J. Dairy Sci. 108:5968–5981
https://doi.org/10.3168/jds.2024-25928

Investigating the effects of concentrate supplement level and type on milk fat production and animal performance of spring-calving grazing dairy cows

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ABSTRACT

The objective of this experiment was to investigate the effect of concentrate supplement level and type on the milk fat production of grazing dairy cows in early to mid-lactation during a high-risk period for reduced milk fat synthesis. Eighty Holstein Friesian dairy cows averaging (mean ± SD) 55 ± 14 DIM were blocked based on their pre-experimental milk production and parity and randomly assigned to 1 of 5 dietary treatments: a pasture-only (P) control supplemented with 0.27 kg of DM/cow per day of a mineral and vitamin pack (P0); P supplemented with 2 kg of DM/cow per day of an industry-standard concentrate (P2); P supplemented with 4 kg of DM/cow per day of an industry-standard concentrate (P4); P supplemented with 4 kg of DM/cow per day of a concentrate containing 10% sodium hydroxide-treated straw (P4S); and P supplemented with 4 kg of DM/cow per day of a concentrate containing 5% calcium salts of fatty acids (P4F). The experiment consisted of an initial 2-wk covariate period, 1 wk of diet acclimatization, and a 12-wk period of data collection. Concentrate supplement level and type had no effect on milk fat concentration. Increasing the concentrate supplementation level linearly increased milk yield, ECM yield, fat yield, protein yield, lactose yield, and milk solids yield. Cows fed P4F had greater milk yield and lactose yield but lower milk protein concentration compared with cows fed P4 and P4S. Compared with the P4S diet, cows fed the P4F diet had greater milk fat yield and tended to produce greater milk solids yield. Cows fed P4F had lower proportions of de novo and mixed fatty acids (FA), as well as greater proportions of preformed FA compared with cows fed P4 and P4S. Cows fed P2 and P4 increased DM and OM intake compared with cows fed P0; however, cows fed P2 and P4 were similar. The total FA intake of cows fed P4 was greatest (400 g/d), cows fed P2 was intermediate (370 g/d), and cows fed P0 was lowest (330 g/d). Changing the concentrate type had no effect on the intakes of total DM, pasture DM, and OM. These results suggest that, although concentrate level and type can affect milk fat yield, they do not affect the milk fat concentration of grazing dairy cows within the conditions investigated in this experiment. Further research is required to determine the nutritional and non-nutritional factors responsible for reducing milk fat concentration in pasture-based systems during the high-risk period.

Key words: dairy cow, milk fat production, concentrate supplementation, grazing

INTRODUCTION

Milk fat contributes substantially to the economic value of milk, as it can lead to the production of highly nutritious food ingredients for human consumption (Mohan et al., 2021). Milk fat is the most variable component of milk and can be affected by several nutritional and non-nutritional factors (Kalač and Samková, 2010; O'Sullivan et al., 2019). Understanding these factors provides an opportunity to increase the economic and environmental sustainability of pasture-based systems. There has been a considerable increase in annual milk fat concentration over the past decade; however, there seems to be a consistent reduction during the late spring and early summer period in pasture-based systems (CSO, 2024). In spring-calving dairy herds, this period coincides with peak milk yield, leading to the inference that the majority of the reduction in milk fat concentration is due to stage of lactation. Carty et al. (2017) reported that the highest prevalence of reduced milk fat concentration occurred during April and May, with 9% of herds experiencing a reduction in milk fat concentration below 3.3%. Notably, this reduction occurred for both spring- and autumn-calving herds, suggesting that time of year

Received October 27, 2024.
Accepted March 15, 2025.
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The list of standard abbreviations for JDS is available at adsa.org/jds-abbreviations-25. Nonstandard abbreviations are available in the Notes.

might have a greater influence on milk fat concentration than the cow's stage of lactation.

The association between reduced milk fat concentration and time of year has stimulated numerous investigations into both environmental (Salfer et al., 2019) and nutritional (Neville et al., 2023) contributing factors. Salfer and Harvatine (2018) reported that annual rhythms could be responsible for a 0.15% to 0.30% fluctuation in milk fat concentration, with the magnitude of the effect possibly varying by latitude and changes in photoperiod length. Rivero and Anrique (2015) suggested that the reduction in milk fat concentration during late spring and early summer might be related to low concentrations of pasture NDF and greater intakes of UFA during this period. Heffernan et al. (2024) recently demonstrated that grassland management practices that maintain the plant in a more immature state could lead to increased concentrations of plant fatty acids. Furthermore, these immature pastures can also have high levels of rapidly fermentable carbohydrates (Dineen et al., 2021a), potentially leading to low rumen pH (O'Grady et al., 2008). These dietary attributes could develop an altered rumen environment and shifts in biohydrogenation pathways leading to the production of CLA isomers (e.g., *trans*-10,*cis*-12 CLA), which have been demonstrated to be potent inhibitors of milk fat synthesis (Bauman and Griinari. 2001). However, in an observational investigation by Neville et al. (2023), the authors did not demonstrate relationships among these pasture chemical composition variables and herds exhibiting reduced milk fat concentration.

During the high-risk period for reduced milk fat synthesis, pasture-based management practices typically provide a considerable level of concentrate supplementation. Ingredients included in such concentrates (e.g., barley, maize meal, soybean meal, maize distillers grains) are typically lower in NDF, higher in rapidly fermentable carbohydrates, and higher in UFA compared with immature pasture. In a review by Bargo et al. (2003), the authors reported a negative relationship among concentrate intake and milk fat concentration, with supplemented cows producing 6% lower milk fat concentration compared with pasture-only diets. The concentration and digestibility of starch in such supplements likely contributes to reduced milk fat concentration (Oba and Allen, 2003; Rugoho et al., 2017; McKay et al., 2019). However, Bargo et al. (2003) highlighted that the negative relationship among concentrate intake and milk fat concentration was equivocal across experiments, which might be related to factors such as level of concentrate supplementation, concentrate ingredients and pasture chemical composition.

There is growing interest in the supplementation of rumen-inert fat ingredients to pasture-fed cows to meet the cow's energy demand (de Souza et al., 2023). Such ingredients can provide a supply of energy without simultaneously increasing the rumen fermentable carbohydrate load, maintaining a more favorable condition for milk fat production. In addition, recent experiments investigating the effects of feeding calcium salts of palm fatty acids (**CaFA**) distillate to cows consuming indoor diets have demonstrated increased milk fat production (Lock and de Souza, 2017) and the ability to alleviate reduced milk fat concentrations (Ramirez-Ramirez et al., 2016). However, limited research is available on the effects of these ingredients when fed to pasture-based cows.

Ultimately, more research is required to understand the nutritional mechanisms that could be responsible for reduced milk fat concentration during the high-risk period in pasture-based systems. Therefore, we designed an experiment to test the hypotheses that (1) increasing concentrate supplementation level would reduce milk fat concentration compared with a pasture-only diet, and (2) at a high concentrate supplementation level, partial replacement of a starch-based ingredient with a fiber- or fat-based ingredient would alleviate reduction in milk fat concentration. Overall, the objective of this experiment was to investigate the effects of concentrate supplement level and type on milk fat production of spring-calving grazing dairy cows during a high-risk period for reduced milk fat synthesis.

MATERIALS AND METHODS

This experiment was conducted with the approval of the Health Products Regulatory Authority (Dublin, Ireland) through the experimental license AE19132-P133, under the European directive 2010/63/EU and Statutory Instrument no. 543 of 2012 (European Union, 2012). The experiment was undertaken at the Dairygold Research Farm (Teagasc, Animal and Grassland Research and Innovation Centre, Moorepark, Fermoy, Co. Cork, Ireland; 52°09′N, 8°16′W) between April and July 2021. Meteorological data were obtained from a weather station located at Moorepark, Fermoy, Co. Cork, Ireland. Daily air temperature (°C), soil temperature (°C at 100-mm depth), and rainfall (mm) were recorded.

Cows, Treatment, and Experimental Design

Twenty-five primiparous (mean ± SD; 57 ± 15 DIM and 451 ± 26 kg of BW) and 55 multiparous (54 ± 14 DIM and 498 ± 53 kg of BW) spring-calving Holstein Friesian dairy cows were enrolled in a 2-wk covariate period. During this period, cows grazed together and received a common diet of pasture and 2.67 kg of DM/cow per day of a dairy concentrate supplement. Data from this covariate period were used to block animals into groups of 5 based on milk yield (26.0 ± 3.72 kg), milk solids

Table 1. Ingredient composition and feeding level of the experimental supplements

Ingredient, g/kg (as-fed basis)	Experimental supplement[1]			
	P2	P4	P4S	P4F
Maize meal	200	200	200	200
Barley	150	150	50	100
Maize gluten	150	150	150	150
Soy hulls	150	150	150	150
Unmolassed beet pulp	76.1	95.8	88.8	97.5
Rapeseed meal extract	70	70	70	70
Liquid sugar cane/beet molasses[2]	70	70	70	70
Maize distillers grains	50	50	50	50
Soybean meal	30.7	27.3	39.4	36.7
Magnesium oxide	30	15	15	15
Mineral and vitamin mix[3]	10	5	5	5
Calcium carbonate	7.4	11.2	10.5	—
Sodium chloride	5.8	5.7	1.3	5.8
Sodium hydroxide-treated straw[4]	—	—	100	—
Calcium salt of fatty acids[5]	—	—	—	50
Feeding level, kg of DM/d	2	4	4	4

[1]P0 = pasture-only control, supplemented with 0.27 kg/d of a mineral and vitamin pack (not presented); P2 = pasture + 2 kg DM concentrate supplement; P4 = pasture + 4 kg DM concentrate supplement; P4S = pasture + 4 kg DM concentrate supplement containing sodium hydroxide-treated straw; P4F = pasture + 4 kg DM concentrate supplement containing a calcium salt of fatty acids.

[2]Molprem (Premier Molasses Co. Ltd.).

