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Investigating the effect of fatty acid supplementation on milk fat production and animal performance in genetically diverse grazing dairy cows during early to mid-lactation

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ABSTRACT

The objective of this experiment was to investigate the effect of fatty acid (FA) supplementation on milk fat production and animal performance in genetically diverse grazing dairy cows during the high-risk period for reduced milk fat synthesis. Forty-five primiparous (mean ± SD; 58 ± 18 DIM and 415 ± 59 kg of BW) and 135 multiparous (45 ± 18 DIM and 497 ± 74 kg of BW) spring-calving dairy cows were blocked based on breed, Economic Breeding Index, parity, pre-experimental milk solids production, and BW and were then randomly assigned to 1 of 3 dietary treatments. The 3 dietary treatments were pasture supplemented with 3.6 kg of DM/cow per day of concentrate containing no supplemental fat (CON); pasture supplemented with 3.6 kg of DM/cow per day of concentrate containing a calcium salt of FA with a FA composition of 58% palmitic acid and 28% oleic acid (MIX); and pasture supplemented with 3.6 kg of DM/cow per day of concentrate containing a fat prill of FA with a FA composition of 97% palmitic acid (PA). The dietary treatments were offered during an 11-wk experimental period, which was then followed by a 6-wk carryover period. During the carryover period, cows received a common diet of pasture and 0.89 kg of DM/cow per day of a dairy concentrate supplement. Each dietary treatment group was comprised of 3 animal genetic groups (AG). Cows fed MIX tended to have greater milk yield, ECM yield, 3.5% FCM yield, fat yield and milk solids yield when compared with cows fed CON, but were similar to cows fed PA. Cows fed PA had greater milk fat concentration when compared with cows fed CON and MIX. Fatty acid supplementation affected the composition of milk fat produced, with cows fed MIX and PA having lower concentrations of de novo FA when compared with cows fed CON. Cows fed PA had greater concentrations of mixed FA when compared with cows fed MIX and CON, whereas cows fed MIX had greater concentrations of preformed FA when compared with cows fed CON and PA. Cows fed CON and PA had similar milk protein concentration but were both greater than cows fed MIX. There was no effect of FA supplementation during the carryover period on the majority of milk production outcomes. Milk fat production was affected by AG; however, there were no major interactions observed between FA supplementation and AG. Fatty acid supplementation of grazing dairy cows can increase milk fat concentration during the high-risk period for reduced milk fat synthesis but it is dependent on the FA composition of the supplemental ingredient. Overall, careful consideration is required when determining the economic sustainability of offering FA supplements to cows consuming high nutritive value pasture.

Key words: dairy cow, milk fat production, palmitic acid, oleic acid, grazing

INTRODUCTION

A major goal of pasture-based dairy production systems is to increase the output of high-fat commodity products, such as butter, cheese, and cream. The demand for these products is predicted to increase globally due to a growing population coupled with increasing standards of living (Alexandratos and Bruinsma, 2012; Mohan et al., 2021). Although the average yearly milk fat concentrations in pasture-based systems, such as Ireland, have increased over the past decade (4.0% to 4.4% from 2013 to 2023, respectively; CSO, 2024), there are opportunities to increase

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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.

this further during the late spring to early summer period. During this high-risk period, milk fat concentration typically reduces before recovering in mid to late summer. The relative reduction observed during this period appears to have increased nationally, from a reduction of 0.25% during 2013 to a reduction of 0.38% during 2023 (CSO, 2024). Understanding the factors influencing the reduction and developing strategies to overcome it are critically important as the high-risk period coincides with peak milk production in spring-calving systems (Timlin et al., 2021).

Changes in pasture chemical composition during this high-risk period, in particular reduced NDF concentrations, have been hypothesized to affect milk fat concentration (Rivero and Anrique, 2015). However, as described by Heffernan et al. (2024) adequate NDF concentrations to maintain milk fat concentration were observed across all harvest dates with no effect of pregrazing herbage mass (869 to 2,364 kg of DM/ha) on pasture NDF concentrations. Reducing the intake of fermentable carbohydrates (from 4.0 to 0.3 kg DM concentrate supplement/d), which could potentially alleviate unfavorable rumen conditions, has also been observed to have no effect on milk fat concentration during the high-risk period (Heffernan et al., 2025). Other nutritional strategies to overcome the reduction in milk fat concentration, such as providing rumen buffers or high NDF ingredients have not been encouraging (Delahoy et al., 2003; Rafferty et al., 2019).

