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

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Short title: Effect of forage particle size on cow performance

Abstract

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

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

Implications

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

Introduction

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

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

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

Materials and Methods

Animals, housing, forages, diets and experimental routine

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

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

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

Cows were milked twice daily at 0700 and 1700 h with milk yield recorded at each milking and samples taken during the final week of each period (two morning and two evening milkings) for subsequent analysis. Body condition score (BCS, Ferguson *et al.,* 1994) and live weight were recorded after the evening milking during the week prior to commencing the study and then at the end of each period. Whole tract apparent digestibility was estimated using acid insoluble ash as an internal marker (Van Keulen and Young, 1977) with faecal samples collected at 1000 and 1600 h for five consecutive days during the final week of each period, and stored at –20℃ prior to subsequent analysis.

Reticular pH and blood collection

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

Particle size distribution and eating behaviour

The PS distribution of the fresh TMR was measured by collecting samples 5 min post-feeding on days 20 to 25 of each period and using a modified Penn State Particle Separator (PSPS) with 5 sieve screens of size 44, 26.9, 19, 8, and 4 mm (Tayyab *et al.,* 2017). A manual shaking procedure was adopted (Kononoff *et al.,* 2003), and each diet was separated into six fractions; >44, 26.9-44, 19-26.9, 8-19, 4-8 and <4 mm. Jaw movement (eating, ruminating and idling) was visually recorded for 48 h commencing at 0530 h on day-18 of each period by instantaneous scan monitoring of all cows at 5 min intervals (Martin and Bateson, 2007). All observers were trained for 1 h before the start of the study with a 96% similarity index achieved. Observations were conducted using 2 observers for a duration of 4 h to minimise fatigue and enhance accuracy (Martin and Bateson, 2007).

Chemical analysis

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

Calculations and statistical analysis

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

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

Results

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

Forage and feed composition

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

Production performance

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

Whole tract digestibility

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

Reticular pH and eating behaviour

Reticular pH was highest prior to the morning feeding in all treatments and then declined with time ($P < 0.001$; Figure 1). There was a time x forage ratio interaction on reticular pH, which was lower in cows fed MS for most of the day except around fresh feed delivery, but there was no effect of GS chop length. When cows were fed the GS based diets the mean minimum reticular pH was 0.1 higher ($P = 0.001$) than when fed the MS based diet (Table 5). Cows fed the MS based diets also spent a longer time at reticular pH levels below pH 6.2 and 6.5 ($P = 0.003$) compared to the GS based diets. Cows spent 1.1 h/day longer eating (P < 0.001) when offered the GS compared to the MS based diets and 0.9 h/day longer ($P = 0.003$) eating the long chop compared to the short chop GS (Table 5). Similarly, eating time (ET) was 4.7 min/kg DM higher when cows were fed the GS compared to the MS based diets ($P < 0.001$), and 2.4 min/kg DMI higher ($P < 0.05$) when fed the longer compared to the shorter GS. There was an interaction ($P < 0.05$) for rumination time (RT; h/day), with the shorter chop length increasing the RT in cows when fed the GS but not the MS based diets, whereas when expressed on a min/kg DMI, a shorter chop length increased RT on the GS and decreased RT on the MS based diets. The PS distribution of fractions 8-19 and 4-8 mm decreased ($P < 0.05$) with time post-feeding, and the DM proportion of the 26.9-44 mm fraction was higher ($P < 0.001$) for diets that contained long chop GS or when mixed with MS (Supplementary Table S1).

Milk fatty acids and blood metabolites

Cows fed the short chop length diets had a 0.04 g/100g higher milk fat C18:3n-3 concentration ($P < 0.001$), whereas, those receiving the long chop length diets had a 0.05 g/100g higher concentration of *cis*-9, *trans*-11 conjugated linoleic acid (CLA; $P = 0.032$; Supplementary Table S2). For cows fed the GS based diets, milk concentrations of C16:0, C16:1n-7, C18:1*c*9 and C18:3n-3 were higher ($P < 0.05$), compared to when the MS based diets were fed. In contrast, milk from cows fed the MS based diets had a higher ($P < 0.01$) concentration of C10:0, C12:0, C14:1, C18:0, C18:1*trans*-8, C18:1*trans*-9, C18:1*trans*-12, C18:2n-6 and total polyunsaturated FA (*P* = 0.015) compared to when fed the GS based diets.

