage (LG); short chop grass silage (SG); long chop grass and maize silages (LM), or short chop grass and maize silages (SM).

Ingredients	LG	SG	LM	SM
Maize silage	0	0	0.323	0.323
Short grass silage	0	0.537	0	0.214
Long grass silage	0.537	0	0.214	0
Rapeseed meal	0.017	0.017	0.064	0.064
Wheat distillers	0.017	0.017	0.064	0.064
Palm kernel cake	0.005	0.005	0.018	0.018
Molasses	0.001	0.001	0.005	0.005
Caustic wheat	0.175	0.175	0.122	0.122
Soya hulls	0.105	0.105	0.083	0.083
Soya bean meal	0.055	0.055	0.086	0.086
Megalac ¹	0.015	0.015	0.004	0.004
Sopralin ²	0.068	0.068	0.009	0.009
Minerals/vitamins ³	0.007	0.007	0.007	0.007
Predicted composition				
ME (MJ/kg DM) ⁴	12.0	12.0	12.1	12.1
MPN (g/kg DM) ⁵	121	121	119	119
MPE (g/kg DM) ⁶	103	103	103	103

¹A rumen protected source of fat (Volac, Royston, UK).

²A rumen protected source of soybean (NWF Agriculture, Cheshire, UK).

³Mineral/vitamins premix (KW Alternative Feeds, Leeds, UK) providing (g/kg) 220 calcium, 30 phosphorus, 80 magnesium, 80 sodium, (mg/kg) 760 copper, 30 selenium, 1000000 IU vitamin A, 300000 IU vitamin D₃, 3,000 IU vitamin E, 2.5 mg/kg vitamin B₁₂, 135 mg/kg biotin.

⁴ME, metabolisable energy.

⁵MPN, metabolisable protein-rumen nitrogen limited.

⁶MPE, metabolisable protein-rumen energy limited.

Table 2 Nutrient composition (g/kg DM), fatty acid profile and particle size of grass silage (long chop, LCG and short chop, SCG), maize silage (MS) and diets fed to cows that contained long chop grass silage (LG); short chop grass silage (SG); long chop grass and maize silages (LM) or short chop grass and maize silages (SM).

	LCG	SCG	MS	LG	SG	LM	SM
DM (g/kg)	198	204	350	308	307	368	380
CP	120	122	81	170	176	176	175
Ash	71	73	39	92	92	71	68
OM	929	927	961	908	908	929	932
NDF	484	490	366	392	384	342	339
ADF	327	331	229	256	261	211	209
ADL	-	-	-	24	25	29	28
Starch	-	-	291	127	133	201	197
ME (MJ/kg)	10.9	10.8	12.0				
<i>Fermentation characteristics (g/kg)</i>							
pH	4.13	4.06	3.80				
NH ₃ -N (g/kg total N)	71	68	62				
Acetate	62.6	26.5	34.6				
Propionate	0.3	0.1	1.1				
Iso-butyrate	0.0	0.0	0.1				
Butyrate	0.3	0.3	0.1				
Lactate	114	140	48				
<i>Fatty acids (g/100 FA)</i>							
C16:0	4.0	3.8	4.8	14.1	15.6	9.1	9.6
C18:0	0.5	0.4	1.2	1.5	1.7	1.3	1.4
C18:1c9	0.3	0.3	3.4	3.5	3.9	4.2	4.4
C18:2n-6	0.5	0.5	1.5	1.2	1.3	2.4	2.4
C18:3n-3	4.7	5.5	0.9	3.3	3.7	2.3	2.3
∑ FA	13.2	13.6	17.4	26.2	28.4	28.2	28.3
<i>Fractions (%DM)</i>							
>44 (mm)	28.6	4.1	-	15.6	-	0.1	-
26.9-44 (mm)	54.7	25.3	-	32.9	16.3	21.0	3.0
19-26.9 (mm)	3.9	5.7	14.0	4.9	4.5	3.7	3.3
8-19 (mm)	9.2	54.1	76.3	17.2	48.2	32.6	52.1
4-8 (mm)	2.3	8.5	8.3	17.1	18.7	19.5	19.6
<4 (mm)	1.3	2.3	1.4	12.3	12.3	23.1	21.9
X _m (mm)	44.2	30.9	12.8	26.9	10.4	8.9	7.5
SD _{gm}	1.15	1.89	1.57	2.5	2.3	2.7	2.2
pef _{>4mm} (%)	98.7	97.7	98.6	87.7	87.7	76.9	78.1
pef _{>8mm} (%)	96.4	89.2	90.3	70.6	69.0	57.4	58.5
peNDF _{>4mm} (%)	47.7	47.8	36.1	34.4	33.6	26.1	26.7
peNDF _{>8mm} (%)	46.6	43.7	33.0	27.7	26.5	19.5	20.0

OM = organic matter; NH₃-N = ammonia nitrogen; ME = metabolisable energy; FA = fatty acid; X_m = geometric mean particle size; SD_{gm} = SD of X_m; pef = physical effectiveness factor; peNDF = physically effective fibre

Table 3 Intake and performance of dairy cows fed diets containing long chop grass silage (LG); short chop grass silage (SG); long chop grass and maize silages (LM), or short chop grass and maize silages (SM).