Although extensive investigations have been conducted into the use of fatty acid (**FA**) supplementation (**FAS**) to manipulate milk fat production (Schroeder et al., 2004; Palmquist and Jenkins, 2017), there is a renewed interest into the effects of specific FA compositions (Rico et al., 2014a; Western et al., 2020a,b; Bales et al., 2024a). High palmitic acid supplements have been demonstrated to increase milk fat concentration (Rico et al., 2014b; Granados-Rivera et al., 2017; de Souza et al., 2019), whereas supplements with higher concentrations of oleic acid have resulted in greater milk yield, BW and BCS gain (de Souza et al., 2021). These responses to FAS have been demonstrated to interact with factors such as stage of lactation (Schroeder et al., 2004), parity (de Souza and Lock, 2018), and milk production level (de Souza et al., 2019). However, the majority of this research has been performed for indoor production systems, which can have a wide range of dietary basal fat concentrations (1.4%–6.8% of DM; dos Santos Neto et al., 2021), differing basal FA composition (i.e., predominantly linoleic acid vs. linolenic acid; Schroeder et al., 2004), and different animal genetics when compared with pasture-based systems.

Altogether, although some work has been performed in pasture-based settings (Batistel et al., 2017; de Souza et al., 2017), there is a lack of experiments investigating the effect of supplementing enriched palmitic acid supplements (>60% palmitic acid) to cows consuming perennial ryegrass during the high-risk period for reduced milk fat synthesis. Therefore, we designed an experiment to test the hypotheses that when compared with a nonfat supplemented control: (1) supplementation with a blend of palmitic and oleic acid would increase milk fat production through increased milk yield and (2) supplementation with palmitic acid would increase milk fat production through increased milk fat concentration and milk yield. These hypotheses were evaluated across 3 groups of genetically diverse dairy cows to investigate the interaction between FA supplementation and animal genotype in pasture-based systems. Overall, the objective of this experiment was to investigate the effect of FA supplementation on milk fat production and animal performance in genetically diverse grazing dairy cows during the high-risk period for reduced milk fat synthesis.

Table 1. Ingredient composition of experimental supplements

Ingredient, g/kg (as-fed basis)	Experimental supplement[1]		
	CON	MIX	PA
Unmolassed beet pulp	226	228	212
Maize meal	200	200	200
Barley	150	150	150
Soya hulls	100	41	50
Maize distillers	75	75	75
Rapeseed meal extract	70	70	70
Liquid sugar cane/beet molasses[2]	70	70	70
Soybean meal	66	78	79
Magnesium oxide	15	15	15
Calcium carbonate	13.9	—	14.6
Defluorinated phosphorus	5.6	5.7	5.7
Sodium chloride	5.4	5.2	5.3
Mineral and vitamin mix[3]	3.3	3.3	3.3
Calcium salt of fatty acids[4]	—	59.2	—
Fat prill of fatty acids[5]	—	—	50

[1]CON = pasture + 3.6 kg DM concentrate supplement; MIX = pasture + 3.6 kg DM concentrate supplement containing a calcium salt of fatty acids; PA = pasture + 3.6 kg DM concentrate supplement containing a fat prill 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 D3 and 8,000 mg/kg of vitamin E.

[4]Mega Max (Volac Wilmar feed ingredients) is a calcium salt of fatty acids containing 84% fat 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.

[5]Mega Fat Extra (Volac Wilmar feed ingredients) is a fat prill of fatty acids containing 99% fat and of the total fatty acids 97% were C16:0 and 3% were defined as others.

