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

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Abstract

This research communication reports the responses to supplementing dairy cattle with a hydrogenated fat-embedded calcium gluconate feed additive. The role of hindgut health in ruminant performance and wellbeing is an area of growing interest. Various prebiotic compounds have been used to promote lower gut health in various non-ruminant species. Calcium gluconate, a prebiotic compound, has previously been observed to increase milk fat yield when fed to ruminants in a form capable of resisting fermentation in the rumen, though the mechanism(s) behind this response remain unclear. The objective of this study was to compare the responses of lactating cattle to two different supplementation levels of a hydrogenated fat-embedded calcium gluconate (HFCG) product to evaluate a potential linear dose response. Forty-six lactating Holstein dairy cattle were used in a 3×3 replicated Latin square design with 28 d periods to evaluate a previously used dose of HFCG (approximately 16 g/d) with both a negative control and a dose of 25 g/d. Supplementation of multiparous animals with 16 g/d HFCG significantly ($P < 0.05$) increased milk fat yield and content relative to the negative control, and subsequently improved gross feed efficiency ($P < 0.05$); additionally, the presence of a potential non-linear dose response was observed for these parameters. Responses when supplemented with 25 g/d HFCG did not differ from the negative control. No production responses were observed in primiparous animals. The mode of action of HFCG, in addition to the potential differential response in primiparous animals remains unclear and warrants further investigation.

It is believed that, due to a variety of physiological and structural differences, the hindgut of ruminants may be more susceptible to factors affecting gut barrier integrity compared to the rumen (Sanz-Fernandez *et al*., 2020). Given what is known about the importance of hindgut health in non-ruminant species, specifically targeting the ruminant hindgut with prophylactic interventions is an opportunity that merits exploration. We have previously observed increases in yields of milk fat (Seymour *et al*., 2020, 2021) and/or protein (Sanz-Fernandez *et al*., 2022; Seymour *et al*., 2022) when supplementing lactating dairy cattle with approximately 16 g/d hydrogenated fat-embedded calcium gluconate (HFCG), a prebiotic compound specifically targeting the increased production of acetate and/or butyrate in the hindgut (Asano *et al*., 1994; Tsukahara *et al*., 2002, 2006). However, the underlying mechanism(s) of these responses remain unclear at present. The objectives of the current study were to evaluate the changes in yields of milk and milk components, and alterations in concentrations of blood and fecal metabolites, in a potentially dose-dependent response to HFCG. We hypothesized that milk fat content and yield, and concentrations of fecal butyrate and plasma non-esterified fatty acids would be increased by HFCG in a linear dose-dependent manner.

Materials and methods

This experiment was conducted at the Trouw Nutrition Agresearch Dairy Facility (Burford, ON) between May and December 2015. All animal procedures were approved by the Institutional Animal Care and Use Committee at Trouw Nutrition Agresearch, in accordance with Canadian Council on Animal Care guidelines. Forty-eight lactating Holstein cattle (9 primiparous, 39 multiparous) were enrolled in the experiment at 21 ± 2 d after calving, however, two multiparous cattle were subsequently removed due to unrelated health

Table 1. Formulation and chemical composition of total mixed ratios

Item	HFCG dose 0 g/d	16 g/d	25 g/d
Ingredient			
Compound feed[a]	40.2	40.4	40.3
Corn silage	26.9	27.0	26.9
Alfalfa haylage	26.2	26.3	26.2
Hay	5.79	5.80	5.79
Premix[b]	0.87	0.56	0.87
Composition[c]			
Dry matter (%)	48.8		
Net energy for lactation (MJ/kg DM)	7.47		
Neutral detergent fiber	35.3		
Acid detergent fiber	23.1		
Non-fiber carbohydrate	35.4		
Crude protein	17.5		
Crude fat	4.14		
Ash	7.67		
K	1.38		
Ca	1.00		
P	0.46		
Na	0.36		
S	0.23		

Values are presented on a percent dry matter basis unless indicated otherwise.
[a]Contained (% as-fed) ground corn (37.4), fine rolled corn (17.3), corn gluten feed (10.4), corn dried distillers grains (8.32), pork meal (4.99), bypass soybean meal (5.08; Top Soy, Trouw Nutrition, Puslinch, ON), bakery waste (3.33), palm fat supplement (2.13; APF+, Trouw Nutrition), fat supplement (1.25; Stay Fat, Darling Ingredients Canada, Cambridge, ON), salt (1.16), sodium sesquicarbonate (1.07), calcium carbonate (1.06), magnesium oxide (0.69), blood meal (0.54), urea (0.33), mineral premix (0.24; Nutri-Plex Micro NS, Trouw Nutrition), organic acid supplement (0.16; RM104, Trouw Nutrition), selenium (0.13), yeast (0.10; BioPower SC10, Lallemand Animal Nutrition, Montréal, QC), methionine (0.09; Alimet, Novus International Inc., St. Charles, MI), monensin (0.02; Rumensin, Elanco Animal Health, Greenfield, IN)
[b]0 g/d: contained (% as-fed) limestone (54.9), rice hulls (42.5), soybean oil (2.60); 16 and 25 g/d: contained limestone (49.7), rice hulls (37.3), hydrogenated fat-embedded calcium gluconate (12.0; Trouw Nutrition) and soybean oil (1.00)
[c]Composition of HFCG-supplemented TMRs did not differ significantly from control