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

Discussion

Nutrient composition and particle length

The current study was conducted to determine the effect of chop length of GS when fed alone or mixed with MS on cow performance, rumen pH, eating behaviour and blood metabolites. The PS of the long chop length GS and MS used in the current study were similar to the mean values fed on UK dairy farms reported by Tayyab *et al.* (2017; 43 and 11 mm respectively), whereas the short chop length GS was within the shortest 5% of the GS surveyed. The DM of the GS was lower than typically reported for 1st cut ryegrass silages (Sinclair *et al.,* 2015), although the chemical composition of both chop length GS was similar, a finding in agreement with previous studies that have altered forage chop length prior to ensiling (Kononoff and Heinrichs, 2003; Yang and Beauchemin, 2007). The lactic acid content was however, higher and the acetic acid content lower in the short chop compared to the long chop length GS, a finding in agreement with others who have reported that a shorter chop length can enhance consolidation in the clamp and improve the fermentation profile (McDonald *et al.,* 1991).

Animal performance

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

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

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

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

Reticular pH and eating behaviour

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

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

Metabolism and milk fatty acids

The higher plasma glucose concentration in cows fed the MS diets in the current study may be due to the higher dietary content of sugar and starch (Oba and Allen, 2003), whereas the lower plasma urea concentration in cows fed the GS based diets may reflect a lower content of rumen degradable N as a greater proportion of dietary N was from rumen-protected protein sources in these diets, although all diets were formulated to have a similar excess of rumen degradable nitrogen. Alternatively, the GS based diets may have resulted in a more suitable rumen microbial environment for the capture of degraded N, as demonstrated by the higher reticular pH.

Overall, the inclusion of MS in the diet altered the FA profiles of the milk more than the GS chop length. Chilliard *et al.* (2000) reviewed the literature on diet and milk FA profile and concluded that cows fed MS based diets had a higher concentration of C10:0, C12:0 and C18:2n-6, due to the higher concentrations in MS compared to GS, a finding in agreement with the current results. Hart *et al.* (2015) also reported a 0.99 g/100g higher milk fat content of C16:0 in cows when fed a 70:30 (DM basis) grass to MS based diets compared to those receiving a 30:70 GS:MS diets, a finding in agreement with the current findings. Soita *et al.* (2005) reported no effect of chop length on milk FA, but in the current study a shorter GS chop length increased the milk fat proportion of C18:3n-3, which may be related to a lower rate and extent of biohydrogenation in the rumen, possibly due to a shorter rumen retention time.

Conclusions

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

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Declaration of interest

None.

Ethics statement

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

Software and data repository resources

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

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

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

[3] Mineral/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 D_3, 3,000 IU vitamin E, 2.5 mg/kg vitamin B_{12}, 135 mg/kg biotin.

[4] ME, metabolisable energy.

[5] MPN, metabolisable protein-rumen nitrogen limited.

[6] MPE, 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				
Fermentation characteristics (g/kg)							
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				
Fatty acids (g/100 FA)							
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
C18:1*c*9	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
Fractions (%DM)							
>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; NH_3-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					*P*-value		
	LG	SG	LM	SM	SED	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; [1]FCM = fat corrected milk

[2]Change 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					*P*-value		
	LG	SG	LM	SM	SED	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					*P*-value		
	LG	SG	LM	SM	SED	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; C×F = interaction between C and F