[3]Mineral and vitamin mix contained the following: 300 mg/kg of selenium, 560 mg/kg of cobalt, 1,000 mg/kg of iodine, 18,800 mg/kg of copper, 22,800 mg/kg of manganese, 32,000 mg/kg of zinc, 2,400,000 IU/kg of vitamin A, 600,000 IU/kg of vitamin D_3 and 8,000 mg/kg of vitamin E.

[4]Nutritionally improved straw (Sundown Products Ltd.).

[5]Mega Max (Volac Wilmar feed ingredients) is a calcium salt of fatty acids, and, of the total fatty acids, 58% were C16:0, 28% were C18:1, 6% were C18:2, 5% were C18:0, and 3% were defined as others.

yield (2.04 ± 0.28 kg), BW (498 ± 53 kg), and parity (2.6 ± 1.7). The cows were then randomly assigned to 1 of 5 dietary treatments in a randomized complete block design (n = 16). The 5 dietary treatments were as follows: a pasture-only (**P**) control, supplemented with 0.27 kg of DM/cow per day of a mineral and vitamin pack (**P0**); P supplemented with 2 kg of DM/cow per day of an industry-standard concentrate (**P2**); P supplemented with 4 kg of DM/cow per day of an industry-standard concentrate (**P4**); P supplemented with 4 kg of DM/cow per day of a concentrate containing 10% sodium hydroxide-treated straw (**P4S**); and P supplemented with 4 kg of DM/cow per day of a concentrate containing 5% CaFA (**P4F**; Table 1). The P0 mineral and vitamin pack, fed at 0.27 kg of DM/cow per day, contained 58% maize meal, 26% magnesium oxide, 8.5% defluorinated phosphate, 5% mineral and vitamin mix, 2% molasses, and 1.2% sodium chloride. The mineral and vitamin mix contained 300 mg/kg of selenium, 560 mg/kg of cobalt, 1,000 mg/kg of iodine, 18,800 mg/kg of copper, 22,800 mg/kg of manganese, 32,000 mg/kg of zinc, 2,400,000 IU/kg of vitamin A, 600,000 IU/kg of vitamin D_3, and 8,000 mg/kg of vitamin E. The industry-standard concentrate was formulated to contain moderate concentrations of both starch and NDF. The concentrates P4S and P4F were similar to the industry-standard concentrates fed in treatments P2 and P4; however, either 100 g/kg or 50 g/kg of barley were replaced with sodium hydroxide-treated straw or a CaFA ingredient, respectively. The CaFA ingredient was a calcium salt (84% fat, 9% Ca, and 7% other), with the fatty acid (**FA**) proportion comprising of 58% palmitic acid, 28% oleic acid, 6% linoleic acid, 5% stearic acid, and 3% others. Concentrate supplements were fed in the parlor manually, twice daily, in 2 equal portions, and refusals were recorded if present. Following a 1-wk acclimatization period to allow animals to transition onto the new dietary treatments, a 12-wk data collection period commenced.

Grazing Management and Sward Measurements

The grazing area consisted of 26.3 ha, permanently subdivided into 16 paddocks, with perennial ryegrass (*Lolium perenne* L.) as the dominant pasture species present. All cows grazed together as a single group and had ad libitum access to fresh water. Cows grazed full-time and were allocated either a 24-h or 36-h residence time within each paddock or until a targeted postgrazing residual compressed sward height of 4 to 4.5 cm was achieved. Postgrazing residual compressed sward height was determined by recording 30 measurements across each grazing allocation using a rising plate meter (di-

ameter 355 mm, and 3.2 kg/m^2; Jenquip, Feilding, NZ). Herbage quantity was monitored weekly and managed in accordance with O'Donovan et al. (2002) with the use of the PastureBase Ireland decision support tool (Hanrahan et al., 2017). Pregrazing herbage yield (kg of DM/ha) was determined twice weekly by cutting 2 strips (1.2 m × 10 m) within each paddock before grazing using an Etesia mower (Etesia UK Ltd.). The harvested material from each strip was weighed and subsampled to determine DM concentration and herbage yield. Dry matter concentration was determined by drying 100 g of the subsampled material for 16 h at 90°C. Sward density was calculated as described by Dineen et al. (2021b). An additional 30 compressed sward height measurements were taken diagonally across each paddock using the previously mentioned rising plate meter to determine pregrazing compressed sward height. The above measurements were used to calculate herbage yield in accordance with Dineen et al. (2021b):

$$\text{Pregrazing herbage yield (kg of DM/ha)} = [\text{Pregrazing compressed sward height (cm)} - 4\text{ (cm)}] \times \text{sward density (kg of DM/cm per ha)}.$$

Pasture allowance was calculated as proposed by Delaby et al. (1998).

Sward and Concentrate Chemical Analysis

Gardena (Accu 60, Gardena International GmbH, Germany) hand shears were used to harvest pasture samples, positioned 4 cm above ground level at 30 representative locations across the entirety of the paddock before grazing. Samples were immediately snap-frozen in liquid nitrogen before being stored at −20°C. Samples were composited on a weekly basis and freeze-dried (LS40+chamber, MechaTech System Ltd.) at a condenser temperature of −55°C for at least 72 h. Concentrate samples were collected weekly and dried at 60°C for 48 h. Dried samples were milled through a 1-mm screen using a Cyclotech 1093 Sample Mill (Foss, DK-3400) and stored subsequent to chemical analysis. Samples were analyzed for ash (AOAC International, 2000, method 942.05), CP using a Leco FP-628 (Leco Australia Pty Ltd., Baulkham Hills, New South Wales Australia; AOAC, 1990, method 990.03), organic matter digestibility (Morgan et al., 1989) using a Fibertec Systems analyzer (Foss, Ballymount, Dublin 12, Ireland), and NDF and ADF using an Ankom 200 Fiber Analyzer (Macedon, NY; AOAC, 1995, method 973.18). The NDF and ADF results are reported inclusive of residual ash. The starch concentration of concentrate supplements was determined by FBA Laboratories (Co. Waterford, Ireland) using the Megazyme Total Starch Assay Procedure (product no. K-TSTA, Megazyme International Ireland Ltd., Co. Wicklow, Ireland). The FA composition of feedstuffs were analyzed via gas chromatography. Fatty acid methyl esters (**FAME**) were extracted in duplicate using the rapid microwave-assisted technique described by Brunton et al. (2015). For base-catalyzed trans-esterification, 10 mL of 2.5% potassium hydroxide in methanol was added, and the Xpress vessels were heated in the MARS 6 (CEM Corporation, Matthews, NC) to 130°C over 4 min and held at this temperature for a further 4 min. The Xpress vessels were removed, and acid-catalyzed esterification was carried out by adding 15 mL of 5% acetyl chloride in methanol before heating to 120°C over 4 min, which was held at this temperature for 2 min. To extract FAME, 10 mL of heptane was added, and the Xpress vessels were inverted 20 times. Furthermore, 20 mL of a saturated sodium chloride solution was added and similarly inverted. Once separation was complete, the top pentane layer was collected and aliquoted into amber GC vials containing sodium sulfate and stored at −20°C. The FAME were analyzed using the Thermo Trace 1600 Series GC (Thermo Fisher Scientific, Waltham, MA) equipped with a flame ionization detector. Injections were carried out using a Triplus RSH autosampler (Thermo Fisher Scientific, Waltham, MA). The injector was a programmable temperature vaporizing inlet, held at 250°C with a split of 50:1, and 1 µL was injected. The flame ionization detector was held at 250°C. The FAME were separated on an RT-2560 fused silica column, containing nonbonded biscyanopropyl polysiloxane phase (100 m × 0.25-mm i.d., 0.2-µm film thickness; Thames Restek UK Ltd.). The initial column temperature was 60°C held for 5 min, then increased to 165°C at a rate of 15°C/min and held for 1 min, then increased to 225°C at 2°C/min and held for 35 min for a total run time of 78 min. The carrier gas was helium and held at a constant pressure of 224,769.1 Pa. Using the 37-component FAME mix, response factors of each

Table 2. Mean air temperature, soil temperature, and total rainfall during the experimental period (April to July 2021) compared with the previous 10-yr average (2011 to 2020)

Item	Month			
	Apr.	May	Jun.	Jul.
Mean air temperature (°C)				
2021	7.4	9.8	14.4	17.2
10-yr average	8.8	11.5	13.9	15.8
Mean soil temperature (°C)				
2021	9.7	12.5	17.0	20.0
10-yr average	10.3	13.7	16.5	18.5
Total rainfall (mm)				
2021	23	131	27	63
10-yr average	73	63	80	62

Table 3. Chemical composition (mean ± SD) of pasture and concentrate feed during the 12-wk experiment