MATERIALS AND METHODS

This experiment was conducted with the approval of the Health Products Regulatory Authority (Dublin, Ire-

Table 2. The mean and SD Economic Breeding Index (EBI), EBI subindicies, and predicted transmitting ability (PTA) for milk production traits of the 3 animal genetic groups enrolled in the 17-wk experiment[1]

Item	Elite	SD	NA	SD	JE	SD
EBI	225	22.3	157	29.7	168	39.5
Subindex, €						
Milk	72	21.4	48	23.5	67	20.7
Fertility	103	23.5	74	28.0	50	26.1
Calving	42	10.0	31	14.2	38	13.0
Beef	−15	7.3	−15	6.0	−52	9.2
Maintenance	15	7.7	14	6.7	52	9.9
Health	6	7.3	3	6.6	5	4.9
Management	2	4.2	2	4.2	8	3.9
PTA						
Milk, kg	25.5	151.4	47.7	144.0	−389.5	164.4
Fat, %	0.20	0.11	0.11	0.10	0.53	0.12
Protein, %	0.13	0.05	0.08	0.05	0.26	0.05
Fat, kg	12.3	4.3	7.9	4.8	13.1	4.7
Protein, kg	8.4	4.2	6.1	4.2	0.9	4.3

[1]Elite = high EBI Holstein Friesian; NA = national average EBI Holstein Friesian; JE = purebred Jersey.

land) through the experimental license (AE19132-P079) 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 August 2022. 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.

Dietary Treatments, Animal Genetic Groups, and Experimental Design

Forty-five primiparous (mean ± SD; 58 ± 18 DIM and 415 ± 59 kg of BW) and 135 multiparous (45 ± 18 DIM and 497 ± 74 kg of BW) spring-calving 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. At the end of the 2-wk covariate period, cows were blocked based on breed, Economic Breeding Index (**EBI**), parity (3.3 ± 2.2), pre-experimental milk solids production (2.0 ± 0.4 kg), and BW (474 ± 83.5 kg) and were then randomly assigned to 1 of 3 dietary treatments investigating FA supplementation (**FAS**; n = 60). The 3 dietary treatments were; pasture supplemented with 3.6 kg of DM/cow per day of concentrate containing no supplemental fat (**CON**); pasture supplemented with 3.6 kg of DM/cow per day of concentrate containing a calcium salt of FA, with a FA composition of 58% palmitic acid and 28% oleic acid (**MIX**); and pasture supplemented with 3.6 kg of DM/cow per day of concentrate containing a fat prill of FA with a FA composition of 97% palmitic acid (**PA;** Table 1). Concentrate supplements were fed in the parlor twice daily in 2 equal portions and refusals were recorded if present. The dietary treatments were offered during an 11-wk experimental period that was then followed by a 6-wk carryover period. During the carryover period, cows received a common diet of pasture and 0.89 kg of DM/cow per day of a dairy concentrate supplement.

Each dietary treatment group was comprised of 3 animal genetic group (**AG**; n = 60). The AG investigated were; Holstein Friesian with high EBI (elite group); Holstein Friesian with the national average EBI (**NA**); and purebred Jersey (**JE**). The 2 genetically divergent groups of Holstein Friesian were characterized by their EBI value (Table 2), which is a monetary measurement applied to Irish dairy cows based on their genetic merit. At the time of the experiment, the cows in the elite group were representative of the top 1% of Irish cows for EBI, whereas the NA group were representative of the average of Irish cows for EBI.

Grazing Management and Sward Measurements

The 180 cows were divided, based on dietary treatment, into 3 grazing groups for both the experimental and carryover periods. Each group grazed their own individual farmlet consisting of 20.7 ha, permanently subdivided into 18 paddocks. Each farmlet was stocked at 2.89 livestock units per hectare. The cows were divided based on dietary treatment to quantify the effect of FAS on grazing dynamics (e.g., postgrazing height) as fat supplementation has previously been demonstrated to affect DMI and pasture substitution rate (Schroeder et al., 2004). However, this approach might have led to confounding among animal group and treatment. Perennial ryegrass (*Lolium perenne* L.) was the dominant pasture species present and cows had ad libitum access to fresh water.