reasons. Animals were individually housed in tie stalls for the duration of the experiment. Both water and feed in the form of a total mixed ration (Table 1) were offered *ad libitum*. Fresh feed was delivered daily at approximately 0900 h, and orts were weighed to determined voluntary dry matter intake (DMI). Animals were milked in place twice daily at approximately 0600 h and 1600 h.

A 3 × 3 replicated Latin square design was used to evaluate performance responses to two levels of HFCG relative to a negative control over three periods of 28 d each. Cattle were blocked by calving date and treatment sequences were randomly assigned to animals within blocks. HFCG supplement (50% hydrogenated palm oil, 40% calcium gluconate, 10% calcium carbonate; Trouw Nutrition) was incorporated into a premix (Table 1) and then added to the base ration to achieve a target dose of either 0 g/d (negative control), 16 g/d, or 25 g/d of HFCG. Samples of blood, milk and feces were collected over the last 3 d of each period, as described in the Supplementary File. Data were analyzed as described in the Supplementary File.

Results and discussion

Responses in DMI and milk production in multiparous cows are presented in Table 2. Milk fat content was significantly increased in response to 16 g/d HFCG (0.19%; $P < 0.05$), resulting in a 6.2% increase in milk fat yield ($P < 0.05$). As DMI was unaffected by treatment, the increased yields resulted in a 6% increase ($P < 0.05$) in gross feed efficiency. Yields of milk fat, 4% fat-corrected and energy-corrected milks, as well as gross feed efficiency and milk fat content, displayed evidence of a potential non-linear dose response to HFCG supplementation, as indicated by the lack-of-fit partition (Table 2). Increased milk fat yield has previously been observed in lactating dairy cattle supplemented with 16 g/d HFCG (Seymour *et al.*, 2020, 2021), which was previously attributed to the increased incorporation of pre-formed fatty acids of endogenous origin. In the present study, no differences were observed in circulating acetate, beta-hydroxybutyrate or non-esterified fatty acids (online Supplementary Table S1). We did not record bodyweight or body condition score to assess the potential mobilization of body reserves. As such, the nature of the milk fat response remains unclear at this point in time. In primiparous animals, no differences were observed in production parameters (online Supplementary Table S2), however, fecal butyrate concentration displayed evidence of a potentially non-linear dose response (online Supplementary Table S3). However, this study lacked sufficient statistical power to make any valid claims pertaining specifically to the responses (or lack thereof) in heifers, and as such these results should be interpreted with caution. All responses observed at 25 g/d HFCG supplementation did not differ from control.

The precise mode of action of the HFCG supplement remains unclear. It is currently hypothesized that gluconic acid salts support the production of acetate and butyrate, which have been implicated in the promotion of gut health and integrity (as reviewed by Liu *et al.*, 2018; Litvak *et al.*, 2018), as well as modulation of whole-body energy metabolism (as reviewed by den Besten *et al.*, 2013). However, the main challenges in evaluating the response to this product are twofold: it is difficult to get an accurate determination of in vivo volatile fatty acid synthesis with spot samples due to their rapid uptake by the gastrointestinal epithelium (den Besten *et al.*, 2013), and the signalling pathways involved are poorly understood across species (den Besten *et al.*, 2013; Litvak *et al.*, 2018; Liu *et al.*, 2018). Due to this, it is difficult to draw conclusions as to why responses to 25 g/d HFCG did not differ from control.

In conclusion, supplementing lactating multiparous dairy cows with 16 g/d HFCG increased both the yield and concentration of milk fat. Yields of milk fat, 4% fat-corrected and energy-corrected milks, as well as gross feed efficiency and milk fat content, displayed evidence of a potential non-linear dose response to HFCG supplementation, contrary to our hypothesis of a linear dose response over this range. Additionally, no responses due to treatment were observed for concentrations of fecal butyric acid or plasma non-esterified fatty acid, contrary to our hypothesis. More work is required to characterize the response to HFCG supplementation both at the level of the gastrointestinal epithelium,

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Table 2. Dry matter intake and production responses in multiparous Holstein cows ($n = 37$) supplemented with 3 levels of hydrogenated fat-embedded calcium gluconate (HFCG)

