

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

Article

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Supplementary Text

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1 **Animal journal**

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

4 U. Tayyab<sup>1</sup>, R. G. Wilkinson<sup>1</sup>, G. L. Charlton<sup>1</sup>, C. K. Reynolds<sup>2</sup>, L. A. Sinclair<sup>1\*</sup>

## 5 **Supplementary materials**

6 *Fatty acid analysis*

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

15 *Particle size determination*

16 The geometric mean PS ( $X_m$ ) was calculated using the method described by ASABE  
17 (2007) as;

$$18 \quad \text{Geometric mean length } (X_m) = \log^{-1} \frac{\sum (M_i \log m X_i)}{\sum M_i} \quad (\text{Equation 1})$$

19 With the standard deviation of  $X_m$  determined as;

$$20 \quad \text{Standard deviation } (SD_{gm}) = \log^{-1} \left[ \frac{\sum M_i (\log m X_i - \log X_g)^2}{\sum M_i} \right]^{1/2} \quad (\text{Equation 2})$$

21 Where;  $X_i$  is diagonal of screen opening of the  $i^{th}$  screen,  $X_{(i-1)}$  is diagonal of screen  
22 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  $i^{\text{th}}$  screen =  $[X_i \times X_{i-1}]^{1/2}$ ,  $M_i$  is mass on  $i^{\text{th}}$
- 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.

Fractions <sup>1</sup>	DM %				SED	Time	P-value		
	LG	SG	LM	SM			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 x F = interaction between C and F.

<sup>1</sup>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				SED	P-value		
	LG	SG	LM	SM		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 t8	0.23	0.22	0.26	0.27	0.009	0.855	<0.001	0.146
C18:1 t9	0.16	0.15	0.20	0.20	0.007	0.799	<0.001	0.229
C18:1 t10	0.35	0.24	0.37	0.39	0.052	0.280	0.030	0.092
C18:1 t11	0.80	0.77	0.76	0.80	0.057	0.771	0.846	0.392
C18:1 t12	0.27	0.28	0.36	0.36	0.011	0.576	<0.001	0.988
C18:1 c9	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 c9, t11	0.50	0.42	0.45	0.43	0.032	0.029	0.367	0.202
CLA t10, c12	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
ΣSFA	74.4	75.5	75.7	74.9	0.70	0.754	0.469	0.054
ΣMUFA	22.1	21.1	20.6	21.3	0.62	0.791	0.149	0.051
ΣPUFA	3.5	3.4	3.7	3.8	0.16	0.720	0.015	0.352

C = chop length; F = forage ratio; C x F = interaction between C and F; CLA = conjugated linoleic acid; EPA = eicosapentaenoic acid; DHA = docosahexaenoic acid; ΣSFA = total saturated FA; ΣMUFA = total monounsaturated FA; ΣPUFA = total polyunsaturated FA