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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 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;

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

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