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# Effect of hydrogenated fat-embedded calcium gluconate on lactation performance in dairy cows

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## Abstract

Hydrogenated fat-embedded calcium gluconate (HFCG), a prebiotic mixture designed to target the hindgut, has improved milk and component yields when supplemented in mid-lactation cows, likely due to improved hindgut health. The objective of this study was to evaluate production responses to HFCG when fed to dairy cattle over a full lactation. Seventy-four Holstein cows (21 primiparous, 53 multiparous) were used in a randomized complete block design comparing supplementation with HFCG (approximately 16 g/day supplement delivering approximately 6.4 g active ingredient) to a negative control from approximately 21 days prior to calving until end of lactation. In multiparous cattle supplemented with HFCG, average daily milk protein yield ( $P = 0.037$ ) was increased during the first 8 weeks of lactation, while average daily yields of milk fat, and fat- and energy-corrected milk tended ( $P \leq 0.075$ ) to increase over the same period of time. Increased yields were likely supported by the concurrent increase in dry matter intake ( $P = 0.036$ ). Future work is needed to characterize the mode of action of this product within both the hindgut lumen and host, as well as investigate the potential differential responses between primiparous and multiparous animals over the course of lactation.

**Key words:** prebiotic, hindgut, milk protein

## Résumé

Le gluconate de calcium incorporé au gras hydrogéné (HFCG — «hydrogenated fat-embedded calcium gluconate»), un mélange prébiotique conçu pour cibler l'intestin postérieur, a amélioré les rendements de lait et des composantes, lorsqu'ajoutés comme supplément auprès des vaches en mi-lactation, probablement imputable à une santé améliorée de l'intestin postérieur. L'objectif de cette étude était d'évaluer les réponses de production au HFCG, lorsque donné aux bovins laitiers sur une lactation complète. Soixante-quatorze vaches holsteins (21 primipares, 53 multipares) ont été utilisées dans un design expérimental à bloc complètement aléatoire comparant la supplémentation avec soit le HFCG (approximativement 16 g/jour du supplément, livrant approximativement 6,4 g de l'ingrédient actif) ou un témoin négatif, à partir de 21 jours avant le vêlage jusqu'à la fin de la lactation. Chez les bovins multipares avec suppléments de HFCG, le rendement moyen quotidien en protéines du lait ( $P = 0,037$ ) était augmenté durant les 8 premières semaines de lactation, tandis que les rendements moyens quotidiens de gras du lait et de lait corrigé pour le gras et l'énergie tendaient ( $P \leq 0,075$ ) à augmenter au cours de la même période de temps. Les rendements améliorés étaient probablement supportés par l'augmentation simultanée de la consommation de matières sèches ( $P = 0,036$ ). Des études ultérieures sont nécessaires afin de caractériser le mode d'action de ce produit dans le lumen de l'intestin postérieur et l'hôte, ainsi que pour étudier les réponses différentielles potentielles entre les animaux primipares et multipares au cours de la lactation. [Traduit par la Rédaction]

**Mots-clés :** prébiotique, intestin postérieur, protéines du lait

## Introduction

Historically, the rumen has been a key focus area for advancements in many aspects of ruminant nutrition; however, there is growing interest in the role of the hindgut in relation to overall animal health and performance. Due to a variety of physiological and functional factors, the hindgut is typically considered to be less robust than the rumen with regard

to maintaining gut barrier integrity (as recently reviewed by Sanz-Fernandez et al. 2020), and is thus more susceptible to dysbiosis, such as that resulting from acidotic events (e.g., Petri et al. 2021; van Gastelen et al. 2021). A preventative nutritional intervention worth exploring is the application of prebiotic compounds (Gibson et al. 2004) specifically targeting the hindgut, translating advancements from human

nutrition and applications used in non-ruminant agricultural species (as reviewed by [Gaggia et al. 2010](#); [Uyeno et al. 2015](#)).

Gluconic acid salts (sodium or calcium gluconate) have previously been shown to elicit a positive performance response in non-ruminant species ([Biagi et al. 2006](#); [Poeikhampha and Bunchasak 2011](#); [Michiels et al. 2020](#)). Gluconic acid has been shown to pass through the small intestine of rats with low levels of absorption ([Asano et al. 1994](#)), supporting the hypothesis that these responses are mediated through a stimulation of volatile fatty acid production, predominantly butyrate, by the hindgut microbiota ([Tsukahara et al. 2002, 2006](#)), though the underlying mechanisms remain poorly characterized (as reviewed by [den Besten et al. 2013](#); [Liu et al. 2018](#)). Our group has described a milk fat response in lactating dairy cattle when calcium gluconate was infused post-rotationally ([McKnight et al. 2019](#); [Doelman et al. 2019b](#)), which was not observed when unprotected calcium gluconate was fermented in the rumen ([McKnight et al. 2019](#)). More recently, we have observed similar responses when feeding a supplement consisting of calcium gluconate embedded in a matrix of hydrogenated fat (HFCG) to mid-lactation cows for 21 days in a Latin square design ([Seymour et al. 2021b](#)). The objective of the present study was to evaluate the milk production response of lactating dairy cattle fed the same HFCG supplement over the course of an entire lactation.

