# *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 Text

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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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## 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 $\mu$m 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;

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

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),

23 $mX_i$ is mean geometric length of particles on $i^{th}$ screen = $[X_i \times X_{i-1}]^{1/2}$ , $M_i$ is mass on $i^{th}$

24 screen.

**Table S1** *Particle size distribution of 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) at 0, 4, 8 and 24h post feeding.*

| | DM % | | | | | *P*-value | | | |
|---|---|---|---|---|---|---|---|---|---|
| Fractions[1] | LG | SG | LM | SM | SED | Time | C | F | C x F |
| >44 mm | | | | | | | | | |
| 0h | 15.6 | - | 0.1 | - | 0.87 | 0.234 | <0.001 | <0.001 | <0.001 |
| 4h | 17.7 | - | 0.1 | - | | | | | |
| 8h | 17.5 | - | 0.1 | - | | | | | |
| 24h | 19.3 | - | 0.2 | - | | | | | |
| 26.9-44 mm | | | | | | | | | |
| 0h | 32.9 | 16.3 | 21.0 | 3.0 | 1.15 | 0.107 | <0.001 | <0.001 | 0.051 |
| 4h | 32.0 | 16.4 | 22.2 | 3.3 | | | | | |
| 8h | 33.4 | 17.1 | 22.8 | 3.4 | | | | | |
| 24h | 33.0 | 19.5 | 23.9 | 2.4 | | | | | |
| 19-26.9 mm | | | | | | | | | |
| 0h | 4.9 | 4.5 | 3.7 | 3.3 | 0.28 | 0.056 | 0.008 | <0.001 | 0.475 |
| 4h | 5.3 | 4.3 | 3.9 | 3.4 | | | | | |
| 8h | 5.1 | 4.1 | 3.5 | 3.4 | | | | | |
| 24h | 5.3 | 4.7 | 4.4 | 3.4 | | | | | |
| 8-19 mm | | | | | | | | | |
| 0h | 17.2 | 48.2 | 32.6 | 52.1 | 0.73 | 0.035 | <0.001 | <0.001 | <0.001 |
| 4h | 16.9 | 48.4 | 32.6 | 50.5 | | | | | |
| 8h | 16.4 | 47.7 | 31.2 | 50.4 | | | | | |
| 24h | 16.1 | 47.3 | 31.0 | 52.4 | | | | | |
| 4-8 mm | | | | | | | | | |
| 0h | 17.1 | 18.7 | 19.5 | 19.6 | 0.66 | <0.001 | 0.019 | <0.001 | 0.217 |
| 4h | 16.0 | 19.2 | 18.2 | 20.0 | | | | | |
| 8h | 15.3 | 18.9 | 17.3 | 19.6 | | | | | |
| 24h | 14.4 | 18.0 | 15.6 | 19.1 | | | | | |
| <4 mm | | | | | | | | | |
| 0h | 12.3 | 12.3 | 23.1 | 21.9 | 0.63 | 0.123 | 0.542 | <0.001 | 0.128 |
| 4h | 12.2 | 12.8 | 22.1 | 22.8 | | | | | |
| 8h | 12.4 | 13.7 | 23.5 | 23.2 | | | | | |
| 24h | 12.3 | 13.0 | 22.8 | 22.6 | | | | | |

C = chop length; F = forage ratio; C × F = interaction between C and F.

[1]Diets were separated into 6 fractions; >44, 26.9-44, 19-26.9, 8-19, 4-8 and <4 mm.

