Supplementation of hydrogenated fat-embedded calcium gluconate improves milk fat content and yield in multiparous Holstein dairy cattle

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SUPPLEMENTARY FILE

Materials and Methods

Samples of all feeds offered were collected weekly and stored at -20°C until analysis. Composite samples of feeds were prepared every 3 weeks and proximate composition determined by near-infrared spectroscopy (Shur-Gain Laboratory, St. Hyacinthe, QC). Milk samples were collected at both milkings on d 26, 27 and 28 of each period and stored at 4°C until analysis for fat, protein and lactose content by mid-infrared spectroscopy (Agriculture and Food Laboratory, University of Guelph, ON). Milk component yields were used to calculate 4% fat-corrected milk yield as 0.4 × milk yield (kg/d) + 15 × fat yield (kg/d; Gaines, 1928) and energy-corrected milk yield as 0.01 × milk yield (kg/d) + 12.2 × fat yield (kg/d) + 7.7 × protein yield (kg/d) + 5.3 × lactose yield (kg/d; Sjaunja et al., 1990). Gross feed efficiency was calculated as the ratio of ECM to DMI.

Blood was collected by venipuncture from the coccygeal vessels at approximately 0900, 1100, 1300 and 1500h on d 27 of each period. Samples were immediately placed on ice and centrifuged for 15 min at 1,500 × *g*. Plasma was pooled by cow within period and stored at -20°C until analysis. Spectrophotometric assays were used to analyze glucose (Glucose GO assay kit, Sigma-Aldrich, Darmstadt, Germany; Raabo and Terkildsen, 1960), non-esterified fatty acids (NEFA-HR(2) kit, Fujifilm Wako Chemicals Europe GmbH, Neuss, Germany; Johnson and

Peters, 1993) and beta-hydroxybutyrate (Cant et al., 1993). At 1300h on d 28 of each period, approximately 150 g of fresh feces were collected directly from the rectum into sealed plastic bag, lyophilized (Guelph Food Technology Centre, University of Guelph, ON) and stored at -20°C until analysis for concentrations of acetic, butyric, and propionic acids by high-pressure liquid chromatography (Shur-Gain Laboratory) using the method previously described by Canale et al. (1984).

All data were analyzed using the GLIMMIX procedure of SAS v9.4 (SAS Institute Inc., Cary, NC) according to the linear model,

$$y_{ijklm} = \mu + parity_i + HFCG_j + parity_i \times HFCG_j + block_k + period_l(block)_k + cow_m(block)_k + \epsilon_{ijklm}$$

where y_{ijkl} is the response of interest, μ is the intercept, $parity_i$ is the fixed effect of parity group i, $HFCG_j$ is the fixed effect of treatment j, $parity_i \times HFCG_j$ is the treatment-parity interaction effect, $block_k$ is the random effect of block k, $period_l(block)_k$ is the random effect of the l^{th} period in the k^{th} block, $cow_m(block)_k$ is the random effect of the m^{th} cow in the k^{th} block, and ε_{ijklm} is the residual error variance. The GLIMMIX procedure was modified by specifying the NOBOUND option to accommodate negative covariance among blocks, in addition to requesting the use of a Newton-Raphson optimization with ridging. For all analyses, the Kenward-Roger correction was used to adjust the denominator degrees of freedom. The presence of potential treatment-parity group interactions were evaluated by testing the simple effect of treatment within parity group using the SLICE option of the LSMEANS statement (Stroup et al., 2018). Contrasts were used to perform specific hypothesis tests comparing both 16 and 25 g/d HFCG to the negative control. Statistical significance was declared where $P < 0.05$.

The presence of a potential linear dose response was evaluated using the GLIMMIX procedure according to the model,

$$y_{iklm} = \beta_{0i} + \beta_{1i}HFCG + \beta_{2i}HFCG^2 + block_k + period_l(block)_k + cow_m(block)_k + \epsilon_{iklm}$$

where y_{iklm} is the response of interest, β_{0i} is the intercept for parity group i, β_{1i} is the linear regression coefficient of HFCG dose for parity group i, β_{2i} is the quadratic regression coefficient of HFCG dose for parity group i, and all other terms are as previously described. As three levels of HFCG were used, a quadratic dose response cannot be conclusively evaluated (Stroup et al., 2018); as such, this was used to evaluate the lack-of-fit of the linear response, i.e., the presence of a potentially higher-order dose response.

References

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Tables

Table S1. Plasma and fecal metabolite responses in multiparous Holstein cows (n = 37) supplemented with 3 levels of hydrogenated fat-embedded calcium gluconate (HFCG).