	Pasture[1]		Concentrate[2]			
Item[3]	G1	G2	P2	P4	P4S	P4F
DM, %	18.7 ± 0.2	19.5 ± 0.4	90.1 ± 0.6	90.2 ± 0.4	89.9 ± 0.3	90.0 ± 0.4
CP, % of DM	21.0 ± 0.6	18.0 ± 2.1	15.3 ± 0.3	15.5 ± 0.3	15.5 ± 0.2	15.0 ± 0.2
NDF, % of DM	36.1 ± 4.2	37.2 ± 2.5	26.2 ± 0.7	29.4 ± 1.6	32.1 ± 1.0	27.5 ± 0.9
ADF, % of DM	18.2 ± 1.0	20.1 ± 0.8	—	—	—	—
Ash, % of DM	8.5 ± 0.5	8.5 ± 1.3	10.6 ± 0.7	9.0 ± 0.1	9.0 ± 0.1	9.0 ± 0.4
Starch, % of DM	—	—	23.2 ± 0.3	23.5 ± 2.9	19.8 ± 0.0	23.0 ± 0.6
OMD, % of OM	86.0 ± 0.6	83.4 ± 0.6	—	—	—	—
Total FA, % of DM	2.02 ± 0.17	1.74 ± 0.23	2.53 ± 0.36	2.47 ± 0.03	2.58 ± 0.10	5.47 ± 0.17
FA (g/100 g FA)						
C14:0	0.54 ± 0.07	0.61 ± 0.08	0.56 ± 0.06	0.48 ± 0.08	0.43 ± 0.01	0.75 ± 0.03
C16:0	24.19 ± 2.06	27.25 ± 2.61	33.71 ± 6.64	27.84 ± 2.00	28.45 ± 1.74	55.83 ± 0.35
C18:0	3.28 ± 0.28	3.53 ± 0.42	4.78 ± 0.08	4.54 ± 0.21	4.61 ± 0.10	4.49 ± 0.07
C18:1	6.87 ± 0.94	8.19 ± 1.15	30.86 ± 0.22	33.68 ± 0.83	33.94 ± 0.46	24.84 ± 0.33
C18:2	9.25 ± 0.59	9.09 ± 0.74	14.25 ± 2.25	18.78 ± 1.68	18.92 ± 1.30	7.16 ± 0.23
C18:3	39.59 ± 4.21	33.13 ± 4.86	0.96 ± 0.11	1.29 ± 0.10	1.23 ± 0.10	0.49 ± 0.05
Total SFA	35.73 ± 3.17	40.48 ± 4.13	46.36 ± 4.72	39.52 ± 1.45	39.57 ± 1.48	64.74 ± 0.22
Total MUFA	8.45 ± 1.11	9.75 ± 1.38	32.94 ± 1.21	35.28 ± 0.61	35.31 ± 0.42	25.54 ± 0.37
Total PUFA	55.82 ± 4.00	49.77 ± 5.37	20.69 ± 3.51	25.2 ± 2.00	25.12 ± 1.83	9.72 ± 0.20

[1]G1 = pasture composition wk 1 to 6; G2 = pasture composition wk 7 to 12.

[2]P2 = 2 kg DM/d feeding rate, standard concentrate supplement; P4 = 4 kg DM/d feeding rate, standard concentrate supplement; P4S = 4 kg DM/d feeding rate, concentrate supplement containing sodium hydroxide-treated straw; P4F = 4 kg DM/d feeding rate, concentrate supplement containing a calcium salt of fatty acid.

[3]OMD = organic matter digestibility.

individual FAME were calculated from the response of the C13 FAME peak. These response factors were used to quantify each FAME peak using the amount of internal standard added to the sample. Results are presented as g/100 g of FA.

Animal Measurements

Cows were milked twice daily at 0730 h and 1500 h, and individual daily milk yields (kg) were recorded using electronic milk meters (Dairymaster, Causeway, Co. Kerry, Ireland). Individual milk fat, crude protein, and lactose concentrations were determined weekly from successive p.m. and a.m. milk samples using a Milkoscan 7 RM (Foss Electric, Hillerød, Denmark). Milk FA were predicted in accordance with Soyeurt et al. (2011). This was performed by the Irish Cattle Breeding Federation (Co. Cork, Ireland) using prediction equations developed as part of the OptiMIR project (Grelet et al., 2014). Milk FA subgroups were calculated similarly to the methods of Benoit et al. (2024) for de novo FA, mixed FA, and preformed FA. The omega, spreadability, and desaturase indices were calculated as described by Timlin et al. (2023). Milk solids (fat [kg] + crude protein [kg]) and ECM (Tyrrell and Reid, 1965) were calculated on a weekly basis. Body weight was recorded once a week using an electronic scale and Winweigh software package (Tru-Test Limited). Body condition score was recorded weekly by 2 trained scorers, using a scale from 1 to 5 (where 1 = emaciated and 5 = extremely fat) with 0.25 increments, as described by Edmonson et al. (1989). Dry matter intake was estimated on an individual cow basis at 2 separate time points (wk 4–5 and 9–10) using the n-alkane technique (Mayes et al., 1986) as modified by Dillon and Stakelum (1989). All cows were dosed twice daily for an 11-d period with a paper bung (Carl Roth, GmbH, Karlsruhe, Germany) containing 760 mg of C32-alkane (n-dotriacontane). Individual fecal samples were collected for 5 d before a.m. and p.m. milkings during d 7 to 11 of the dosing period and stored at −20°C. Fecal samples were later thawed and pooled per cow before being dried at 40°C until completely dry and milled through a 1-mm screen. Herbage samples, representative of that consumed by the grazing animals, were harvested manually using Gardena hand shears during the 5-d fecal collection period. Herbage samples were stored at −20°C before being bowl chopped, freeze-dried, and milled through a 1-mm screen. Following extraction, the n-alkane concentrations for feces, herbage, and concentrate samples were analyzed by gas chromatography (Varian 3400 series GC; Dove and Mayes, 2006) using direct saponification (Dillon, 1993). Intake was estimated based on the ratio of naturally occurring tritriacontane (C33) in herbage and concentrate compared with the dosed C32 using the following equation:

$$\text{Daily herbage DMI} = [(F_i/F_j) \times (D_j + I_s \times S_j) - I_s \times S_i] /[P_i - (F_i/F_j \times P_j)],$$

where F_i, S_i, and P_i are the concentrations (mg/kg of DM) of odd-chain n-alkanes, and F_j, S_j, and P_j are the concentrations (mg/kg of DM) of even-chain n-alkanes naturally occurring in feces, concentrates, and herbage, respectively: D_j is the daily dosage rate (mg/d) of even-chain n-alkane, and I_s is the daily concentrate intake (kg of DM/d). Animal behavior, in the form of feeding, ruminating, and resting times, was measured using a 3-axis accelerometer in the MooMonitor+ collar device (Dairymaster, Causeway, Co. Kerry, Ireland). Blood samples were collected at 3 time points during the experiment (wk 5, 8, and 10) immediately after morning milking from the coccygeal vessels of cows, using 21-gauge Vacutainer needles (Becton Dickson, Plymouth, UK). The blood samples were collected into lithium heparin Vacutainer tubes. Blood tubes were centrifuged (1,922 × *g* for 15 min at 4°C) before plasma harvesting and decanted into 3.5-mL plasma tubes and stored at −20°C. Blood plasma samples were analyzed in duplicate for nonesterified fatty acids (**NEFA**) and BHB concentration using enzymatic colorimetry (ELx808 Ultra Microplate Reader; Bio-Tek Instruments Inc.). Analysis was performed using the manufacturer's instructions on the respective commercially available kits (NEFA HR-2 kit, Wako Chemicals GmbH, Neuss, Germany; RB1008 kit, Randox Laboratories Ltd., Crumlin, Co. Antrim, UK). Plasma urea nitrogen analysis was conducted at University College Dublin (Dublin, Ireland) using enzymatic tests (UR3825 kit, Randox Laboratories Ltd.). Rumen fluid samples were collected at 2 separate time points (wk 5 and 10) using a Flora Rumen Scoop (Prof-Products, Guelph, Ontario, Canada; Geishauser et al., 2012). After collection, the rumen fluid was strained through 2 layers of large-pore polyethylene cheesecloth (Graytec, GD Textile, Manchester, UK), and rumen pH was measured immediately. Subsamples were also collected into either an empty 15-mL screw-cap tube or a tube containing 2 mL of 500 g/L trichloroacetic acid before being snap-frozen in liquid nitrogen and stored at −20°C. Samples were subsequently analyzed for rumen VFA (Varian CP-3000 GC analyzer, Varian Inc., Palo Alto, CA) and ammonia N concentrations (ABX Horiba Pentra 400 chemistry analyzer, Horiba-ABX Diagnostics, Kyoto, Japan).

Statistical Analysis

All data were analyzed using a linear mixed effects model within the MIXED procedure in SAS version 9.4 (SAS Institute Inc., Cary, NC, 2002), as follows:

$$Y_{ijklm} = \mu + T_i + W_j + TW_{ij} + BX_{ki} + P_l + C_k + B_m + \varepsilon_{ijklm},$$

where Y_{ijklm} = dependent variable, μ = intercept, T_i = fixed effect of treatment (i = 1 to 5), W_j = fixed effect of week (j = 1 to 12), TW_{ij} = fixed interaction effect of treatment i and week j, BX_{ki} = the covariate adjustment for each cow k, P_l = fixed effect of parity l, C_k = random effect of cow k, B_m = random effect of block m, and ε_{ijklm} = residual error. A first-order autoregressive covariance structure was applied to the repeated measurements taken on a

Table 4. Effects of concentrate supplement level and type on milk production and composition in early- to mid-lactation grazing dairy cows

	Diet[1]						*P*-value[2]		
Item[3]	P0	P2	P4	P4S	P4F	SEM	Diet	Lin	Quad
Milk yield, kg/d	22.9^{d}	24.7^{c}	26.1^{b}	26.1^{b}	27.6^{a}	0.45	<0.01	<0.01	0.71
ECM yield, kg/d	26.5^{c}	28.6^{b}	30.0^{a}	30.0^{a}	31.1^{a}	0.45	<0.01	<0.01	0.57
Fat, %	4.41	4.36	4.29	4.23	4.20	0.08	0.32	0.25	0.93
De novo, g/100 g of fat	26.0^{bc}	26.6^{b}	27.4^{a}	27.4^{a}	25.5^{c}	0.27	<0.01	<0.01	0.83
Mixed, g/100 g of fat	29.7^{c}	30.4^{b}	31.1^{a}	31.0^{a}	30.2^{bc}	0.21	<0.01	<0.01	0.90
Preformed, g/100 g of fat	42.4^{ab}	41.2^{bc}	39.8^{d}	40.1^{cd}	42.7^{a}	0.49	<0.01	<0.01	0.76
Protein, %	3.63^{c}	3.66^{bc}	3.74^{ab}	3.75^{a}	3.65^{c}	0.03	0.02	0.01	0.55
Lactose, %	4.83^{bc}	4.81^{c}	4.88^{ab}	4.85^{abc}	4.90^{a}	0.02	0.02	<0.01	0.18
Fat yield, kg/d	1.00^{c}	1.08^{b}	1.11^{ab}	1.10^{b}	1.15^{a}	0.02	<0.01	<0.01	0.38
Protein yield, kg/d	0.83^{c}	0.91^{b}	0.98^{a}	0.97^{a}	1.01^{a}	0.02	<0.01	<0.01	0.85
Lactose yield, kg/d	1.11^{d}	1.19^{c}	1.28^{b}	1.26^{b}	1.36^{a}	0.02	<0.01	<0.01	0.91
Milk solids yield, kg/d	1.83^{c}	1.98^{b}	2.09^{a}	2.08^{a}	2.15^{a}	0.03	<0.01	<0.01	0.55

[a–d]Means within row with different superscripts refer to a difference for diet ($P \leq 0.05$).