## Materials and methods

All procedures involving animals in the present study were approved by the animal care committee of Trouw Nutrition Canada (Guelph, ON, Canada) in compliance with Canadian Council for Animal Care guidelines. Animals were housed in the Dairy Research Facility at Trouw Nutrition Agresearch (Burford, ON, Canada) between December 2015 and October 2017. The response in lactation performance to supplementation with HFCG was evaluated using a randomized complete block design. Statistical power dimensioning was based on a herd average milk fat yield of 1265 g/day, a standard deviation of 190 g/day, an expected treatment response of 130 g/day ([Doelman et al. 2019b](#); [McKnight et al. 2019](#)), and a type I error rate of 0.05. Accordingly, it was estimated that 35 animals per treatment would achieve a statistical power of 0.8. Animals were blocked by parity, milk fat yield, and expected calving date. For multiparous animals, the milk fat yield of the previous lactation was used as blocking criteria, whereas the expected milk fat yield based on genetic evaluation was used for primiparous animals. Beginning at  $21 \pm 3$  days prior to expected calving, animals within blocks were randomly assigned to either the negative control (CON) or HFCG treatments. At the outset of the experiment, 11 primiparous and 29 multiparous animals were assigned to each treatment group; due to adverse health events unrelated to the study, 2 multiparous animals were removed from the control group, and 5 animals (1 primiparous, 4 multiparous) were removed from the HFCG group, resulting in a final animal number of 21 primiparous and 53 multiparous cattle.

Animals assigned to the HFCG treatment were offered a total mixed ration (TMR; [Table 1](#)) with the HFCG supplement (Trouw Nutrition, Amersfoort, the Netherlands). The supple-

ment was composed of 50% hydrogenated palm fat, 40% calcium gluconate, and 10% calcium carbonate, and was included at a rate of 4.3 kg/tonne in the dry period compound feed and 1.37 kg/tonne in the lactation compound feed, to achieve a targeted intake of the HFCG product of approximately 16 g/day based on a previous pilot study ([Doelman et al. 2019a](#)). Animals assigned to the CON treatment were offered the same basal TMRs without the supplement. Animals were enrolled in the study for the entirety of their lactation. Fifty-nine animals reached the predetermined endpoint of 44 weeks of lactation (i.e.,  $307 \pm 4$  days in milk (DIM)), while 15 animals (2 primiparous, 13 multiparous) underwent involuntary dry-off after 40 weeks of lactation ( $280 \pm 4$  DIM). One additional primiparous animal underwent involuntary dry-off at 215 DIM and was excluded from subsequent analyses.

During the dry period, animals were housed in group pens with ad libitum access to both feed and water, with individual daily feed intake monitored using a Calan Broadbent Feeding System (American Calan, Northwood, NH, USA). After calving, animals were moved to the lactating barn where they were housed in individual tie stalls. During lactation, daily individual feed intake was measured by manually weighing refusals when fresh feed was delivered the following day. Dry matter (DM) content of feeds was determined weekly and used to estimate DMI, and samples of all feedstuffs were collected every 2 months and analyzed for nutrient content by near-infrared spectroscopy (forages) or wet chemistry (ingredients; Shur-Gain Laboratory, St. Hyacinthe, QC, Canada). Bodyweight and body condition score were evaluated monthly over the course of each animal's lactation.

Animals were milked in place twice daily at approximately 0500 and 1600 h, and milk weights were recorded at each milking. Milk samples were collected for the determination of milk components at weekly intervals during the first month of lactation (i.e., weeks 1–4), followed by monthly intervals for the remainder of lactation (i.e., weeks 8–44). During sampling weeks, milk samples were collected at both milkings on the last 3 days of that week using the autosampler of the milking equipment to ensure sample homogeneity from that milking. Samples were stored at 4 °C until analyzed for milk fat, protein, and lactose concentration by mid-infrared spectroscopy at the University of Guelph Agriculture and Food Laboratory (Guelph, , Canada). Average milk component concentrations were used to estimate average milk component yields for each sampling week; milk component yields were subsequently used to estimate 4% fat-corrected milk (FCM) yield as  $0.4 \times \text{milk yield (kg/day)} + 15 \times \text{milk fat yield (kg/day)}$  ([Gaines 1928](#)) and energy-corrected milk (ECM) yield as  $0.01 \times \text{milk yield (kg/day)} + 12.2 \times \text{milk fat yield (kg/day)} + 7.7 \times \text{milk protein yield (kg/day)} + 5.3 \times \text{milk lactose yield (kg/day)}$  ([Sjaunja et al. 1990](#)). Gross feed efficiency (GFE) was calculated as the ratio of ECM (kg/day) to DMI (kg/day).

To evaluate cumulative differences in milk and component yields over the entire lactation, 305-d yields of milk, fat, and protein, and the average concentrations of fat and protein were retrieved from dairy herd improvement records reported by Lactanet (Guelph, ON, Canada). Using these records, total lactation FCM yield was calculated as previously de-

**Table 1.** Average formulation and chemical composition of basal total mixed rations offered over the experimental period.