[1] Average percentage of time cows spent below each pH level

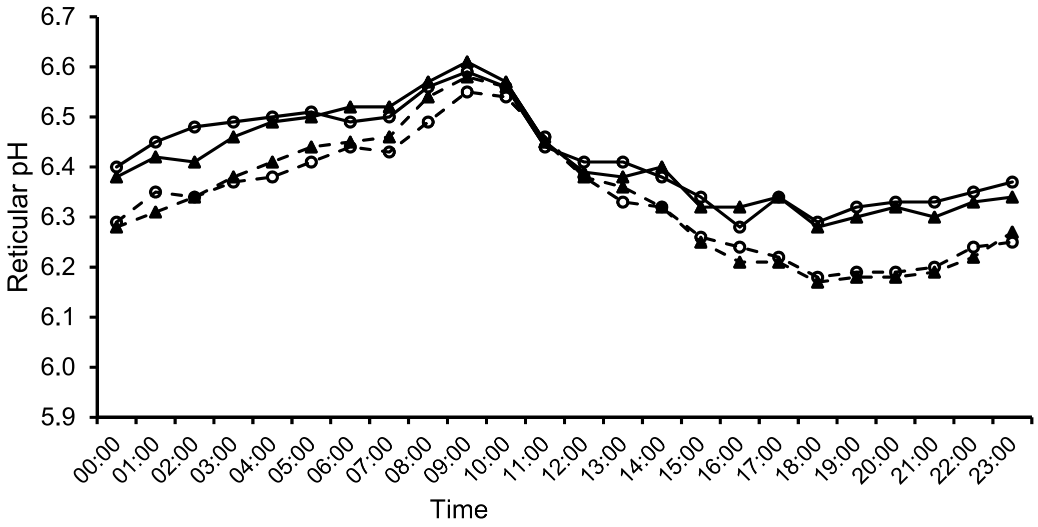

Figure 1 Hourly reticular pH in cows fed diets containing long chop grass silage (LG; ●); short chop grass silage (SG; –▲–); long chop grass and maize silages (LM; --**o**--), or short chop grass and maize silages (SM; --▲--). (SED, 0.042; Time, *P*<0.001; forage ratio, *P*=0.003; Time×F, *P*<0.001).