[1]P0 = pasture-only control; P2 = pasture + 2 kg DM concentrate supplement; P4 = pasture + 4 kg DM concentrate supplement; P4S = pasture + 4 kg DM concentrate supplement containing sodium hydroxide-treated straw; P4F = pasture + 4 kg DM concentrate supplement containing a calcium salt of fatty acids.

[2]Lin and Quad refer to the pre-planned contrasts included to evaluate the linear and quadratic effects, respectively, of concentrate level (i.e., P0, P2, and P4).

[3]ECM estimated according to Tyrrell and Reid (1965); milk solids = kg of fat + crude protein; de novo = fatty acids C4 to C14; mixed = fatty acids C16, C16:1; preformed = fatty acids greater than or equal to C17.

Table 5. Effects of concentrate supplement level and type on intake of DM, OM, NDF, and fatty acids in early- to mid-lactation dairy cows grazing perennial ryegrass

	Diet[1]						*P*-value[2]		
Item[3]	P0	P2	P4	P4S	P4F	SEM	Diet	Lin	Quad
Intake, kg/d									
Total DM	19.14^{b}	20.38^{a}	21.03^{a}	21.30^{a}	20.79^{a}	0.35	<0.01	<0.01	0.54
DMI, % of BW	3.90^{b}	4.12^{a}	4.20^{a}	4.15^{a}	4.20^{a}	0.08	0.03	<0.01	0.49
Pasture DM	18.87^{a}	18.38^{a}	17.03^{b}	17.30^{b}	16.79^{b}	0.35	<0.01	<0.01	0.37
Supplement DM	0.27	2.00	4.00	4.00	4.00	—	—	—	—
OM	17.42^{c}	18.57^{b}	19.27^{ab}	19.52^{a}	19.06^{ab}	0.32	<0.01	<0.01	0.60
NDF	6.66^{c}	6.94^{bc}	7.15^{ab}	7.38^{a}	7.01^{b}	0.12	<0.01	<0.01	0.79
FA	0.33^{d}	0.37^{c}	0.40^{b}	0.40^{b}	0.50^{a}	0.006	<0.01	<0.01	0.25
C16:0	0.09^{d}	0.10^{c}	0.11^{b}	0.11^{b}	0.20^{a}	0.007	<0.01	<0.01	0.04
C18:1	0.03^{e}	0.04^{d}	0.06^{c}	0.06^{b}	0.08^{a}	0.002	<0.01	<0.01	0.12
SFA	0.12^{d}	0.14^{c}	0.15^{b}	0.15^{b}	0.25^{a}	0.002	<0.01	<0.01	0.02
MUFA	0.03^{e}	0.05^{d}	0.06^{c}	0.06^{b}	0.08^{a}	0.001	<0.01	<0.01	0.51
PUFA	0.17^{b}	0.18^{ab}	0.18^{a}	0.19^{a}	0.17^{b}	0.003	0.01	0.04	0.46
FA intake, % of DMI	1.74^{c}	1.83^{d}	1.89^{c}	1.90^{b}	2.46^{a}	0.001	<0.01	<0.01	<0.01

$^{a–e}$Means within row with different superscripts refer to a difference for diet ($P \leq 0.05$).

[1]P0 = pasture-only control; P2 = pasture + 2 kg DM concentrate supplement; P4 = pasture + 4 kg DM concentrate supplement; P4S = pasture + 4 kg DM concentrate supplement containing sodium hydroxide-treated straw; P4F = pasture + 4 kg DM concentrate supplement containing a calcium salt of fatty acids.

[2]Lin and Quad refer to the pre-planned contrasts included to evaluate the linear and quadratic effects, respectively, of concentrate level (i.e., P0, P2, and P4).

[3]Estimated using the n-alkane technique during wk 4–5 and 9–10.

cow over the 12-wk period. Respective pre-experimental variables were applied as covariate measurements for analysis. Dry matter intake and blood and rumen metabolites were also analyzed using the MIXED procedure in SAS version 9.4. The model included the fixed effects of treatment, parity, and sampling time point, as well as the random effect of cow. All means were generated using the LSMEANS statement. When appropriate, the LSD post hoc mean separation test was used to determine differences between LSM. Pre-planned contrasts were also included to evaluate the linear and quadratic effects of concentrate level (i.e., P0, P2, and P4). Statistical significance was considered if $P \leq 0.05$ and statistical trend if $0.05 < P \leq 0.10$.

RESULTS

Climatic conditions in the form of mean air and soil temperatures (°C) along with total rainfall (mm) are reported on a monthly basis (April to July 2021) in Table 2.

Pasture Chemical Composition and Grazing Management

Pregrazing DM yield during the experimental period was 1,564 ± 426 kg DM/ha. Pre- and postgrazing compressed sward heights were 8.61 ± 1.69 and 4.37 ± 0.35 cm, respectively. Pasture allowance (>4 cm) was 18.0 ± 5.74 kg DM/cow per day on average across the 12-wk experimental period. Pasture chemical composition is presented in Table 3 for grazing period 1 (**G1**; wk 1 to 6) and grazing period 2 (**G2**; wk 6 to 12). Pasture CP concentration was 19.4% ± 2.4% across the 12-wk experiment; however, variability was observed between the 2 grazing periods. Pasture NDF increased from G1 to G2, peaking during wk 6 (44%), before gradually reducing for the remainder of the experiment. Organic matter digestibility averaged 84.7% ± 1.4% across the experimental period. Organic matter digestibility was greater during G1 (peaking at 86.7% in wk 5) compared with G2 (low of 82.6% in wk 10). Total FA concentration reduced (−0.28% of DM) between G1 and G2 with a lower proportion of PUFA (−6.05 g/100 g FA) and a greater proportion of SFA (+4.75 g/100 g FA) observed. Furthermore, C18:3 was reduced (−6.46 g/100 g FA), whereas C16:0 and C18:1 increased (+3.06 and +1.32 g/100 g FA, respectively).

Milk Production and Milk Composition

All milk production variables were affected by diet, except for milk fat concentration (Table 4). Increasing the concentrate supplementation level (i.e., P0, P2, and P4) linearly increased milk yield, ECM yield, fat yield, protein yield, lactose yield, and milk solids yield ($P < 0.01$). Cows fed P2 and P4 had greater milk fat yield (+0.08 and +0.11 kg/cow per day, respectively) compared with cows fed P0 ($P < 0.01$); however, cows fed

Table 6. Effects of concentrate supplement level and type on BW, BCS, feeding, resting, and ruminating times in early- to mid-lactation dairy cows grazing perennial ryegrass

	Diet[1]						*P*-value[2]		
Item	P0	P2	P4	P4S	P4F	SEM	Diet	Lin	Quad
BW, kg	510	511	515	522	520	5.4	0.40	0.58	0.78
BW change, kg/wk	1.41	1.36	1.68	2.06	1.18	0.50	0.74	0.80	0.89
BCS	3.03	2.99	3.06	3.00	3.02	0.03	0.45	0.34	0.09
BCS change in score per week	0.002	0.002	0.003	0.006	0.005	0.005	0.95	0.95	0.84
Feeding time, min/d	628	615	608	613	612	14.2	0.85	0.22	0.83
Resting time, min/d	293	307	306	299	283	16.3	0.79	0.56	0.72
Ruminating time, min/d	464	471	466	464	462	16.3	0.99	0.84	0.72
Ruminating time, min/kg of OM per day	26	26	25	24	24	0.9	0.34	0.23	0.94
Ruminating time, min/kg of NDF per day	69	69	67	64	67	2.5	0.53	0.59	0.77

[1]P0 = pasture-only control; P2 = pasture + 2 kg DM concentrate supplement; P4 = pasture + 4 kg DM concentrate supplement; P4S = pasture + 4 kg DM concentrate supplement containing sodium hydroxide-treated straw; P4F = pasture + 4 kg DM concentrate supplement containing a calcium salt of fatty acids.

[2]Lin and Quad refer to the pre-planned contrasts included to evaluate the linear and quadratic effects, respectively, of concentrate level (i.e., P0, P2, and P4).

P2 and P4 were similar to one another ($P = 0.14$). Cows fed P4 had greater proportions of de novo and mixed FA, whereas the proportion of preformed FA was lower compared with cows fed P0 and P2 ($P < 0.01$). Cows fed P4 had greater milk protein concentrations compared with cows fed P0 but were similar to cows fed P2.

At the higher concentrate feeding level (4 kg of DM/cow per day), changing the concentrate type (i.e., P4, P4S, and P4F) affected milk production and composition (Table 4). Cows fed P4F had greater milk yield and lactose yield but lower milk protein concentration compared with cows fed P4 and P4S. Compared with the P4S diet, cows fed the P4F diet had greater milk fat yield ($P = 0.04$) and tended to produce greater milk solids yield ($P = 0.09$). Cows fed P4F had lower proportions of de novo and mixed FA, as well as greater proportions of preformed FA compared with cows fed P4 and P4S ($P < 0.01$). There was an effect of concentrate feeding level and type on several other milk FA concentrations and indices (Supplemental Table S1, see Notes).