	HFCG Dose				*P*-values[1]			
Metabolite	0 g/d	16 g/d	25 g/d	SED[2]	16 g/d	25 g/d	LIN	LOF
Plasma								
Glucose (mM)	3.45	3.48	3.37	0.102	0.808	0.390	0.511	0.495
NEFA[3] (µM)	138	155	160	13.5	0.212	0.108	0.240	0.497
BHB[4] (mM)	0.899	0.878	1.004	0.1003	0.832	0.306	0.411	0.466
Acetate (µM)	121	117	118	6.4	0.626	0.667	1.000	1.000
Feces								
Acetic acid (mmol/g)	0.241	0.244	0.240	0.128	0.838	0.897	0.768	0.744
Propionic acid (mmol/g)	0.0835	0.0848	0.0817	0.00432	0.759	0.677	0.584	0.514
Butyric acid (mmol/g)	0.0555	0.0578	0.0571	0.00220	0.255	0.443	0.342	0.458

[1]16 g/d: 0 g HFCG/d vs. 16 g HFCG/d; 25 g/d: 0 g HFCG/d vs. 25 g HFCG/d; LIN: linear dose response; LOF: lack-of-fit of linear dose response
[2]Standard error of the difference
[3]Non-esterified fatty acid
[4]Beta-hydroxybutyrate

Table S2. Dry matter intake and production responses in primiparous Holstein heifers (n = 9) supplemented with 3 levels of hydrogenated fat-embedded calcium gluconate (HFCG). Values are presented in units of kg/d unless indicated otherwise.

	HFCG Dose				*P*-values[1]			
Response	0 g/d	16 g/d	25 g/d	SED[2]	16 g/d	25 g/d	LIN	LOF
DMI[3]	20.4	20.0	21.0	1.25	0.711	0.623	0.511	0.435
Milk yield	36.2	35.3	34.5	1.14	0.613	0.341	0.894	0.905
Milk fat yield	1.38	1.34	1.38	0.110	0.721	0.997	0.680	0.682
Milk protein yield	1.05	1.02	0.99	0.047	0.435	0.180	0.792	0.927
Milk lactose yield	1.54	1.49	1.49	0.073	0.480	0.482	0.631	0.770
FCM yield[4]	35.1	34.0	34.9	2.17	0.608	0.914	0.590	0.613
ECM yield[5]	33.6	32.3	33.1	1.94	0.497	0.802	0.509	0.556
GFE[6] (kg ECM/kg DMI)	1.64	1.65	1.60	0.123	0.927	0.765	0.802	0.743
Milk fat content (%)	3.85	4.00	3.94	0.191	0.442	0.630	0.512	0.601
Milk protein content (%)	2.96	2.93	2.92	0.069	0.603	0.573	0.739	0.855
Milk lactose content (%)	5.00	5.01	5.00	0.058	0.537	0.925	0.883	0.904

[1]16 g/d: 0 g HFCG/d vs. 16 g HFCG/d; 25 g/d: 0 g HFCG/d vs. 25 g HFCG/d LIN: linear dose response; LOF: lack-of-fit of linear dose response
[2]Standard error of the difference
[3]Dry matter intake
[4]4% fat-corrected milk: 0.4 × milk yield (kg/d) + 15 × fat yield (kg/d; Gaines, 1928)
[5]Energy-corrected milk: 0.01 × milk yield (kg/d) + 12.2 × fat yield (kg/d) + 7.7 × protein yield (kg/d) + 5.3 × lactose yield (kg/d; Sjaunja et al., 1990)
[6]Gross feed efficiency

87 Table S3. Plasma and fecal metabolite responses in primiparous Holstein heifers (n = 9)

88 supplemented with 3 levels of hydrogenated fat-embedded calcium gluconate (HFCG).

	HFCG Dose				*P*-values[1]			
Metabolite	0 g/d	16 g/d	25 g/d	SED[2]	16 g/d	25 g/d	LIN	LOF
Plasma								
Glucose (mM)	4.16	4.15	4.05	0.210	0.957	0.588	0.847	0.738
NEFA[3] (µM)	134	169	178	28.6	0.216	0.123	0.487	0.778
BHB[4] (mM)	0.832	0.851	0.864	0.2006	0.923	0.874	0.971	0.996
Acetate (µM)	133	123	122	13.3	0.449	0.431	1.000	1.000
Feces								
Acetic acid (mmol/g)	0.269	0.303	0.260	0.026	0.195	0.712	0.098	0.082
Propionic acid (mmol/g)	0.0913	0.0976	0.0923	0.00883	0.484	0.910	0.453	0.474
Butyric acid (mmol/g)	0.0587	0.0672	0.0568	0.00448	0.060	0.673	0.020	0.015

[1]16 g/d: 0 g HFCG/d vs. 16 g HFCG/d; 25 g/d: 0 g HFCG/d vs. 25 g HFCG/d; LIN: linear dose response; LOF: lack-of-fit of linear dose response

[2]Standard error of the difference

[3]Non-esterified fatty acid

[4]Beta-hydroxybutyrate

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