	Dry	Lactating
Ingredient		
Corn silage	41.4 ± 3.17	27.0 ± 2.66
Compound feed	25.5 ± 1.78 <sup>a</sup>	43.4 ± 4.46 <sup>b</sup>
Haylage	14.9 ± 4.04	26.5 ± 2.84
Wheat straw	12.0 ± 2.83	0.63 ± 0.798
Grass hay	6.28 ± 6.65	2.48 ± 1.889
Composition		
Dry matter (%)	52.1 ± 4.06	55.2 ± 3.59
Net energy for lactation (NE <sub>L</sub> ; Mcal/kg)	1.43 ± 0.052	1.80 ± 0.029
CP	15.5 ± 0.24	17.1 ± 0.58
NFC	26.3 ± 3.88	35.9 ± 4.09
NDF	39.5 ± 1.97	32.2 ± 3.25
ADF	28.0 ± 0.97	21.0 ± 0.87
Ether extract	2.77 ± 0.292	4.53 ± 0.078
Ash	4.73 ± 0.729	5.94 ± 0.889
Ca	1.13 ± 0.039	0.959 ± 0.2125
P	0.367 ± 0.129	0.419 ± 0.0359
Na	0.141 ± 0.0186	0.387 ± 0.0132
K	1.15 ± 0.074	1.37 ± 0.067
Vitamin A (IU/kg DM)	7210 ± 157.5	4910 ± 10.7
Vitamin D (IU/kg DM)	2090 ± 46.1	1340 ± 1.3
Vitamin E (IU/kg DM)	100 ± 2.2	35.6 ± 0.03

**Note:** Values are presented as mean ± standard deviation in percentage DM unless indicated otherwise.

<sup>a</sup>Contained on average (% product basis) corn dried distiller's grains (28.5), corn gluten meal (26.5), oat hulls (20.8), mineral premix (6.46; Propulsion, Trouw Nutrition, Puslinch, ON, Canada), calcium carbonate (5.60), feather meal (3.89), magnesium oxide (1.86), methionine (1.67; Alimet, Novus International, Saint Charles, MO, USA), fat supplement (1.47; Stay Fat, Darling Ingredients Canada, Cambridge, ON, Canada), calcium sulfate (1.15), sodium chloride (1.07), dicalcium phosphate (0.932), and monensin (0.0340; Rumensin, Elanco, Greenfield, IN, USA).

<sup>b</sup>Contained on average (% product basis) ground corn (33.6), corn gluten feed (12.9), wheat shorts (9.22), bypass soybean meal (9.09; Top Soy, Trouw Nutrition), corn dried distiller's grains (8.46), bakery waste (6.11), fine rolled corn (6.09), pork meal (2.52), feather meal (2.13), palmitic acid (1.86; APF+, Trouw Nutrition), calcium carbonate (1.58), blood meal (1.33), fat supplement (1.32; Stay Fat, Darling Ingredients Canada), sodium chloride (1.23), sodium sesquicarbonate (1.06), magnesium oxide (0.536), mineral premix (0.244; Nutri-Plex Dairy Mineral 2, Trouw Nutrition), urea (0.215), rumen modifier (0.173; RM104, Trouw Nutrition), methionine (0.135; Alimet, Novus International), selenium (0.132; Selenium FSP 500, Trouw Nutrition), yeast (0.102; BioPower SC10ME, Lallemand Animal Nutrition, Montréal, QC, Canada), and monensin (0.0187; Rumensin, Elanco).

scribed. As lactose content is not reported as part of the dairy herd improvement program, fat- and protein-corrected milk (FPCM) yield was calculated instead of ECM using the formula  $FPCM = 0.25 \times \text{milk yield (kg)} + 12.2 \times \text{milk fat yield (kg)} + 7.7 \times \text{milk protein yield (kg)}$ ; [Sjaunja et al. 1990](#)).

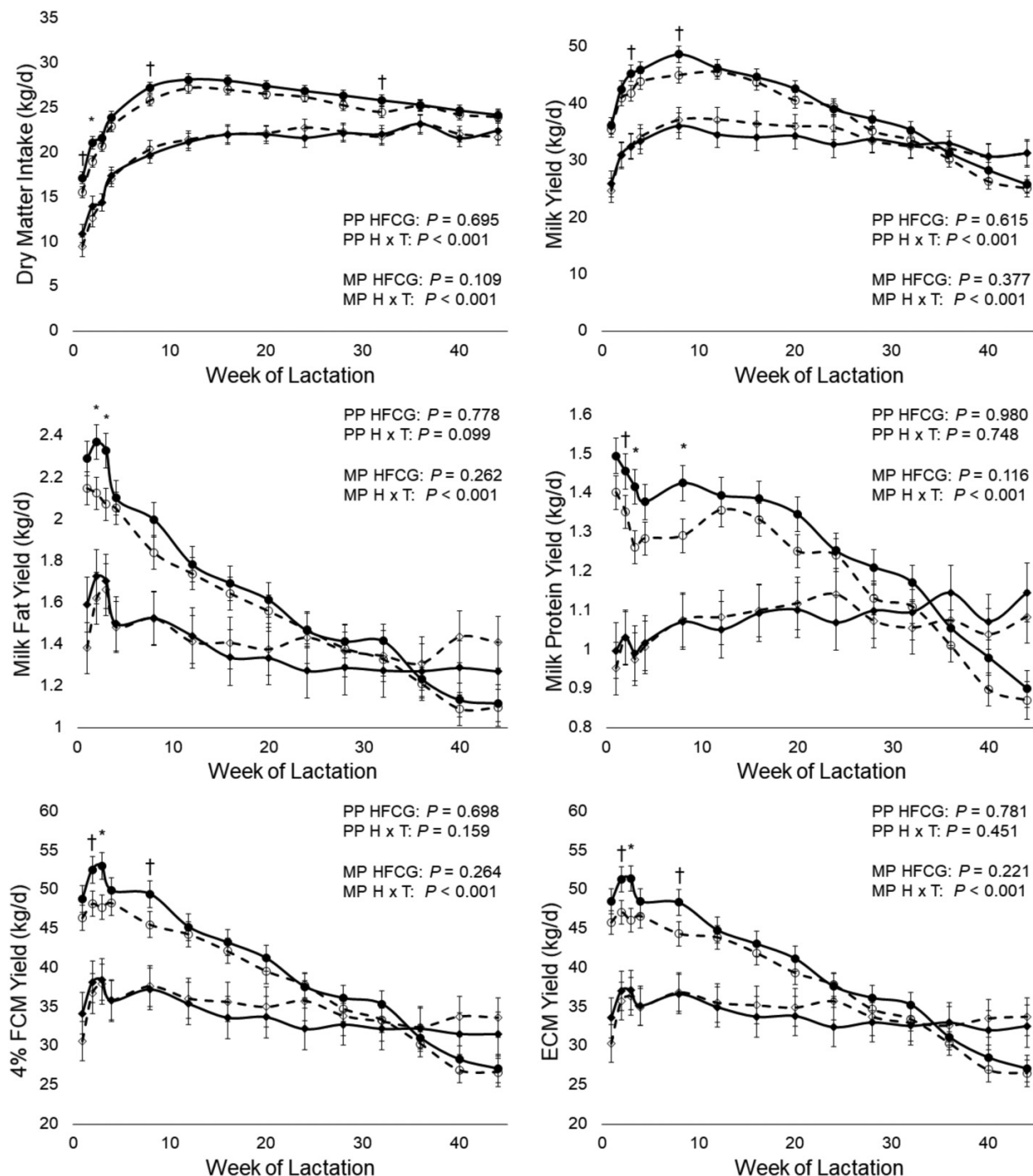
Individual lactation curves were modeled using the equation of [Dijkstra et al. \(1997\)](#):