Cows grazed full time and were assigned either a 24 h or 36 h residence time within each paddock or until a target postgrazing compressed sward height residual of 4 to 4.5 cm was achieved. Herbage quantity was recorded weekly using PastureBase Ireland (Hanrahan et al., 2017) and managed in accordance with O'Donovan et al. (2002). Pregrazing herbage yield (kg of DM/ha) was determined twice weekly by removing 2 herbage strips (1.2 m × 10 m) within each paddock 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 in Dineen et al. (2021a). Additionally, 30 compressed sward height measurements were taken diagonally across each paddock to determine pregrazing compressed sward height using a rising plate meter (diameter 355 mm and 3.2 kg/m^2 Jenquip, Feilding, New Zealand). The above measurements were used to calculate pregrazing herbage yield in accordance with Dineen et al. (2021a):

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

Postgrazing residual compressed sward height was determined by recording 30 measurements across each grazing allocation using a rising plate meter.

Sward and Concentrate Chemical Analysis

A Gardena (Accu 60, Gardena International GmbH, Germany) hand shears was 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 were freeze-dried (LS40+chamber, MechaTech System Ltd.) at −55°C for at least 72 h. Concentrate samples were composited weekly and dried at 60°C for 48 h. Once dried, samples were milled through a 1-mm screen using a Cyclotech 1093 Sample Mill (Foss, DK-3400) and stored before chemical analysis. Samples were analyzed for organic matter digestibility (Morgan et al., 1989) using the Fibertec Systems analyzer (Foss, Ballymount, Dublin 12, Ireland), CP using a Leco FP-628 (Leco Australia Pty Ltd., Baulkham Hills, New South Wales, Australia; AOAC, 1990; method 990.03), ash (AOAC International, 2000; method 942.05), NDF and ADF using ANKOM technology (Macedon, NY; Lee and Prosky, 1995; method 973.18). The results for NDF and ADF are reported inclusive of 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). Fatty acid composition of feedstuffs were analyzed via gas chromatography as described in Heffernan et al. (2025).

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 FT6000 (Foss Electric, Hillerød, Denmark). Milk solids [fat (kg) + crude protein (kg)], ECM (Tyrrell and Reid, 1965) and 3.5% FCM were calculated on a weekly basis as descibed in de Souza et al. (2019). Milk FA were predicted in accordance with Soyeurt et al. (2011). This was performed by the Irish Cattle Breeders Federation (Co. Cork, Ireland) using prediction equations developed as part of the OptiMIR project (Grelet et al., 2014). Milk FA subgroups were calculated similar to Benoit et al. (2024) for de novo FA, mixed FA and preformed FA. The omega, spreadability, and desaturase index were calculated as described in Timlin et al. (2023). 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 1 to 5 scale (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 during the experimental period (wk 4–5 and wk 9–10) and once during the carryover period (wk 15–16). The n-alkane technique (Mayes et al., 1986), as modified by Dillon and Stakelum (1989) was used to measure DMI and is further described in Heffernan et al. (2025). Blood samples were collected at 3 time points during the experiment (wk 5, 10, and 15) immediately after morning milking from the coccygeal vessels of cows using 21-gauge vacutainer needles. The blood samples were collected into lithium heparin vacutainer tubes (Becton Dickson, Plymouth, UK). 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 instructions on the respective commercially available kits (NEFA HR-2 kit; Wako Chemicals GmbH,

Table 3. Chemical composition (mean ± SD) of pasture and concentrate feed during the 11-wk experimental period