DMI, Feeding Behavior, BW, and BCS

The effects of concentrate supplement level and type on intake of DM, OM, NDF, and FA are presented in Table 5. Cows fed P2 and P4 increased DM and OM intake compared with cows fed P0 ($P < 0.01$); however, cows fed P2 and P4 were similar to each other. Accordingly, DMI as a percentage of BW was greater for cows fed P2 and P4 compared with cows fed P0, but cows fed P2 and P4 were similar ($P = 0.42$). Cows fed P4 had lower pasture DMI compared with cows fed P0 and P2 ($P < 0.01$). The NDF intake of cows fed P4 was greater than that of cows fed P0 ($P < 0.01$) but was similar to cows fed P2. Cows fed P0 and P2 had similar NDF intakes. The FA intake of cows fed P4 was greatest (400 g/d); cows fed P2 were intermediate (370 g/d); and cows fed P0 were lowest (330 g/d). A similar effect was observed for the intakes of C16:0, C18:1, SFA, and MUFA. The PUFA intake of cows fed P4 was greater than that of cows fed P0 ($P < 0.01$) but similar to cows fed P2.

Changing the concentrate type had no effect on the intakes of total DM, pasture DM, and OM. Cows fed P4S had greater NDF intake compared with cows fed P4F but were similar to cows fed P4. Cows fed P4F had greater intakes of FA, C16:0, C18:1, SFA, and MUFA compared with cows fed P4S and P4. Cows fed P4F had lower PUFA intake compared with cows fed P4 and P4S.

Concentrate supplement level or type had no effect on BW or BCS across the experimental period (Table 6). Similarly, concentrate level or type had no effect on feeding, resting, and ruminating times (Table 6).

Rumen Fermentation Characteristics and Blood Metabolites

The effects of concentrate supplement level and type on rumen fermentation characteristics and blood metabolites are presented in Tables 7 and 8. No effect of diet was detectable for the majority of the rumen fermentation outcomes evaluated. However, concentrate supplement type did affect rumen ammonia N concentration, whereby cows fed P4F had lower rumen ammonia N compared with cows fed P4 and P4S. Furthermore, concentrate supplement level tended to increase rumen propionate concentrations, as cows fed P4 tended to have greater propionate concentration than cows fed P0 ($P = 0.09$). Accordingly, cows fed P4 had a lower acetate:propionate ratio compared with cows fed P2 and P0. Cows fed P0 had greater NEFA concentration

Table 7. Effects of concentrate supplement level and type on rumen pH and concentrations of rumen ammonia N and VFA in early- to mid-lactation dairy cows grazing perennial ryegrass

	Diet[1]						*P*-value[2]		
Item[3]	P0	P2	P4	P4S	P4F	SEM	Diet	Lin	Quad
Rumen pH	6.25	6.14	6.07	6.16	6.21	0.07	0.33	0.07	0.80
Ammonia N, mg/dL	7.0^{a}	7.4^{a}	7.7^{a}	7.3^{a}	5.8^{b}	0.36	<0.01	0.14	0.93
VFA concentration, mmol									
Total VFA	134.2	142.9	147.9	146.5	143.8	5.39	0.40	0.06	0.77
Acetate	86.1	92.4	93.9	94.0	92.0	3.51	0.47	0.09	0.56
Propionate	29.4^{x}	30.7^{xy}	34.0^{y}	32.7^{xy}	32.6^{xy}	1.30	0.09	0.01	0.52
Butyrate	15.5	16.3	16.2	16.3	15.7	0.70	0.88	0.48	0.53
Isobutyrate	0.8	0.8	0.8	0.8	0.7	0.03	0.42	0.28	0.89
Valerate	1.4	1.6	1.7	1.6	1.7	0.13	0.57	0.11	0.84
Isovalerate	1.1	1.1	1.1	1.1	1.1	0.05	0.80	0.18	0.92
Acetate:propionate ratio	2.9^{ab}	2.9^{a}	2.7^{c}	2.8^{abc}	2.8^{bc}	0.05	0.02	0.04	0.03
SCFA	3.2	3.5	3.7	3.5	3.5	0.18	0.56	0.06	0.88

$^{a–c}$Means within row with different superscripts refer to a difference for diet ($P \leq 0.05$).

^{x,y}Means within row with different superscripts refer to a tendency to differ for diet ($0.05 > P > 0.1$).

[1]P0 = pasture-only control; P2 = pasture + 2 kg DM concentrate supplement; P4 = pasture + 4 kg DM concentrate supplement; P4S = pasture + 4 kg DM concentrate supplement containing sodium hydroxide-treated straw; P4F = pasture + 4 kg DM concentrate supplement containing a calcium salt of fatty acids.

[2]Lin and Quad refer to the pre-planned contrasts included to evaluate the linear and quadratic effects, respectively, of concentrate level (i.e., P0, P2, and P4).

[3]SCFA = short-chain fatty acids.

compared with cows fed P2 and P4 ($P < 0.01$). Changing the concentrate type had no effect on NEFA concentrations. Finally, there was no effect of diet on plasma urea nitrogen or BHB concentrations.

DISCUSSION

The economic viability of pasture-based production systems relies on the production of milk with high fat and protein concentrations using low-cost fed sources (Dillon et al., 2005). During late spring to early summer, milk fat concentrations can reduce, leading to concern on behalf of the producer, lower overall profitability, and a greater environmental footprint per kilogram of fat- and protein-corrected milk. This experiment investigated the effect of several nutritional factors on the milk fat production of pasture-fed lactating dairy cows during the high-risk period for reduced milk fat synthesis.

Milk Production and Milk Composition

Increasing the level of concentrate supplementation in the current experiment had no effect on milk fat concentration. Therefore, our first hypothesis was not supported. In a review of concentrate supplementation of pasture-based diets, Bargo et al. (2003) observed a negative association among concentrate intake and milk fat concentration. However, in that review, the majority of concentrate supplements contained high levels of starch-based ingredients. Rugoho et al. (2017) demonstrated that supplementing pasture-fed cows with 4 kg DM/d of a wheat and corn grain-based concentrate led to reduction in milk fat concentration and yield. The authors attributed this reduction to altered biohydrogenation of FA, possibly driven by low rumen pH. In the current experiment, an industry-standard concentrate was used to investigate the effect of concentrate supplementation level on milk fat concentration. The industry-standard concentrate contained more moderate starch (~23% of DM) and higher NDF concentrations (~27.5% of DM) compared with previous investigations (Bargo et al., 2003; Rugoho et al., 2017). These attributes of lower starch and greater NDF concentrations in the concentrate supplement might have alleviated the effect of concentrate supplementation on milk fat concentration that is typically observed (Bargo et al., 2003). In the current experiment, there was no effect of concentrate supplement level on rumen pH, suggesting that the industry-standard concentrate may not have substantially altered the rumen environment. It is important to highlight that the rumen pH data were obtained using a trans-esophageal rumen sampling device and were collected at 2 specific time points during the experiment. The trans-esophageal rumen sampling method has been demonstrated to contain some measurement bias (Geishauser and Gitzel, 1996; Duffield et al., 2004), and, therefore, care is advised when interpreting the current rumen pH data. The level of concentrate supplementation investigated in the current experiment (i.e., up to 4 kg DM/cow) might also have been too low to affect

Table 8. Effect of concentrate supplement level and type on PUN, NEFA and BHB concentrations of early to mid-lactation dairy cows grazing perennial ryegrass

	Diet[1]						*P*-value[2]		
Item[3]	P0	P2	P4	P4S	P4F	SEM	Diet	Lin	Quad
PUN, mg/dL	10.29	10.75	11.45	11.33	10.29	0.41	0.10	0.04	0.80
NEFA, mmol/L	0.29[a]	0.25[b]	0.22[bc]	0.18[c]	0.20[bc]	0.02	<0.01	0.02	0.74
BHB, mmol/L	0.52	0.59	0.53	0.59	0.58	0.03	0.12	0.79	0.06

[a–c]Means within row with different superscripts refer to a difference for diet ($P \leq 0.05$).

[1]P0 = pasture-only control; P2 = pasture + 2 kg DM concentrate supplement; P4 = pasture + 4 kg DM concentrate supplement; P4S = pasture + 4 kg DM concentrate supplement containing sodium hydroxide-treated straw; P4F = pasture + 4 kg DM concentrate supplement containing a calcium salt of fatty acids.

[2]Lin and Quad refer to the pre-planned contrasts included to evaluate the linear and quadratic effects, respectively, of concentrate level (i.e., P0, P2, and P4).

[3]PUN = plasma urea nitrogen; NEFA = nonesterified fatty acids.

milk fat concentration. However, other experiments at similar concentrate supplementation levels with high-starch ingredients have observed reductions in milk fat concentration (Delaby et al., 2001; McKay et al., 2019).

Although concentrate supplementation level had no effect on milk fat concentration in the current experiment, a linear increase in milk fat yield occurred. A milk fat yield response to supplementation (calculated as the difference in milk fat production between non-supplement and supplemented treatments, divided by the DMI of concentrate supplement) of +0.04 and +0.03 kg of milk fat/kg of concentrate DM was observed for cows fed P2 and P4, respectively. These responses are slightly greater than the +0.02 kg of fat/kg of concentrate DM observed by McEvoy et al. (2008) but within the range (+0.02 to +0.07 kg fat/kg concentrate DM) observed by Kennedy et al. (2008). Similar to milk fat yield, concentrate supplementation level increased milk yield, ECM yield, milk protein concentration, milk protein yield, and milk solids yield. The milk yield response to concentrate supplementation was + 0.9 and +0.8 kg milk/kg concentrate DM for P2 and P4, respectively. This was lower than the average of 1 kg milk/kg concentrate DM reported by Bargo et al. (2003). Although these outcomes will increase milk revenue, consideration is required as to whether overall profitability is increased in pasture-based systems (Ramsbottom et al., 2015).