$$(1) \quad M = M_0 \exp \left\{ \frac{\mu_T [1 - \exp(-k_2 t)]}{k_2} - \lambda t \right\}$$

where  $M$  is the daily milk yield (kg/day) on day in milk  $t$ ;  $M_0$  is the theoretical milk yield (kg/day) at the onset of lactation (i.e.,  $t = 0$ );  $\mu_T$  is the specific rate of secretory cell proliferation ( $d^{-1}$ ) at parturition (i.e.,  $t = 0$ );  $k_2$  is the specific rate of decay of cell proliferation ( $d^{-1}$ ); and  $\lambda$  is the specific

rate of secretory cell apoptosis ( $d^{-1}$ ), which is also indicative of the terminal decline in daily milk yield in late lactation ( $\% d^{-1}$ ; [Seymour et al. 2021a](#)). Parameters were estimated using the NLIN procedure of SAS v 9.4 (SAS Institute Inc., Cary, NC) using the Marquardt estimation method with a maximum of 3000 iterations and 300 subiterations. A range of initial values were used for each parameter as follows: 0.5–30 by 5 for  $M_0$ ; 0.01–0.1 by 0.01 for both  $\mu_T$  and  $k_2$ ; and 0.001–0.01 by 0.001 for  $\lambda$  ([Pot 2020](#); [Seymour et al. 2021a](#)). All parameter estimates were constrained to be greater than zero. After excluding lactation curves that could not be adequately fit with the available data or with parameter estimates that violated the greater-than-zero constraint (7 multiparous, 2 primiparous), 65 valid sets of lactation curve parameters were used for subsequent analyses. As described by [Dijkstra et al. \(1997\)](#), days to peak milk yield ( $t_p$ , d) for each lactation

**Fig. 1.** Temporal responses in dry matter intake and yields of milk and milk components in primiparous ( $n = 21$ ; diamonds) and multiparous ( $n = 53$ ; circles) Holstein dairy cattle supplemented with hydrogenated fat-embedded calcium gluconate for at least 40 weeks of lactation. PP, effect within primiparous group; MP, effect within multiparous group; HFCG, effect of treatment given parity group;  $H \times T$ , treatment-by-time interaction effect given parity group. 4% FCM, 4% fat-corrected milk yield; ECM, energy-corrected milk yield. Error bars represent standard errors of the means. Filled shapes with solid lines: hydrogenated fat-embedded calcium gluconate; empty shapes with dashed lines: negative control; \*:  $P < 0.05$ ; †:  $0.05 \leq P < 0.15$ .