	Pasture[1]			Concentrate		
Item	CON	MIX	PA	CON	MIX	PA
DM, %	20.2 ± 3.1	20.9 ± 2.9	20.3 ± 2.8	89.8 ± 0.5	89.6 ± 0.5	89.6 ± 0.3
CP, % of DM	18.5 ± 1.5	18.2 ± 1.5	18.2 ± 1.7	15.6 ± 0.2	15.8 ± 0.5	15.8 ± 0.4
NDF, % of DM	37.4 ± 2.6	36.9 ± 1.7	37.3 ± 2.5	26.3 ± 0.4	23.4 ± 0.9	22.6 ± 0.7
ADF, % of DM	19.6 ± 1.0	19.8 ± 1.1	19.8 ± 1.2	—	—	—
Ash, % of DM	8.2 ± 0.9	8.3 ± 0.9	8.4 ± 1.2	9.0 ± 0.3	8.8 ± 0.3	9.1 ± 0.3
Starch, % of DM	—	—	—	28.1 ± 1.5	26.8 ± 1.7	26.7 ± 1.6
OMD,[2] % of OM	84.5 ± 0.3	84.7 ± 0.4	84.3 ± 0.7	—	—	—
Total FA, % of DM	3.2 ± 0.4	3.1 ± 0.4	3.0 ± 0.3	2.1 ± 0.2	5.8 ± 0.3	6.6 ± 0.9
FA (g/100 g FA)						
C14:0	0.32 ± 0.03	0.33 ± 0.05	0.34 ± 0.03	0.59 ± 0.07	1.10 ± 0.06	0.72 ± 0.09
C16:0	14.03 ± 0.77	14.26 ± 0.8	14.96 ± 1.27	27.19 ± 0.66	57.22 ± 0.65	77.68 ± 0.89
C18:0	1.70 ± 0.08	1.72 ± 0.09	1.87 ± 0.17	3.93 ± 0.12	5.68 ± 0.07	1.16 ± 0.05
C18:1	3.02 ± 0.54	3.11 ± 0.49	3.31 ± 0.75	31.19 ± 0.46	22.47 ± 0.12	8.61 ± 0.31
C18:2	9.50 ± 4.45	9.36 ± 4.38	11.24 ± 0.4	21.65 ± 1.3	6.30 ± 0.3	6.60 ± 0.3
C18:3	60.52 ± 2.45	60.33 ± 2.01	58.77 ± 3.02	1.44 ± 0.03	0.43 ± 0.01	0.44 ± 0.01
Total SFA	20.2 ± 3.55	20.59 ± 3.62	21.65 ± 3.77	38.38 ± 6.88	67.84 ± 14.64	81.91 ± 19.98
Total MUFA	3.92 ± 0.85	3.99 ± 0.89	4.12 ± 0.94	33.43 ± 8.95	23.47 ± 6.46	9.61 ± 2.46
Total PUFA	75.87 ± 16.19	75.4 ± 16.29	74.22 ± 15.76	28.19 ± 5.74	8.69 ± 1.67	8.48 ± 1.76

[1]CON = pasture + 3.6 kg DM concentrate supplement; MIX = pasture + 3.6 kg DM concentrate supplement containing a calcium salt of fatty acids with a fatty acid composition of 58% palmitic acid and 28% oleic acid; PA = pasture + 3.6 kg DM concentrate supplement containing a fat prill of fatty acids with a fatty acid composition of 97% palmitic acid.

[2]OMD = organic matter digestibility.

Neuss, Germany; RB1008 kit; Randox Laboratories Ltd., Crumlin, Co. Antrim, UK). Plasma urea nitrogen (**PUN**) analysis was conducted at University College Dublin (Dublin, Ireland) using enzymatic tests (UR3825 kit; Randox Laboratories Ltd.). Animal behavior in the form of feeding, ruminating and resting time was measured using a 3-axis accelerometer in the MooMonitor+ collar device (Dairymaster, Causeway, Co. Kerry, Ireland).

Statistical Analysis

The cow was the experimental unit to which we applied the treatments for all variables. The power of the study was determined retrospectively using milk fat yield and milk yield values as the outcome variables. The mean and standard deviation values for milk fat yield and milk yield were 1.19, 0.07, 25.1 and 1.83 kg, respectively. To have an 80% chance of detecting, as significant at the 5% level, a difference of 0.04 kg for milk fat yield and 0.9 kg for milk yield, 60 and 66 cows per group were required. All data were analyzed using a linear mixed effects model within the MIXED procedure in SAS version 9.4 (SAS Institute Inc., Cary, NC):

$$Y_{ijklm} = + \mu + T_i + G_j + W_k + TG_{ij} + TW_{ik} + GW_{jk} + TGW_{ijk} + C_{lb} + BX_l + P_m + \varepsilon_{ijklm},$$