At the higher concentrate feeding level (i.e., 4 kg DM/cow), partial replacement of barley with sodium hydroxide-treated straw (P4S) or CaFA (P4F) did not affect milk fat concentration. Therefore, our second hypothesis was also not supported. The objective of the inclusion of sodium hydroxide-treated straw was to increase NDF and reduce the starch concentrations of the concentrate supplement. As discussed previously, the industry-standard concentrate base formulation (P4) might already have been an optimal chemical composition to maintain milk fat concentrations (Rustomo et al., 2006). In addition, the pasture offered as part of the basal diet might also have had sufficient NDF concentrations to maintain milk fat concentration. Although Rivero and Anrique (2015) and Carty et al. (2017) suggested that late-spring to early-summer pastures might be deficient in NDF concentration, in the current experiment adequate pasture NDF concentrations greater than 35% were observed (Stockdale et al., 1987). This is in agreement with McEvoy et al. (2009), who noted sufficient dietary NDF to support normal rumen function and to maintain milk fat concentration across their experiment. Furthermore, Heffernan et al. (2024) reported sufficient concentrations of pasture NDF (>35%) across a range of herbage masses spanning the high-risk period for reduced milk fat synthesis. It is important to consider that a lack of an effect on milk fat concentration might also have been due to the relatively low partial replacement of starch with sodium hydroxide-treated straw (i.e., 100 g/kg replacement; 400 g DM/d). However, the practicality and cost implications of including higher quantities within a pasture-based system are challenging. Overall, the partial replacement of barley with sodium hydroxide-treated straw did not affect any milk production or milk composition outcomes. Within the conditions of the current experiment, sodium hydroxide-treated straw can replace barley while maintaining animal performance.

In the current experiment, partial replacement of barley with CaFA did not affect milk fat concentration. Freeman and Kirkland (2015) fed a quantity of a CaFA similar to the current experiment and also found no effect on milk fat concentration. In contrast, Garnsworthy (1990) demonstrated that when CaFA are included in the concentrate supplement fed to grazing dairy cows, milk fat concentration is increased. However, in that experiment, CaFA were included at 125 g/kg of the concentrate supplement (as opposed to 50 g/kg in the current experiment). In experiments where cows consumed indoor diets, mixed results have also been observed for

the effect of FA supplementation on milk fat concentration. Lock and Van Amburgh (2012) suggested that the effect can be dependent on the specific FA composition and the amount of FA supplementation. Palmitic acid, in particular, has been shown to increase milk fat concentration and yield (de Souza and Lock, 2018); however, Shepardson and Harvatine (2021) demonstrated that the response is dependent on the inclusion level of palmitic acid. For cows fed a basal diet of elephant grass and 8 kg of concentrate DM/cow per day, de Souza et al. (2017) demonstrated that the addition of calcium salts of soybean fatty acids reduced milk fat concentration, whereas supplementation with calcium salts of palm fatty acids maintained milk fat concentration. In the current experiment, the FA composition of the calcium salts of palm fatty acid distillate was 58% palmitic acid, 28% oleic acid, 6% linoleic acid, 5% stearic acid, and 3% others. Further experiments to evaluate whether FA supplements with higher palmitic acid concentrations can increase the milk fat concentrations from pasture-fed cows are warranted.

Partial replacement of barley with CaFA increased milk yield and tended to increase milk solids yield; however, reduced milk protein concentration was observed. These findings are generally in agreement with previous experiments investigating the supplementation of CaFA to pasture-fed cows (Erickson et al., 1989; Freeman and Kirkland, 2015; de Souza et al., 2017) and with a recent review by dos Santos Neto et al. (2021). The increased milk yield is likely due to an increase in total energy intake (Rico et al., 2015; de Souza and Lock, 2018). It is difficult to describe the mechanisms involved in the reduction in milk protein concentration. Western et al. (2020) also observed a reduction in milk protein concentration, whereas de Souza et al. (2021) observed a tendency for increased milk protein concentration and a significant increase in milk protein yield. In the current experiment, the reduced milk protein concentration could be linked to the lower rumen ammonia N concentrations observed in cows fed P4F. Overall, the equivocal effects of FA supplementation on pasture-fed cows warrants further investigation to understand the suitability of such ingredients in pasture-based systems.

DMI and Substitution Rate

Bargo et al. (2003) reported that cows fed concentrate supplement increase their DMI by 24% (across a range of 1.8 to 10 kg of concentrate/cow per day) compared with cows fed a pasture-only diet. In the current experiment, cows fed P2 and P4 increased DMI by 6% and 10%, respectively, compared with cows fed P0. Although concentrate supplementation can lead to increased DMI, a reduction in pasture DMI is often observed, with Bargo et al. (2003) reporting a reduction in pasture DMI of 13% when cows were offered concentrate supplementation. In the current experiment, cows fed P4 reduced pasture DMI by 10% compared with cows fed P0; however, cows fed P2 had similar pasture DMI compared with cows fed P0. These findings are in agreement with Dillon et al. (1997) and McEvoy et al. (2008), who both observed reduced pasture DMI only at higher concentrate feeding levels. The reduction in pasture DMI relative to the amount of concentrate DMI can be defined as the substitution rate (Kellaway and Porta 1993). Although pasture DMI was not statistically lower when cows were fed P2 compared with cows fed P0, a substitution rate of 0.24 kg of pasture DM per kilogram of concentrate DM was observed. When the concentrate feeding level was increased, in the current experiment, the substitution rate increased to 0.45 kg of pasture DM per kilogram of concentrate DM. A negative relationship exists between substitution rate and milk yield response, with a greater substitution rate ultimately leading to lower increases in DMI and a lower milk yield response. These responses to concentrate supplementation could explain, at least in part, the numerically reducing milk yield response in the current experiment when concentrate supplementation level was increased. Interestingly, cows fed concentrates had lower NEFA concentrations compared with cows fed the pasture-only diet. This could be due to a greater DMI being achieved and potentially greater energy partitioning toward maintaining body adipose tissue stores; however, no effect on BCS was observed.

Increasing the level of concentrate supplementation in the current experiment linearly increased the intakes of FA, C16:0, C18:1, SFA, and MUFA. In addition, the intake of PUFA was greater for cows fed P4 compared with cows fed P0 but similar to that of cows fed P2. Concentrate supplements typically have greater concentrations of linoleic FA compared with pasture (Elgersma, 2015), and, when combined with greater intake of rapidly fermentable carbohydrates, this could lead to a reduction in milk fat synthesis (Harvatine and Allen, 2006). During periods of altered rumen function (e.g., low rumen pH), the incomplete biohydrogenation of linoleic FA can create bioactive isomers (*trans*-10,*cis*-12 CLA) that can downregulate milk fat synthesis (Palmquist and Jenkins 2017). Although cows fed P4 had greater FA and PUFA intakes compared with cows fed P0, there was no effect on milk fat concentration. This was likely due to the stable rumen environment (i.e., similar rumen pH and total VFA concentrations) observed across the concentrate feeding levels in the current experiment. This emphasizes the importance of identifying the optimal concentrate formulation strategy to effectively supplement pasture-based diets, which

can have moderate to high concentrations of FA with rapid FA availability (Glasser et al., 2013).

Partial replacement of barley with sodium hydroxide-treated straw or CaFA did not affect DM, OM, or NDF intake. Although cows fed P4F did not have greater DMI than cows fed P4, they did have greater FA intake, leading to greater energy density of the diet (Bargo et al., 2003; Schroeder et al., 2004). This is the likely mechanism for cows fed P4F to have greater milk yield than cows fed P4. A further mechanism that could be responsible for the increased milk yield is the reduced de novo FA synthesis and increased preformed FA incorporation into milk when cows were fed P4F, which de Souza et al. (2017) discussed as being a more energetically favorable process. Nonetheless, the increased animal performance achieved in the current experiment when cows were supplemented with CaFA was modest and raises the question surrounding the economic suitability of such ingredients in pasture-based systems.

Environmental factors such as circadian rhythms have recently been associated with fluctuations in milk fat concentration (Salfer et al., 2019). Consistent annual patterns in milk composition have been observed within non-seasonal calving systems, with the highest milk fat concentration occurring during winter and the lowest during summer. Salfer et al. (2019) reported a range in milk fat concentration amplitude (i.e., peak to mean) from 0.07% to 0.14%, with an increase in amplitude at greater latitudes. Due to Ireland's relatively high latitude, further research should investigate the possible association of environmental factors and milk fat concentration during the high-risk period of reduced milk fat synthesis.

CONCLUSIONS

Within pasture-based systems, milk fat concentration appears to be consistently reduced during late spring and early summer. This provides an opportunity to increase the economic and environmental sustainability of pasture-based systems. In the current experiment, increasing concentrate supplement level or partial replacement of barley with either sodium hydroxide-treated straw or CaFA did not affect the milk fat concentration of early- to mid-lactation grazing dairy cows. Increasing the concentrate supplement level did increase milk fat yield, as well as milk yield, ECM yield, milk protein concentration, milk protein yield, and milk solids yield. Partial replacement of barley with CaFA increased milk yield. Overall, other nutritional factors (e.g., increasing the saturation of FA ingredients) and non-nutritional factors (e.g., genetics, day length) should be explored to determine the mechanisms that reduce milk fat concentration in pasture-based systems during the high-risk period.

NOTES

Financial support from the Dairy Research Ireland Dairy Levy Trust (Dublin, Ireland) is gratefully acknowledged. The technical assistance of Michelle Liddane, David Flynn, Pat O'Connor, Andy McGrath, Christina Fleming, Jim Flynn, Stuart Kirwan, Aaron Casey, David Ryan, and the entire farm staff at the Dairygold Research Farm (Teagasc, Animal and Grassland Research and Innovation Centre, Moorepark, Fermoy, Co. Cork, Ireland) is greatly appreciated. The authors thank Colman Purcell of Dairygold (Lombardstown, Co. Cork, Ireland) for supporting the manufacturing of the concentrate supplements, and Barry Sheehan of the Irish Cattle Breeding Federation (Co. Cork, Ireland) for milk spectral analysis. Supplemental material for this article is available at https://doi.org/10.17632/dwv9bdz9sd.1. This experiment was conducted with the approval of the Health Products Regulatory Authority (Dublin, Ireland) through the experimental license AE19132-P133, under the European directive 2010/63/EU and Statutory Instrument no. 543 of 2012. The authors have not stated any conflicts of interest.