curve was calculated as

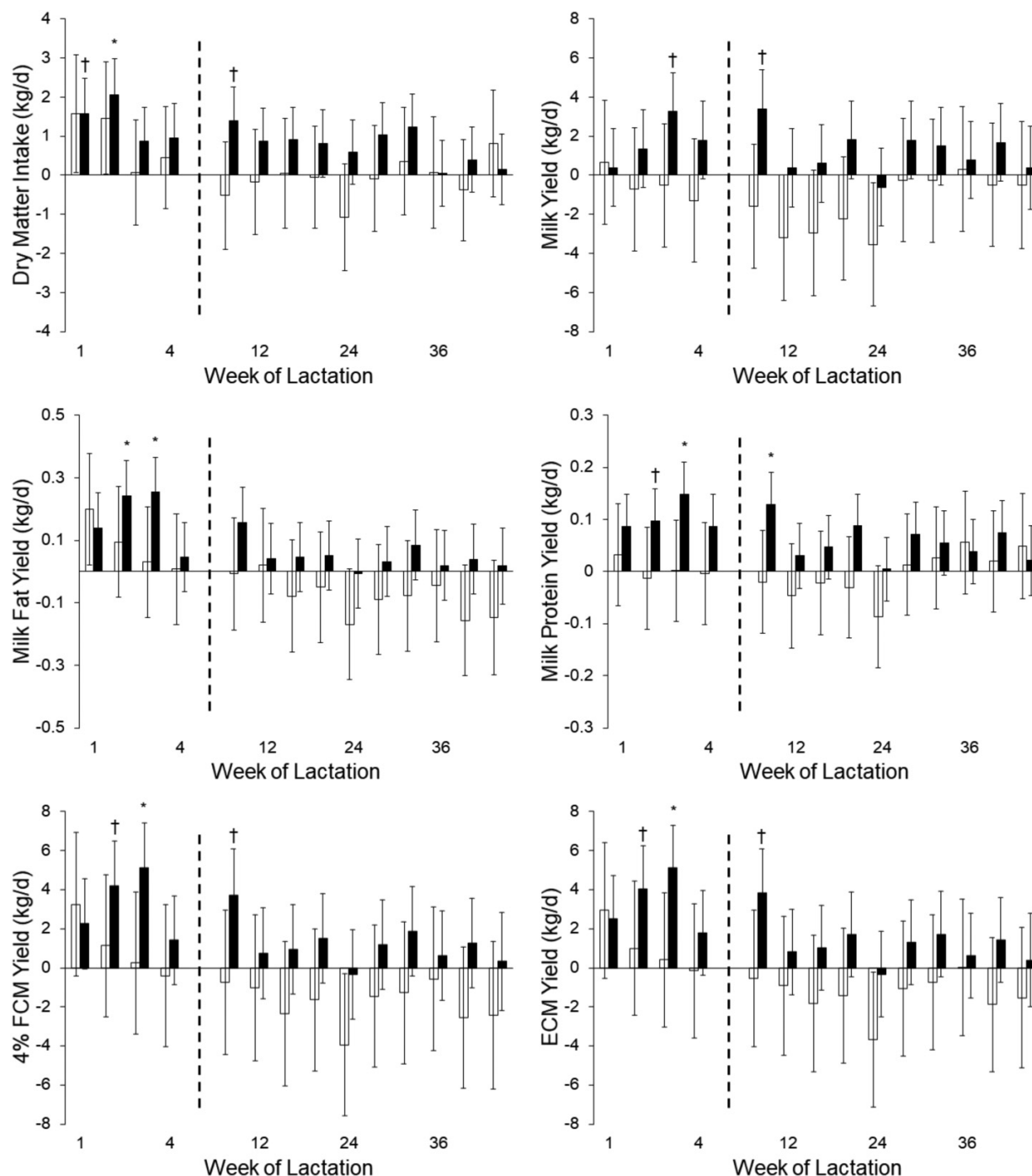
$$(2) \quad t_p = \frac{\ln(\mu_T/\lambda)}{k_2}$$

while peak milk yield ( $M_p$ , kg/day) was calculated as

$$(3) \quad M_p = M_0 \left( \frac{\lambda}{\mu_T} \right)^{\lambda/k_2} \exp \left( \frac{\mu_T - \lambda}{k_2} \right)$$



**Fig. 2.** Relative responses in dry matter intake and yields of milk and milk components in primiparous ( $n = 21$ ; white bars) and multiparous ( $n = 53$ ; black bars) Holstein dairy cattle supplemented with hydrogenated fat-embedded calcium gluconate for at least 40 weeks of lactation. Bars represent response relative to negative control within parity. Error bars represent standard error of the difference. Dashed line represents transition from weekly to monthly sampling. 4% FCM, 4% fat-corrected milk yield; ECM, energy-corrected milk yield. \*:  $P < 0.05$ ; †:  $0.05 \leq P < 0.15$ .



All data were analyzed using the GLIMMIX procedure of SAS using a Newton–Raphson optimization with ridging. Preliminary analyses indicated the presence of potential treatment–parity group interactions (e.g., Fig. 1). As such, responses evaluated over the course of lactation (e.g., daily milk and component yields, dry matter intake.) were modelled as a factorial arrangement of the fixed effects of par-

ity group (primiparous or multiparous), treatment (CON or HFCG), and week of lactation. The effects of block and animal within block were considered random. Correlated errors due to repeated sampling of animals within blocks were modelled using a spatial power covariance structure. For outcomes measured once per animal (i.e., 305-d milk and component yields, lactation curve parameters), the effect of week

of lactation and associated interactions were removed from the model, and a compound symmetry residual (co)variance structure was used to accommodate negative covariance between blocks. Denominator degrees of freedom for comparisons were computed using the Kenward–Roger correction. Tests of simple effects and differences in least-squares means within each parity group were requested using the SLICE and SLICEDIFF options, respectively, of the LSMEANS statement. Pairwise comparisons of HFCG versus CON within parity group and sampling week were requested using the SLICEDIFF option. Preliminary analyses suggested that the magnitude of production responses was greatest in the first 8 weeks of lactation; best linear unbiased estimates for each parity group–treatment combination during this period were constructed, and treatment differences within parity group during this time period were evaluated using orthogonal contrasts. Statistical significance was declared where  $P < 0.05$ , and responses were considered trends where  $0.05 \leq P < 0.15$ .