where Y_{ijklm} = dependent variable, μ = intercept, T_i = fixed effect of FAS i, G_j = fixed effect of AG j, W_k = fixed effect of wk k, TG_{ij} = fixed interaction effect of FAS i and AG j, TW_{ik} = fixed interaction effect of FAS i and wk k, GW_{jk} = fixed interaction effect of AG j and wk k, TGW_{ijk} = interaction effect of FAS i, AG j and Wk k, C_{lb} = random effect of cow l within block b, BX_l = the covariate adjustment for each cow l, P_m = fixed effect of parity m, and ε_{ijklm} = residual error. An autoregressive AR(1) covariance structure was applied to the repeated measurements taken on individual cows over the experimental and carryover periods. Respective pre-experimental variables were applied as covariate measurements for analysis. The data relating to the 11-wk experimental and 6-wk carryover periods were analyzed separately using the same statistical model. Data relating to DMI and plasma metabolites were analyzed using an adjusted version of the model described above with the repeated effect removed and the fixed effect of week being replaced by sampling time point. All means were generated using the LSMEANS statement. When appropriate, the LSD post hoc mean separation test was used to determine differences between LSM. Statistical significance was considered if $P \leq 0.05$ and statistical trend if $0.05 < P \leq 0.10$.

RESULTS

Total monthly rainfall (mm) and mean monthly air and soil temperatures (°C) for the experimental and carryover periods (April to August), along with the corresponding previous 10-yr averages, are reported in Supplemental Table S1 (see Notes).

Table 4. Effect of concentrate fatty acid supplementation (FAS) and animal genetic group (AG) on milk production and composition during the 11-wk experimental period

	FAS[1]				AG[2]				*P*-value		
Item[3]	CON	MIX	PA	SEM	Elite	NA	JE	SEM	FAS	AG	FAS × AG
Milk yield, kg/d	24.6[y]	25.5[x]	24.7[xy]	0.24	26.6[A]	26.8[A]	21.4[B]	0.28	0.06	<0.01	0.74
ECM yield, kg/d	29.9[y]	30.7[x]	30.4[xy]	0.29	31.6[A]	30.3[B]	29.2[C]	0.29	0.10	<0.01	0.98
3.5% FCM yield, kg/d	29.6[y]	30.6[x]	30.2[xy]	0.31	31.3[A]	30.1[B]	29.1[C]	0.31	0.08	<0.01	0.96
Fat, %	4.82[b]	4.81[b]	4.96[a]	0.03	4.63[B]	4.25[C]	5.72[A]	0.04	0.02	<0.01	0.91
De novo, g/100 g of fat	27.2[a]	25.9[b]	26.1[b]	0.13	26.0[B]	25.7[B]	27.4[A]	0.13	<0.01	<0.01	0.11
Mixed, g/100 g of fat	32.9[b]	32.8[b]	34.0[a]	0.12	32.5[B]	31.8[C]	35.4[A]	0.13	<0.01	<0.01	0.23
Preformed, g/100 g of fat	38.7[b]	40.1[a]	38.7[b]	0.21	40.2[B]	41.2[A]	36.1[C]	0.21	<0.01	<0.01	0.03
Protein, %	3.79[a]	3.73[b]	3.82[a]	0.02	3.68[B]	3.53[C]	4.13[A]	0.02	<0.01	<0.01	0.79
Lactose, %	4.88	4.87	4.86	0.01	4.84[C]	4.87[B]	4.90[A]	0.01	0.41	<0.01	0.45
Fat yield, kg/d	1.17[y]	1.21[x]	1.20[xy]	0.01	1.22[A]	1.14[B]	1.22[A]	0.01	0.07	<0.01	0.98
Protein yield, kg/d	0.92	0.94	0.93	0.01	0.97[A]	0.94[B]	0.88[C]	0.01	0.22	<0.01	0.88
Lactose yield, kg/d	1.20[y]	1.24[x]	1.20[y]	0.01	1.28[A]	1.31[A]	1.05[B]	0.01	0.06	<0.01	0.62
Milk solids yield, kg/d	2.09[y]	2.15[x]	2.14[xy]	0.01	2.19[A]	2.08[B]	2.10[B]	0.02	0.08	<0.01	0.96

[a,b]Means within row with different lowercase superscripts refer to a difference for FAS ($P < 0.05$)

[x,y]Means within row with different lowercase superscripts refer to a tendency to differ for FAS ($0.1 > P > 0.05$).