Nonstandard abbreviations used: CaFA = calcium salts of palm fatty acids; FA = fatty acids; FAME = fatty acid methyl esters; G1, G2 = grazing periods 1 (wk 1–6) and 2 (wk 6–12), respectively; NEFA = nonesterified fatty acids; OMD = organic matter digestibility; P = pasture; P0 = pasture-only control, supplemented with 0.27 kg of DM/cow per day of minerals and vitamins; P2 = P + 2 kg of DM/cow per day of industry-standard concentrate; P4 = P + 4 kg of DM/cow per day of industry-standard concentrate; P4S = P + 4 kg of DM/cow per day of concentrate containing 10% sodium hydroxide-treated straw; P4F = P + 4 kg of DM/cow per day of concentrate containing 5% CaFA; PUN = plasma urea nitrogen.

REFERENCES

AOAC. 1990. Official Methods of Analysis. 15th ed. AOAC International.

AOAC. 1995. Official Methods of Analysis. 14th ed. AOAC International.

AOAC International. 2000. Official Methods of Analysis. 17th ed. AOAC International.

Bargo, F., L. D. Muller, E. S. Kolver, and J. E. Delahoy. 2003. Invited review: Production and digestion of supplemented dairy cows on pasture. J. Dairy Sci. 86:1–42. https://doi.org/10.3168/jds.S0022-0302(03)73581-4.

Bauman, D. E., and J. M. Griinari. 2001. Regulation and nutritional manipulation of milk fat: Low-fat milk syndrome. Livest. Prod. Sci. 70:15–29. https://doi.org/10.1016/S0301-6226(01)00195-6.

Benoit, A. C., J. M. dos Santos Neto, and A. L. Lock. 2024. Mammary gland responses to altering the supply of de novo fatty acid substrates and preformed fatty acids on the yields of milk components and milk fatty acids. J. Dairy Sci. 107:10653–10666. https://doi.org/10.3168/jds.2024-24982.

Brunton, N. P., C. Mason, and M. J. Collins Jr. 2015. Rapid microwave-assisted preparation of fatty acid methyl esters for the analysis of fatty acid profiles in foods. J. Anal. Chem. 70:1218–1224. https://doi.org/10.1134/S1061934815100044.

Carty, C. I., A. G. Fahey, M. R. Sheehy, S. Taylor, I. J. Lean, C. G. McAloon, L. O'Grady, and F. J. Mulligan. 2017. The prevalence, temporal and spatial trends in bulk tank equivalent milk fat depression in Irish milk recorded herds. Ir. Vet. J. 70:14. https://doi.org/10.1186/s13620-017-0092-y.

CSO (Central Statistics Office). 2024. Agriculture Milk Statistics. Accessed Feb. 21, 2024. https://data.cso.ie/table/AKM01.

de Souza, J., F. Batistel, and F. A. P. Santos. 2017. Effect of sources of calcium salts of fatty acids on production, nutrient digestibility, energy balance, and carryover effects of early lactation grazing dairy cows. J. Dairy Sci. 100:1072–1085. https://doi.org/10.3168/jds.2016-11636.

de Souza, J., F. Batistel, and F. A. P. Santos. 2023. Enhancing the recovery of human-edible nutrients in milk and nitrogen efficiency throughout the lactation cycle by feeding fatty acid supplements. Front. Sustain. Food Syst. 7:1186454. https://doi.org/10.3389/fsufs.2023.1186454.

de Souza, J., and A. L. Lock. 2018. Long-term palmitic acid supplementation interacts with parity in lactating dairy cows: Production responses, nutrient digestibility, and energy partitioning. J. Dairy Sci. 101:3044–3056. https://doi.org/10.3168/jds.2017-13946.

de Souza, J., C. M. Prom, and A. L. Lock. 2021. Altering the ratio of dietary palmitic and oleic acids affects production responses during the immediate postpartum and carryover periods in dairy cows. J. Dairy Sci. 100:1072–1085. https://doi.org/10.3168/jds.2020-19311.

Delaby, L., J.-L. Peyraud, A. Bouttier, and J.-R. Peccatte. 1998. Effet d'une réduction simultanée de la fertilisation azotée et du chargement sur les performances des vaches laitières et la valorisation du pâturage. Ann. Zootech. 47:17–39. https://doi.org/10.1051/animres:19980102.

Delaby, L., J. L. Peyraud, and R. Delagarde. 2001. Effect of the level of concentrate supplementation, herbage allowance and milk yield at turn-out on the performance of dairy cows in mid lactation at grazing. Anim. Sci. 73:171–181. https://doi.org/10.1017/S1357729800058161.

Dillon, P. 1993. The use of n-alkanes as markers to determine intake, botanical composition of available or consumed herbage in studies of digesta kinetics with dairy cows. PhD dissertation. School of Food and Agricultural Science, National University of Ireland, Dublin, Ireland

Dillon, P., S. Crosse, and B. O'Brien. 1997. Effect of concentrate supplementation of grazing dairy cows in early lactation on milk production and milk processing quality. Ir. J. Agric. Food Res. 36:145–159.

Dillon, P., J. R. Roche, L. Shalloo, and B. Horan. 2005. Optimising financial return from grazing in temperate pastures. Pages 131–147 in Utilisation of Grazed Grass in Temperate Animal Systems: Proceedings of a Satellite Workshop of the International Grassland Congress, July 2005, Cork, Ireland. https://doi.org/10.3920/9789086865543_011.

Dillon, P., and G. Stakelum. 1989. Herbage and dosed alkanes as a grass measurement technique for dairy cows. Isr. J. Agric. Res. 28:104.

Dineen, M., B. McCarthy, P. Dillon, F. Coughlan, N. Galvin, and M. E. Van Amburgh. 2021b. The effect of concentrate supplement type on milk production, nutrient intake, and total-tract nutrient digestion in mid-lactation, spring-calving dairy cows grazing perennial ryegrass (*Lolium perenne* L.) pasture. J. Dairy Sci. 104:11593–11608. https://doi.org/10.3168/jds.2021-20148.

Dineen, M., B. McCarthy, D. Ross, A. Ortega, P. Dillon, and M. E. Van Amburgh. 2021a. Characterization of the nutritive value of perennial ryegrass (*Lolium perenne* L.) dominated pastures using updated chemical methods with application for the Cornell Net Carbohydrate and Protein System. Anim. Feed Sci. Technol. 272:114752. https://doi.org/10.1016/j.anifeedsci.2020.114752.

dos Santos Neto, J. M., J. de Souza, and A. L. Lock. 2021. Effects of calcium salts of palm fatty acids on nutrient digestibility and production responses of lactating dairy cows: A meta-analysis and meta-regression. J. Dairy Sci. 104:9752–9768. https://doi.org/10.3168/jds.2020-19936.

Dove, H., and R. W. Mayes. 2006. Protocol for the analysis of n-alkanes and other plant-wax compounds and for their use as markers for quantifying the nutrient supply of large mammalian herbivores. Nat. Protoc. 1:1680–1697. https://doi.org/10.1038/nprot.2006.225.

Duffield, T., J. C. Plaizier, A. Fairfield, R. Bagg, G. Vessie, P. Dick, J. Wilson, J. Aramini, and B. McBride. 2004. Comparison of techniques for measurement of rumen pH in lactating dairy cows. J. Dairy Sci. 87:59–66. https://doi.org/10.3168/jds.S0022-0302(04)73142-2.

Edmonson, A. J., I. J. Lean, L. D. Weaver, T. Farver, and G. Webster. 1989. A body condition scoring chart for Holstein dairy cows. J. Dairy Sci. 72:68–78. https://doi.org/10.3168/jds.S0022-0302(89)79081-0.

Elgersma, A. 2015. Grazing increases the unsaturated fatty acid concentration of milk from grass-fed cows: A review of the contributing factors, challenges and future perspectives. Eur. J. Lipid Sci. Technol. 117:1345–1369. https://doi.org/10.1002/ejlt.201400469.

Erickson, P., M. Murphy, and J. Clark. 1989. Calcium salts of long chain fatty acids and nicotinic acid supplementation of diets fed to Holstein cows in early lactation. J. Dairy Sci. 72:483.

European Union. 2012. Directive 2010/63/EU of the European Parliament and of the Council of 22 September 2010 on the protection of animals used for scientific purposes. Off. J. Eur. Union L 276:33.

Freeman, M. J., and R. M. Kirkland. 2015. The effect of supplementation with a rumen-protected fat through an extended period of lactation on milk production of dairy cows in a pasture-based dairy system in Tasmania. Page 190 in Proc. Adv. Anim. Biosci. Cambridge University Press.

Garnsworthy, P. C. 1990. Feeding calcium salts of fatty acids in high-starch or high-fibre compound supplements to lactating cows at grass. Anim. Sci. 51:441–447. https://doi.org/10.1017/S0003356100012472.

Geishauser, T., and A. Gitzel. 1996. A comparison of rumen fluid sampled by oro ruminal probe versus rumen fistula. Small Rumin. Res. 21:63–69. https://doi.org/10.1016/0921-4488(95)00810-1.

Geishauser, T., N. Linhart, A. Neidl, and A. Reimann. 2012. Factors associated with ruminal pH at herd level. J. Dairy Sci. 95:4556–4567. https://doi.org/10.3168/jds.2012-5380.

Glasser, F., M. Doreau, G. Maxin, and R. Baumont. 2013. Fat and fatty acid content and composition of forages: A meta-analysis. Anim. Feed Sci. Technol. 185:19–34. https://doi.org/10.1016/j.anifeedsci.2013.06.010.

Grelet, C., J. A. Fernandez Pierna, H. Soyeurt, F. Dehareng, N. Gengler, and P. Dardenne. 2014. Creation of universal MIR calibrations by standardization of milk spectra: Example of fatty acids. Pages 108–108 in Book of Abstracts of the 65th Annual Meeting of the European Association for Animal Production. Wageningen Academic Publishers. https://doi.org/10.3920/9789086867998_068.