**Table S2** *Milk fatty acids content (g/100g FA) in 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 |
| C4:0 | 1.36 | 1.70 | 1.32 | 1.33 | 0.103 | 0.021 | 0.009 | 0.031 |
| C6:0 | 1.20 | 1.38 | 1.22 | 1.22 | 0.073 | 0.070 | 0.198 | 0.084 |
| C8:0 | 0.89 | 0.94 | 0.98 | 0.96 | 0.039 | 0.436 | 0.069 | 0.224 |
| C10:0 | 2.11 | 2.13 | 2.58 | 2.50 | 0.050 | 0.389 | <0.001 | 0.224 |
| C12:0 | 3.01 | 3.08 | 3.93 | 3.80 | 0.090 | 0.730 | <0.001 | 0.111 |
| C14:0 | 12.26 | 12.44 | 13.62 | 13.18 | 0.199 | 0.350 | <0.001 | 0.034 |
| C14:1 | 1.02 | 1.03 | 1.16 | 1.11 | 0.039 | 0.627 | <0.001 | 0.240 |
| C15:0 | 1.32 | 1.28 | 1.38 | 1.31 | 0.050 | 0.131 | 0.268 | 0.733 |
| C16:0 | 42.20 | 42.78 | 40.53 | 39.83 | 0.716 | 0.905 | <0.001 | 0.215 |
| C16:1 | 0.52 | 0.52 | 0.50 | 0.52 | 0.015 | 0.324 | 0.257 | 0.392 |
| C16:1n-7 | 1.23 | 1.19 | 1.15 | 1.15 | 0.036 | 0.551 | 0.026 | 0.441 |
| C17:0 | 0.66 | 0.60 | 0.59 | 0.60 | 0.027 | 0.278 | 0.093 | 0.106 |
| C17:1 | 0.21 | 0.21 | 0.21 | 0.21 | 0.014 | 0.890 | 0.934 | 0.696 |
| C18:0 | 9.15 | 9.86 | 9.38 | 9.94 | 0.298 | 0.381 | 0.008 | 0.084 |
| C18:1 *t*8 | 0.23 | 0.22 | 0.26 | 0.27 | 0.009 | 0.855 | <0.001 | 0.146 |
| C18:1 *t*9 | 0.16 | 0.15 | 0.20 | 0.20 | 0.007 | 0.799 | <0.001 | 0.229 |
| C18:1 *t*10 | 0.35 | 0.24 | 0.37 | 0.39 | 0.052 | 0.280 | 0.030 | 0.092 |
| C18:1 *t*11 | 0.80 | 0.77 | 0.76 | 0.80 | 0.057 | 0.771 | 0.846 | 0.392 |
| C18:1 *t*12 | 0.27 | 0.28 | 0.36 | 0.36 | 0.011 | 0.576 | <0.001 | 0.988 |
| C18:1 *c*9 | 17.33 | 16.47 | 15.60 | 16.31 | 0.546 | 0.847 | 0.020 | 0.052 |
| C18:2 n-6 | 2.02 | 2.02 | 2.20 | 2.29 | 0.067 | 0.383 | <0.001 | 0.373 |
| C18:3 n-3 | 0.46 | 0.51 | 0.37 | 0.40 | 0.014 | <0.001 | <0.001 | 0.397 |
| C20:0 | 0.19 | 0.19 | 0.18 | 0.19 | 0.005 | 0.701 | 0.447 | 0.464 |
| C20:3 n-3 | 0.27 | 0.07 | 0.28 | 0.21 | 0.077 | 0.027 | 0.167 | 0.260 |
| C20:3 n-6 | 0.12 | 0.11 | 0.11 | 0.12 | 0.009 | 0.753 | 0.949 | 0.716 |
| C22:0 | 0.04 | 0.04 | 0.03 | 0.03 | 0.003 | 0.686 | 0.118 | 0.580 |
| CLA *c*9, *t*11 | 0.50 | 0.42 | 0.45 | 0.43 | 0.032 | 0.029 | 0.367 | 0.202 |
| CLA *t*10, *c*12 | 0.04 | 0.05 | 0.05 | 0.07 | 0.010 | 0.069 | 0.037 | 0.788 |
| EPA | 0.05 | 0.06 | 0.04 | 0.05 | 0.008 | 0.120 | 0.065 | 0.746 |
| DHA | 0.08 | 0.14 | 0.20 | 0.20 | 0.065 | 0.503 | 0.050 | 0.588 |
| ∑SFA | 74.4 | 75.5 | 75.7 | 74.9 | 0.70 | 0.754 | 0.469 | 0.054 |
| ∑MUFA | 22.1 | 21.1 | 20. 6 | 21.3 | 0.62 | 0.791 | 0.149 | 0.051 |
| ∑PUFA | 3.5 | 3.4 | 3.7 | 3.8 | 0.16 | 0.720 | 0.015 | 0.352 |

C = chop length; F = forage ratio; C × F = interaction between C and F; CLA = conjugated linoleic acid; EPA = eicosapentaenoic acid; DHA = docosahexaenoic acid; ∑SFA = total saturated FA ∑MUFA = total monounsaturated FA; ∑PUFA = total polyunsaturated FA