	HFCG dose				*P*-values[a]			
Response	0 g/d	16 g/d	25 g/d	SED[b]	16 g/d	25 g/d	LIN	LOF
DMI[c]	23.9	23.5	24.0	0.57	0.519	0.809	0.393	0.363
Milk yield	45.4	46.0	44.9	0.84	0.477	0.583	0.284	0.228
Milk fat yield	1.61	1.71	1.60	0.052	0.041	0.840	0.015	0.014
Milk protein yield	1.33	1.34	1.30	0.023	0.763	0.287	0.397	0.263
Milk lactose yield	1.83	1.85	1.79	0.036	0.428	0.394	0.191	0.129
FCM yield[d]	42.3	44.1	42.0	1.04	0.067	0.752	0.025	0.022
ECM yield[e]	39.9	41.4	39.6	0.93	0.090	0.643	0.031	0.025
GFE[f] (kg ECM/kg DMI)	1.68	1.78	1.64	0.055	0.043	0.589	0.011	0.008
Milk fat content (%)	3.54	3.73	3.59	0.079	0.039	0.578	0.035	0.052
Milk protein content (%)	2.91	2.89	2.88	0.034	0.603	0.381	0.707	0.886
Milk lactose content (%)	4.75	4.78	4.76	0.028	0.290	0.646	0.317	0.383

Values are presented in units of kg/d unless indicated otherwise
[a]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.
[b]Standard error of the difference.
[c]Dry matter intake.
[d]4% fat-corrected milk: 0.4 × milk yield (kg/d) + 15 × fat yield (kg/d).
[e]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).
[f]Gross feed efficiency.

as well as the downstream signalling pathways in the host animal. Both the nature of the non-linear dose response and potential parity-by-treatment interactions require additional work to confirm.

Supplementary material. The supplementary material for this article can be found at https://doi.org/10.1017/S0022029922000851

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References

Asano T, Yuasa K, Kunugita K, Teraji T and Mitsuoka T (1994) Effects of gluconic acid on human fecal bacteria. *Microbial Ecology in Health and Disease* **7**, 247–256.

den Besten G, van Eunen K, Groen AK, Venema K, Reijngoud DJ and Bakker BM (2013) The role of short-chain fatty acids in the interplay between diet, gut microbiota, and host energy metabolism. *Journal of Lipid Research* **54**, 2325–2340.

Litvak Y, Byndloss MX and Baumler AJ (2018) Colonocyte metabolism shapes the gut microbiota. *Science (New York, N.Y.)* **362**, 1–6.

Liu H, Wang J, He T, Becker S, Zhang G, Li D and Ma X (2018) Butyrate: a double-edged sword for health? *Advances in Nutrition* **9**, 21–29.

Sanz-Fernandez MV, Daniel JB, Seymour DJ, Kvidera SK, Bester Z, Doelman J and Martin-Tereso J (2020) Targeting the hindgut to improve health and performance in cattle. *Animals* **10**, 1817–1835.

Sanz-Fernandez MV, Seymour DJ, Daniel JB, Doelman J and Martín-Tereso J (2022) Effect of hydrogenated fat-embedded calcium gluconate on lactation performance in commercial settings. *Journal of Dairy Science* **105**, 154–155.

Seymour DJ, Carson M, Daniel JB, Sanz-Fernandez MV, Martín-Tereso J and Doelman J (2020) Effect of fat-embedded calcium gluconate on lactation performance in high-yielding multiparous dairy cows in a commercial dairy setting. *Journal of Animal Science* **98**, 146–147.

Seymour DJ, Sanz-Fernandez MV, Daniel JB, Martín-Tereso J and Doelman J (2021) Effects of supplemental calcium gluconate embedded in a hydrogenated fat matrix on lactation, digestive, and metabolic variables in dairy cattle. *Journal of Dairy Science* **104**, 7845–7855.

Seymour DJ, McKnight L, Carson M, Sanz-Fernandez MV, Daniel JB, Metcalf JA, Martín-Tereso J and Doelman J (2022) Effect of hydrogenated fat-embedded calcium gluconate on lactation performance in dairy cows. *Canadian Journal of Animal Science* **102**, 518–527.

Tsukahara T, Koyama H, Okada M and Ushida K (2002) Stimulation of butyrate production by gluconic acid in batch culture of pig cecal digesta and identification of butyrate-producing bacteria. *Journal of Nutrition* **132**, 2229–2234.

Tsukahara T, Hashizume K, Koyama H and Ushida K (2006) Stimulation of butyrate production through the metabolic interaction among lactic acid bacteria, *Lactobacillus acidophilus*, and lactic acid-utilizing bacteria, *Megasphaera elsdenii*, in porcine cecal digesta. *Animal Science Journal* **77**, 454–461.