	Treatments				SED	P-value		
	LG	SG	LM	SM		C	F	C × F
DM intake (kg/day)	20.0	20.5	22.8	24.0	0.56	0.035	<0.001	0.335
Milk yield (kg/day)	37.3	39.1	41.1	40.5	0.63	0.179	<0.001	0.011
4% FCM ¹ (kg/day)	37.3	37.5	40.1	38.9	1.11	0.477	0.012	0.376
Milk fat (g/kg)	40.1	38.5	39.5	38.6	0.93	0.090	0.560	0.418
Milk fat (kg/day)	1.49	1.50	1.60	1.55	0.044	0.477	0.012	0.376
Milk protein (g/kg)	30.9	30.7	32.3	32.4	0.28	0.738	<0.001	0.461
Milk protein (kg/day)	1.16	1.20	1.33	1.31	0.023	0.432	<0.001	0.085
Milk lactose (g/kg)	45.8	46.2	45.5	45.7	0.26	0.095	0.058	0.709
Milk lactose (kg/day)	1.72	1.81	1.87	1.85	0.033	0.122	<0.001	0.029
Live weight (kg)	668	671	683	693	4.6	0.065	<0.001	0.339
Live weight change (kg/day) ¹	-0.35	-0.41	0.15	0.79	0.277	0.144	<0.001	0.078
Body condition score	2.41	2.52	2.51	2.74	0.060	<0.001	<0.001	0.138
Body condition score change ²	-0.07	-0.09	-0.12	0.16	0.120	0.145	0.256	0.088

C = chop length; F = grass to maize silage ratio; C×F = interaction between C and F; ¹FCM = fat corrected milk

²Change over the 28-day period.

Table 4 Diet digestibility (kg/kg) in dairy cows fed diets containing long chop grass silage (LG); short chop grass silage (SG); long chop grass and maize silages (LM), or short chop grass and maize silages (SM).

	Treatments				SED	P-value		
	LG	SG	LM	SM		C	F	C × F
DM	0.659	0.739	0.639	0.629	0.0257	0.063	0.001	0.019
OM	0.677	0.754	0.656	0.645	0.0262	0.084	0.001	0.022
N	0.709	0.772	0.737	0.719	0.0177	0.082	0.326	0.003
NDF	0.614	0.666	0.418	0.407	0.0290	0.323	<0.001	0.140
ADF	0.582	0.681	0.417	0.389	0.0243	0.149	<0.001	0.014

C = chop length; F = grass to maize silage ratio; C×F = interaction between C and F; OM = organic matter; N = nitrogen

Table 5 Reticular pH and eating behaviour of dairy cows fed diets containing long chop grass silage (LG); short chop grass silage (SG); long chop grass and maize silages (LM), or short chop grass and maize silages (SM).

	Treatments				SED	P-value		
	LG	SG	LM	SM		C	F	C x F
Daily minimum pH	5.99	5.98	5.90	5.87	0.039	0.421	0.001	0.594
Daily maximum pH	6.82	6.84	6.76	6.82	0.038	0.175	0.128	0.497
Mean pH	6.42	6.41	6.33	6.34	0.035	0.998	0.001	0.775
% time <5.8 pH ¹	0.93	0.41	0.42	0.37	0.471	0.401	0.422	0.492
% time <6.0 pH	4.91	3.85	5.42	6.37	2.863	0.979	0.460	0.622
% time <6.2 pH	14.5	17.1	27.0	27.8	5.11	0.643	0.003	0.795
% time <6.5 pH	63.9	65.9	81.0	77.9	6.27	0.902	0.003	0.572
Eating (h/d)	5.8	4.9	4.6	4.0	0.30	0.003	<0.001	0.463
Eating (min/kg DMI)	17.3	14.5	12.2	10.3	0.96	0.021	<0.001	0.520
Rumination (h/d)	9.3	10.0	10.1	10.0	0.23	0.084	0.013	0.029
Rumination (min/kg DMI)	28.1	29.2	26.8	25.3	0.79	0.709	<0.001	0.026
Chews/bolus (n)	54	65	59	69	2.3	<0.001	0.011	0.768

C = chop length; F = forage ratio; CxF = interaction between C and F

¹Average percentage of time cows spent below each pH level

488 **Figure 1** Hourly reticular pH in cows fed diets containing long chop grass silage (LG;
489 —●—); short chop grass silage (SG; —▲—); long chop grass and maize silages (LM; --●--
490 -), or short chop grass and maize silages (SM; --▲--). (SED, 0.042; Time, $P < 0.001$;
491 forage ratio, $P = 0.003$; Time \times F, $P < 0.001$).

492

493 **Figure 2** Plasma glucose (a), plasma β -hydroxybutyrate (3-OHB) (b) and plasma urea
494 (c) concentrations in cows fed diets containing long chop grass (LG;—●—); short chop
495 grass silage (SG, —▲—); long chop grass and maize silages (LM; --●--), or short chop
496 grass and maize silages (SM; --▲--). For plasma glucose; SED, 0.108; Time, $P <$
497 0.001; F, $P = 0.008$. For plasma 3-OHB; SED, 0.112; Time, $P < 0.001$. For plasma urea;
498 SED, 0.265; Time, $P = 0.004$; chop length, $P = 0.093$; forage ratio, $P < 0.001$.