## Results

$P$ -values for the parity–treatment–week interaction effect for all outcomes were above 0.900. As outlined by [Stroup et al. \(2018\)](#), the statistical tests of interaction effects are often drastically underpowered due to the multiple degrees of freedom involved, which warrants the investigation of simple effects (e.g., treatment given parity group) to properly evaluate the presence of potential underlying effects that are tested with fewer degrees of freedom. Following such an analysis, differential responses were observed between primiparous and multiparous animals, as well as across time. The magnitude of responses appeared to be greatest during the first 8 weeks of lactation, as highlighted in [Figs. 1 and 2](#). No differences in milk component concentrations, GFE, bodyweight, or body condition score were observed due to treatment ( $P \geq 0.184$ ) within either parity group.

Production responses over the first 8 weeks of lactation in multiparous cows are presented in [Table 2](#). Milk protein yield was increased by 8.2% ( $P = 0.037$ ) and milk fat yield tended to increase by 8.2% ( $P = 0.056$ ). This increase in milk component yields drove tendencies for increased yields of both FCM (7.0%;  $P = 0.075$ ) and ECM (7.5%;  $P = 0.055$ ). To support these increased yields, DMI increased by 6.6% ( $P = 0.036$ ) in early lactation. No statistically significant differences between treatment groups were observed in full lactation yields of milk or milk components, nor parameters describing individual lactation curves ([Table 3](#)).

Production responses over the first 8 weeks of lactation in primiparous animals are presented in [Table 4](#). Overall, no statistically significant responses due to treatment were observed for any outcome. While a significant treatment-by-time interaction was observed for most outcomes, it was determined that this was due to the effect of time within treatment (due to the natural shape of the lactation curve), as pairwise comparisons of HFCG versus control animals at each week of lactation were not statistically significant. No differences were observed in full lactation milk and component yields ([Table 5](#)), nor in estimates of lactation curve parameters.

**Table 2.** Early lactation (weeks 1–8) production responses in multiparous Holstein cattle ( $n = 53$ ) supplemented with hydrogenated fat-embedded calcium gluconate for at least 40 weeks of lactation.

Variable	Treatment <sup>a</sup>			$P$ value
	CON	HFCG	SED <sup>b</sup>	
DMI	20.7	22.1	0.64	0.036
Milk yield	42.0	44.1	1.75	0.247
Fat content (%)	5.00	5.09	0.160	0.570
Protein content (%)	3.25	3.33	0.065	0.195
Lactose content (%)	4.74	4.77	0.047	0.567
Fat yield	2.06	2.23	0.067	0.056
Protein yield	1.33	1.44	0.052	0.037
Lactose yield	2.00	2.11	0.085	0.225
FCM yield <sup>c</sup>	47.7	51.0	1.87	0.075
ECM yield <sup>d</sup>	46.4	49.9	1.79	0.055
GFE <sup>e</sup> (kg/kg)	2.40	2.36	0.077	0.606
BW	731	741	17.6	0.567
BCS	3.00	2.98	0.050	0.800

**Note:** Values are in units of kg/day unless noted otherwise.

<sup>a</sup>CON: negative control; HFCG: hydrogenated fat-embedded calcium gluconate.

<sup>b</sup>Standard error of the difference.

<sup>c</sup>4% fat-corrected milk yield:  $0.4 \times \text{milk yield} + 15 \times \text{fat yield}$ .

<sup>d</sup>Energy-corrected milk yield:  $0.01 \times \text{milk yield} + 12.2 \times \text{fat yield} + 7.7 \times \text{protein yield} + 5.3 \times \text{lactose yield}$ .

<sup>e</sup>ECM yield (kg/day): DMI (kg/day).

## Discussion

In the present study, the responses in yields of both milk fat and protein were larger in multiparous animals during the first 8 weeks of lactation, partially explained by an increase in DMI. During this period, animals supplemented with HFCG consumed an additional 1.37 kg of DM per day relative to control, estimated to supply approximately 62 g of additional fat. As milk fat yield tended to increase by 168 g/day during this period, additional factors likely influenced this response. The increase in milk fat yield above that supplied by the diet may be the result of increased incorporation of pre-formed fatty acids of endogenous origin, as demonstrated in previous work where HFCG supplementation also resulted in increased circulating NEFA ([Seymour et al. 2021b](#)). Animals would likely have been mobilizing body reserves to support lactation during this time ([Drackley 2016](#); [Phuong et al. 2016](#); [Seymour et al. 2020a](#)); however, the partitioning of mobilized fatty acids between milk fat and oxidation by peripheral tissues remains unclear. No differences in bodyweight or body condition scores were observed between treatment groups, though these measures likely would not accurately capture potential changes in body fat reserves. In contrast, the observed increase in milk protein yield was most likely supported by increased DMI, which is estimated to have supplied an additional 234 g crude protein per day relative to control, though it is possible that the mobilization of endogenous protein sources contributed to milk protein yield in the first weeks of lactation ([Bell 1995](#)). The lack of statistical difference between treatment groups when examining full lactation milk and component yields serves to support our observa-

**Table 3.** Responses in full lactation milk and component yields ( $n = 51$ ), and differences in lactation curve parameter estimates ( $n = 46$ ) in multiparous Holstein cattle supplemented with hydrogenated fat-embedded calcium gluconate for least 40 weeks of lactation.