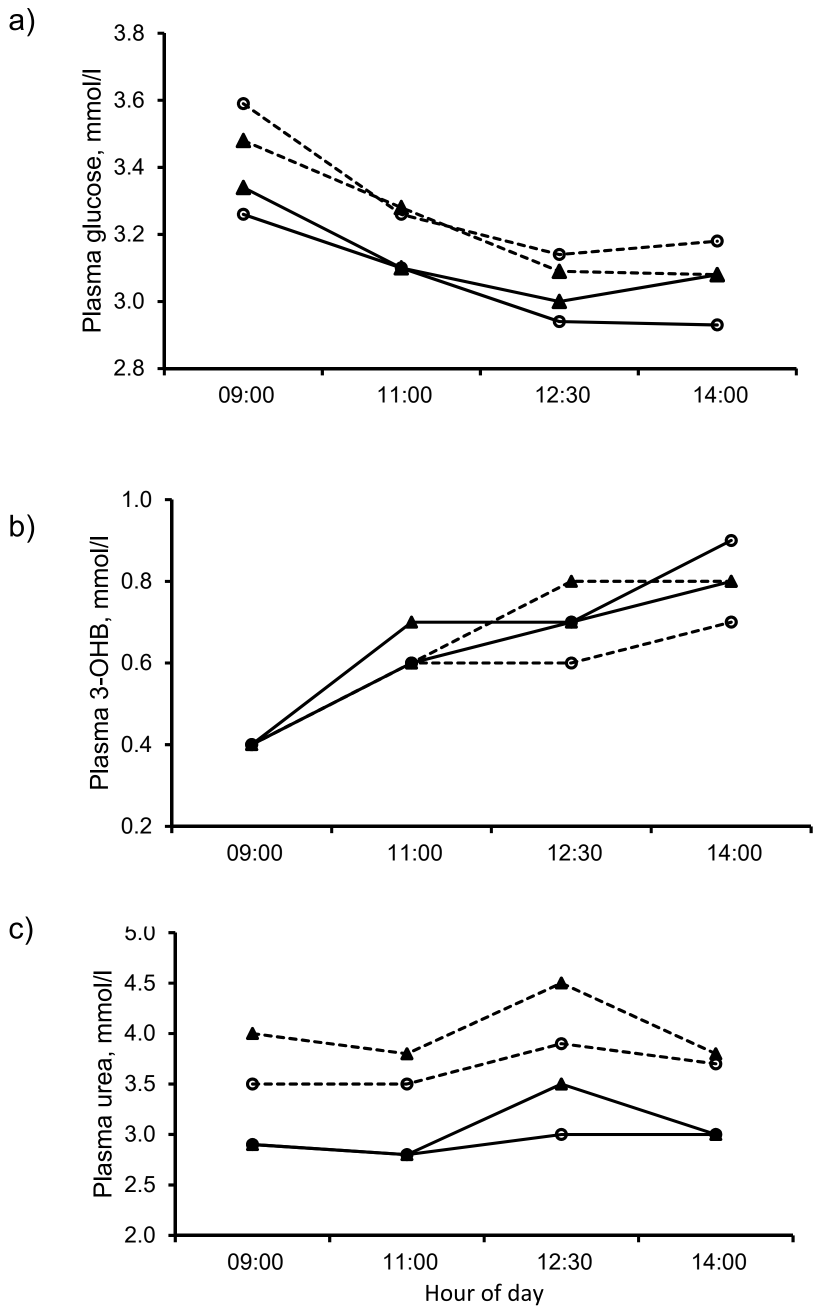

Figure 2 Plasma glucose (a), plasma β-hydroxybutyrate (3-OHB) (b) and plasma urea (c) concentrations in cows fed diets containing long chop grass (LG;–**o**–); short chop grass silage (SG, –▲–); long chop grass and maize silages (LM; --**o**--), or short chop grass and maize silages (SM; --▲--). For plasma glucose; SED, 0.108; Time, $P <$

0.001; F, P=0.008. For plasma 3-OHB; SED, 0.112; Time, P<0.001. For plasma urea; SED, 0.265; Time, P=0.004; chop length, P=0.093; forage ratio, P<0.001.

***Animal* journal**

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

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Supplementary materials

Fatty acid analysis

Fatty acid methyl esters (FAME) in hexane were prepared from milk by the method of Feng *et al.* (2004). Individual FAME were determined by GC (Hewlett Packard 6890, Wokingham, UK) fitted with a CP-Sil 88 column (100 m x 0.25 mm i.d. x 0.2 μm film). Fatty acid (FA) identification and recoveries were determined using pure methyl ester standards (Nu-Chek Prep, Elysian, MN; Natural ASA, Hovdebygda, Norway), and a mixed reference standard was used as a routine check for recoveries and correction factors for individual FA. Feed FA were determined by the procedure described by Jenkins (2010).

Particle size determination

The geometric mean PS (X_m) was calculated using the method described by ASABE (2007) as;

$$Geometric\ mean\ length\ (Xm)\ = \log^{-1}\ \frac{\sum(Mi\ \log mXi)}{\sum Mi} \qquad \text{(Equation 1)}$$

With the standard deviation of X_m determined as;

$$Standard\ deviation\ \ (SDgm) = \log^{-1}\ [\frac{\sum Mi\,(\,\log mXi - logXg)2}{\sum Mi}]^{1/2} \qquad \text{(Equation 2)}$$

Where; X_i is diagonal of screen opening of the i^{th} screen, $X_{(i-1)}$ is diagonal of screen opening in the next larger than the i^{th} screen, X_m is geometric length (particle size), mX_i is mean geometric length of particles on i^{th} screen = $[X_i \times X_{i-1}]^{1/2}$, M_i is mass on i^{th} screen.