Hanrahan, L., A. Geoghegan, M. O'Donovan, V. Griffith, E. Ruelle, M. Wallace, and L. Shalloo. 2017. PastureBase Ireland: A grassland decision support system and national database. Comput. Electron. Agric. 136:193–201. https://doi.org/10.1016/j.compag.2017.01.029.

Harvatine, K. J., and M. S. Allen. 2006. Effects of fatty acid supplements on ruminal and total tract nutrient digestion in lactating dairy cows. J. Dairy Sci. 89:1092–1103. https://doi.org/10.3168/jds.s0022-0302(06)72177-4.

Heffernan, C., M. Egan, C. Fleming, T. F. O'Callaghan, and M. Dineen. 2024. Investigating the effects of herbage mass and harvest date on perennial ryegrass nutritive value. In Why grasslands? Proceedings of the 30th General Meeting of the European Grassland Federation. Grassland Science in Europe, Leeuwarden, the Netherlands.

Kalač, P., and E. Samková. 2010. The effects of feeding various forages on fatty acid composition of bovine milk fat: A review. Czech J. Anim. Sci. 55:521–537. https://doi.org/10.17221/2485-CJAS.

Kellaway, R., and S. Porta. 1993. Feeding Concentrates: Supplements for Dairy Cows. Agmedia, East Melbourne, Australia.

Kennedy, E., M. O'Donovan, L. Delaby, and F. P. O'Mara. 2008. Effect of herbage allowance and concentrate supplementation on dry matter intake, milk production and energy balance of early lactating dairy

cows. Livest. Sci. 117:275–286. https://doi.org/10.1016/j.livsci.2007.12.025.
Lock, A. L., and J. de Souza. 2017. Update on fatty acid digestion and metabolism and impacts on milk production. Tri-State Nutrition Conference. CABI Digital Library.
Lock, A. L., and M. E. Van Amburgh. 2012. Feeding for milk components. WCDS Adv. Dairy Technol. 24:265–277.
Mayes, R. W., C. S. Lamb, and P. M. Colgrove. 1986. The use of dosed and herbage n-alkanes as markers for the determination of herbage intake. J. Agric. Sci. 107:161–170. https://doi.org/10.1017/S0021859600066910.
McEvoy, M., E. Kennedy, J. P. Murphy, T. M. Boland, L. Delaby, and M. O'Donovan. 2008. The effect of herbage allowance and concentrate supplementation on milk production performance and dry matter intake of spring-calving dairy cows in early lactation. J. Dairy Sci. 91:1258–1269. https://doi.org/10.3168/jds.2007-0710.
McEvoy, M., M. O'Donovan, E. Kennedy, J. P. Murphy, L. Delaby, and T. M. Boland. 2009. Effect of pregrazing herbage mass and pasture allowance on the lactation performance of Holstein-Friesian dairy cows. J. Dairy Sci. 92:414–422. https://doi.org/10.3168/jds.2008-1313.
McKay, Z. C., M. B. Lynch, F. J. Mulligan, G. Rajauria, C. Miller, and K. M. Pierce. 2019. The effect of concentrate supplementation type on milk production, dry matter intake, rumen fermentation, and nitrogen excretion in late-lactation, spring-calving grazing dairy cows. J. Dairy Sci. 102:5042–5053. https://doi.org/10.3168/jds.2018-15796.
Mohan, M. S., T. F. O'Callaghan, P. Kelly, and S. A. Hogan. 2021. Milk fat: Opportunities, challenges and innovation. Crit. Rev. Food Sci. Nutr. 61:2411–2443. https://doi.org/10.1080/10408398.2020.1778631.
Morgan, D., G. Stakelum, and J. Dwyer. 1989. Modified neutral detergent cellulase digestibility procedure for use with the 'Fibertec' system. Ir. J. Agric. Res. 91–92.
Neville, O. B., A. G. Fahey, and F. J. Mulligan. 2023. Comparison of milk and grass composition from grazing Irish dairy herds with and without milk fat depression. Ir. Vet. J. 76:5. https://doi.org/10.1186/s13620-023-00230-3.
O'Donovan, M., P. Dillon, M. Rath, and G. Stakelum. 2002. A comparison of four methods of herbage mass estimation. Ir. J. Agric. Food Res. 41:17–27.
O'Grady, L., M. L. Doherty, and F. J. Mulligan. 2008. Subacute ruminal acidosis (SARA) in grazing Irish dairy cows. Vet. J. 176:44–49. https://doi.org/10.1016/j.tvjl.2007.12.017.
O'Sullivan, M., B. Horan, K. M. Pierce, S. McParland, K. O'Sullivan, and F. Buckley. 2019. Milk production of Holstein-Friesian cows of divergent Economic Breeding Index evaluated under seasonal pasture-based management. J. Dairy Sci. 102:2560–2577. https://doi.org/10.3168/jds.2018-15559.
Oba, M., and M. S. Allen. 2003. Effects of corn grain conservation method on ruminal digestion kinetics for lactating dairy cows at two dietary starch concentrations. J. Dairy Sci. 86:184–194. https://doi.org/10.3168/jds.S0022-0302(03)73599-1.
Palmquist, D. L., and T. C. Jenkins. 2017. A 100-Year Review: Fat feeding of dairy cows. J. Dairy Sci. 100:10061–10077. https://doi.org/10.3168/jds.2017-12924.
Ramirez-Ramirez, H. A., E. Castillo Lopez, C. J. R. Jenkins, N. D. Aluthge, C. Anderson, S. C. Fernando, K. J. Harvatine, and P. J. Kononoff. 2016. Reduced-fat dried distillers grains with solubles reduces the risk for milk fat depression and supports milk production and ruminal fermentation in dairy cows. J. Dairy Sci. 99:1912–1928. https://doi.org/10.3168/jds.2015-9712.
Ramsbottom, G., B. Horan, D. P. Berry, and J. R. Roche. 2015. Factors associated with the financial performance of spring-calving, pasture-based dairy farms. J. Dairy Sci. 98:3526–3540. https://doi.org/10.3168/jds.2014-8516.
Rico, D. E., S. H. Preston, J. M. Risser, and K. J. Harvatine. 2015. Rapid changes in key ruminal microbial populations during the induction of and recovery from diet-induced milk fat depression in dairy cows. Br. J. Nutr. 114:358–367. https://doi.org/10.1017/S0007114515001865.
Rivero, M. J., and R. Anrique. 2015. Milk fat depression syndrome and the particular case of grazing cows: A review. Acta Agric. Scand. A Anim. Sci. 65:42–54. https://doi.org/10.1080/09064702.2015.1052545.
Rugoho, I., L. Cheng, W. Aizimu, R. H. Bryant, and G. R. Edwards. 2017. Effects of post-grazing herbage height and concentrate feeding on milk production and major milk fatty acids of dairy cows in mid-lactation. Grass Forage Sci. 72:211–219. https://doi.org/10.1111/gfs.12244.
Rustomo, B., O. AlZahal, N. E. Odongo, T. F. Duffield, and B. W. McBride. 2006. Effects of rumen acid load from feed and forage particle size on ruminal pH and dry matter intake in the lactating dairy cow. J. Dairy Sci. 89:4758–4768. https://doi.org/10.3168/jds.S0022-0302(06)72525-5.
Salfer, I. J., C. D. Dechow, and K. J. Harvatine. 2019. Annual rhythms of milk and milk fat and protein production in dairy cattle in the United States. J. Dairy Sci. 102:742–753. https://doi.org/10.3168/jds.2018-15040.
Salfer, I. J., and K. J. Harvatine. 2018. Seasonal variation in milk composition. Pages 157–166 in Proc. Tri-State Dairy Nutrition Conference. CABI Digital Library.
Schroeder, G. F., G. A. Gagliostro, F. Bargo, J. E. Delahoy, and L. D. Muller. 2004. Effects of fat supplementation on milk production and composition by dairy cows on pasture: A review. Livest. Prod. Sci. 86:1–18. https://doi.org/10.1016/S0301-6226(03)00118-0.
Shepardson, R. P., and K. J. Harvatine. 2021. Effects of fat supplements containing different levels of palmitic and stearic acid on milk production and fatty acid digestibility in lactating dairy cows. J. Dairy Sci. 104:7682–7695. https://doi.org/10.3168/jds.2020-19665.
Soyeurt, H., F. Dehareng, N. Gengler, S. McParland, E. Wall, D. P. Berry, M. Coffey, and P. Dardenne. 2011. Mid-infrared prediction of bovine milk fatty acids across multiple breeds, production systems, and countries. J. Dairy Sci. 94:1657–1667. https://doi.org/10.3168/jds.2010-3408.
Stockdale, C. R., A. Callaghan, and T. E. Trigg. 1987. Feeding high energy supplements to pasture fed dairy cows. Effects of stage of lactation and level of supplement. Aust. J. Agric. Res. 38:927–940. https://doi.org/10.1071/AR9870927.
Timlin, M., E. Fitzpatrick, K. McCarthy, J. T. Tobin, E. G. Murphy, K. M. Pierce, J. P. Murphy, D. Hennessy, M. O'Donovan, N. Harbourne, A. Brodkorb, and T. F. O'Callaghan. 2023. Impact of varying levels of pasture allowance on the nutritional quality and functionality of milk throughout lactation. J. Dairy Sci. 106:6597–6622. https://doi.org/10.3168/jds.2022-22921.
Tyrrell, H., and J. Reid. 1965. Prediction of the energy value of cow's milk. J. Dairy Sci. 48:1215–1223. https://doi.org/10.3168/jds.S0022-0302(65)88430-2.
Western, M. M., J. de Souza, and A. L. Lock. 2020. Milk production responses to altering the dietary ratio of palmitic and oleic acids vary with production level in dairy cows. J. Dairy Sci. 103:11472–11482. https://doi.org/10.3168/jds.2020-18936.

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