	Treatment <sup>a</sup>		SED <sup>b</sup>	P value
	CON	HFCG		
Lactation yields				
Milk yield (kg)	11 700	12 200	466	0.320
Fat content (%)	3.74	3.83	0.164	0.609
Protein content (%)	3.10	3.11	0.043	0.923
Fat yield (kg)	434	465	21.7	0.166
Protein yield (kg)	360	377	14.4	0.248
FCM <sup>c</sup> yield (kg)	11 200	11 800	461	0.164
FPCM <sup>d</sup> yield (kg)	11 000	11 600	433	0.150
Lactation curve parameters <sup>e</sup>				
M <sub>0</sub> (kg/day)	27.5	25.3	3.34	0.513
μ <sub>T</sub> (d <sup>-1</sup> )	0.097	0.111	0.0276	0.636
k <sub>2</sub> (d <sup>-1</sup> )	0.0699	0.0702	0.01974	0.986
λ (d <sup>-1</sup> )	0.00076	0.00353	0.000879	0.439
M <sub>P</sub> (kg/day)	47.5	49.1	1.91	0.399
t <sub>P</sub> (days)	59.4	56.7	10.00	0.788

<sup>a</sup>CON: negative control; HFCG: hydrogenated fat-embedded calcium gluconate.

<sup>b</sup>Standard error of the difference.

<sup>c</sup>4% fat-corrected milk yield:  $0.4 \times \text{milk yield} + 15 \times \text{fat yield}$ .

<sup>d</sup>Fat- and protein-corrected milk yield:  $0.25 \times \text{milk yield} + 12.2 \times \text{milk fat yield} + 7.7 \times \text{milk protein yield}$ .

<sup>e</sup> $M_0$ : theoretical milk yield at onset of lactation;  $\mu_T$ : specific rate of secretory cell proliferation;  $k_2$ : rate of decay of cell proliferation;  $\lambda$ : specific rate of secretory cell apoptosis;  $M_P$ : peak milk yield;  $t_P$ : days to peak milk yield.

**Table 4.** Early lactation (weeks 1–8) production responses in primiparous Holstein cattle ( $n = 21$ ) supplemented with hydrogenated fat-embedded calcium gluconate for at least 40 weeks of lactation.

Variable	Treatment <sup>a</sup>		SED <sup>b</sup>	P-value
	CON	HFCG		
DMI	14.7	15.3	1.02	0.555
Milk yield	32.3	31.6	2.77	0.802
Fat content (%)	4.86	5.13	0.253	0.299
Protein content (%)	3.20	3.25	0.103	0.667
Lactose content (%)	4.76	4.82	0.075	0.446
Fat yield	1.54	1.60	0.138	0.638
Protein yield	1.01	1.01	0.083	0.994
Lactose yield	1.54	1.53	0.135	0.943
FCM yield <sup>c</sup>	36.0	36.7	2.96	0.814
ECM yield <sup>d</sup>	35.1	35.8	2.85	0.797
GFE <sup>e</sup> (kg/kg)	2.56	2.40	0.122	0.184
BW	614	639	27.6	0.380
BCS	3.03	3.01	0.079	0.777

**Note:** Values are in units of kg/day unless noted otherwise.

<sup>a</sup>CON: negative control; HFCG: hydrogenated fat-embedded calcium gluconate.

<sup>b</sup>Standard error of the difference.

<sup>c</sup>4% fat-corrected milk yield:  $0.4 \times \text{milk yield} + 15 \times \text{fat yield}$ .

<sup>d</sup>Energy-corrected milk yield:  $0.01 \times \text{milk yield} + 12.2 \times \text{fat yield} + 7.7 \times \text{protein yield} + 5.3 \times \text{lactose yield}$ .

<sup>e</sup>ECM yield (kg/day): DMI (kg/day).



**Table 5.** Responses in full lactation milk and component yields ( $n = 21$ ), and differences in lactation curve parameter estimates ( $n = 19$ ) in primiparous Holstein cattle supplemented with hydrogenated fat-embedded calcium gluconate for least 40 weeks of lactation.

	Treatment <sup>a</sup>		SED <sup>b</sup>	P-value
	CON	HFCG		
Lactation yields				
Milk yield (kg)	10 600	10 000	727	0.450
Fat content (%)	3.81	3.76	0.254	0.855
Protein content (%)	3.10	3.18	0.067	0.250
Fat yield (kg)	399	376	33.8	0.507
Protein yield (kg)	326	330	22.3	0.851
FCM <sup>c</sup> yield (kg)	10 200	9650	719	0.430
FPCM <sup>d</sup> yield (kg)	10 000	9640	676	0.561
Lactation curve parameters <sup>e</sup>				
M <sub>0</sub> (kg/day)	19.5	17.1	5.21	0.645
μ <sub>T</sub> (d <sup>-1</sup> )	0.101	0.131	0.0435	0.499
k <sub>2</sub> (d <sup>-1</sup> )	0.0783	0.0974	0.03 098	0.546
λ (d <sup>-1</sup> )	0.00 132	0.00 076	0.001 366	0.683
M <sub>P</sub> (kg/day)	39.8	36.1	2.98	0.221
t <sub>P</sub> (days)	68.2	85.6	15.62	0.278

<sup>a</sup>CON: negative control; HFCG: hydrogenated fat-embedded calcium gluconate.

<sup>b</sup>Standard error of the difference.

<sup>c</sup>4% fat-corrected milk yield:  $0.4 \times \text{milk yield} + 15 \times \text{fat yield}$ .

<sup>d</sup>Fat- and protein-corrected milk yield:  $0.25 \times \text{milk yield} + 12.2 \times \text{milk fat yield} + 7.7 \times \text{milk protein yield}$ .

<sup>e</sup> $M_0$ : theoretical milk yield at onset of lactation;  $\mu_T$ : specific rate of secretory cell proliferation;  $k_2$ : rate of decay of cell proliferation;  $\lambda$ : specific rate of secretory cell apoptosis;  $M_P$ : peak milk yield;  $t_P$ : days to peak milk yield.

tions of a positive response predominantly in early lactation, rather than a consistent response across the entire lactation.

The responses in milk fat and protein yield in early lactation observed in the present study were more pronounced than those in a similar study using multiparous cows over the same time frame; when comparing the response to HFCG supplementation in both the dry period and early lactation (Seymour et al. 2020b), average daily yields of milk fat and protein increased numerically by 1.65% and 0.33%, respectively, in the first 8 weeks of lactation, in contrast to the approximately 8% increase in both milk fat and protein observed presently over the same period. It is possible that differences in climate, genetic potential, and management practices contributed to this differential response to HFCG supplementation, as yields of milk fat and protein in negative control animals were approximately 149% and 65% greater, respectively, than the negative control animals in Seymour et al. (2020b); however, this can neither be confirmed nor rejected with the available data.

Calcium gluconate has been observed to alter volatile fatty acid production, particularly increasing the synthesis of butyrate, in the hindgut (Asano et al. 1994; Tsukahara et al. 2002, 2006). McKnight et al. (2019) observed differential responses between feeding unprotected calcium gluconate and post-ruminal delivery of the same compound, highlighting the need for calcium gluconate to circumvent extensive fermentation in the rumen. Given the production responses in multiparous animals in this study and other studies with the same HFCG supplement (Doelman et al. 2019a; Seymour et

al. 2021b) are similar to those observed with post-ruminal infusion of calcium gluconate (Doelman et al. 2019b; McKnight et al. 2019), this suggests that the fat matrix of the HFCG supplement is able to provide protection from the rumen environment to allow a sufficient amount of calcium gluconate to reach the lower parts of the digestive tract intact. Dietary butyrate has been implicated in improvements in gut health and epithelial integrity, as well as in responses in whole body lipid and energy metabolism (as reviewed by den Besten et al. (2013) and Liu et al. (2018)). Additionally, improvements in gut barrier function could potentially reduce the infiltration of xenobiotic compounds and thus reduce the energy spent mounting an immune response (Fox et al. 2005; Kvidera et al. 2017). The HFCG supplement contained 10% calcium carbonate, which could potentially act as a buffer in the gut lumen and also support improved gut health through modulation of luminal pH. However, without additional data pertaining to blood and (or) tissue metabolite concentrations, these hypotheses remain speculative at present.

Lactation curve parameter estimates, while not statistically different between treatment groups, were similar to those previously reported for both primiparous and multiparous Holstein dairy cattle (Dematawewa et al. 2007; Dijkstra et al. 2010; Seymour et al. 2021a). It is of note, however, that the model evaluated in the present study does not account for changes in milk component yields. The model proposed by Dijkstra et al. (1997) has previously been found to poorly fit yields of FCM and ECM in primiparous animals due to the changes in milk component concentrations over time; the

high content of fat and protein in early lactation with decreasing concentrations as lactation progresses serves to flatten the ECM and FCM curves of primiparous animals, resulting in issues generating accurate parameter estimates from these curves (Seymour et al. 2021a). The response, or lack thereof, in primiparous animals is currently unclear; as this study was not designed to properly evaluate potential response differences between parity groups, these findings are likely the result of insufficient statistical power due to either the small number of primiparous animals used, responses too small to detect given the variation in the primiparous group, or a combination of these factors. On a biological basis, this may be due to the naturally lower levels of production in heifers, as well as differences in the regulation of nutrient partitioning between the concurrent demands of lactation and growth. Additionally, the inclusion rate of HFCG (approximately 0.07% of DMI) was based on intakes of multiparous animals. Primiparous animals assigned the HFCG treatment consumed approximately 10.7 g/day of the HFCG supplement. It is possible that this inclusion rate is insufficient to achieve an effective daily intake of the active ingredient for primiparous animals. This warrants further work evaluating the supplementation of HFCG in primiparous cows specifically.

## Conclusions

Supplementation with an HFCG product increased milk protein yields and tended to increase milk fat yield during the first 8 weeks of lactation in multiparous Holstein cows, driving trends for increased yields of 4% fat-corrected and energy-corrected milks during the same period. This increase in milk production was at least partially supported by increased dry matter intake during this time, with likely contributions from endogenous sources (i.e., body reserves of fat and protein). Model parameter estimates describing individual lactation curves did not differ between treatment groups. The responses in primiparous cattle cannot be confirmed presently due to the lack of statistical power to properly evaluate the responses.

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### Data availability

The data collected in this study are not publicly available due to commercial restrictions.

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### Competing interests

All authors are employees of Trouw Nutrition, which has a commercial interest in the product under investigation.

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