[A–C]Means within row with different uppercase superscripts refer to a difference for AG ($P < 0.05$).

[1]CON = pasture + 3.6 kg DM concentrate supplement; MIX = pasture + 3.6 kg DM concentrate supplement containing a calcium salt of fatty acids with a fatty acid composition of 58% palmitic acid and 28% oleic acid; PA = pasture + 3.6 kg DM concentrate supplement containing a fat prill of fatty acids with a fatty acid composition of 97% palmitic acid.

[2]Elite = high EBI Holstein Friesian; NA = national average EBI Holstein Friesian and JE = purebred Jersey.

[3]ECM estimated according to Tyrrell and Reid, (1965); 3.5% FCM = [(0.4324 × kg of milk) + (16.216 × kg of milk fat)]; de novo = fatty acids C4 to C14; mixed = fatty acids C16, C16:1; preformed = fatty acids greater than or equal to C17; milk solids = kg of fat + CP.

Grazing Characteristics and Diet Chemical Composition

During the experimental period, pregrazing herbage mass was 1,510 ± 345, 1,591 ± 360, and 1,569 ± 411 kg of DM/ha for CON, MIX, and PA, respectively. Pregrazing compressed sward height was 8.8 ± 1.90, 9.1 ± 2.01, and 9.0 ± 1.98 cm, whereas postgrazing compressed sward height was 4.2 ± 0.62, 4.3 ± 0.34 and 4.2 ± 0.73 cm for CON, MIX, and PA, respectively. During the carryover period, pregrazing herbage mass was 1,934 ± 606, 1,922 ± 523, and 1,968 ± 448 kg of DM/ha for CON, MIX, and PA, respectively. Pregrazing compressed sward height was 8.9 ± 1.49, 8.9 ± 1.78, and 9.1 ± 1.31 cm, whereas postgrazing compressed sward height was 4.3 ± 0.39, 4.3 ± 0.36, and 4.3 ± 0.37 cm for CON, MIX, and PA, respectively. The herbage and concentrate chemical composition during the experimental and carryover periods are presented in Table 3 and Supplemental Table S2 (see Notes).

Effect of FAS and AG on Milk Production and Milk Composition

Cows fed MIX tended to have greater milk yield, ECM yield, 3.5% FCM yield, fat yield, and milk solids yield when compared with cows fed CON, but were similar to cows fed PA (Table 4). Cows fed PA had greater milk fat concentration (4.96%) when compared with cows fed CON (4.82%) and MIX (4.81%; $P = 0.02$). Fatty acid supplementation affected the composition of milk fat produced, with cows fed MIX and PA having a lower concentration of de novo FA when compared with cows fed CON (25.9, 26.1, and 27.2 g/100 g of fat, respectively; $P < 0.01$). Cows fed PA had a greater concentration of mixed FA when compared with cows fed MIX and CON (34.0, 32.8 and 32.9 g/100 g of fat, respectively; $P < 0.01$). Cows fed MIX had a greater concentration of preformed FA when compared with cows fed CON and PA (40.1, 38.7 and 38.7 g/100 g of fat, respectively; $P < 0.01$). There was an effect of FAS on several other milk FA concentrations and indices (Supplemental Table S3, see Notes). Cows fed CON and PA had similar milk protein concentration but were both greater than cows fed MIX ($P < 0.01$). There was no effect of FAS on lactose concentration or protein yield. During the carryover period, there was no effect of FAS on the majority of milk production and milk composition outcomes; however, there were some carryover effects on several milk FA concentrations and indices (Table 5 and Supplemental Table S4, see Notes).

During both the experimental and carryover periods, all milk production outcomes were affected by AG (Tables 4 and 5, Supplemental Tables S3 and S4). There was no FAS by AG interaction on milk production outcomes, except for preformed FA concentrations. During the experimental period, elite cows fed MIX had greater preformed FA concentrations when compared with elite

Table 5. Effect of concentrate fatty acid supplementation (FAS) and animal genetic group (AG) on milk production and composition during the 6-wk carryover period

	FAS[1]				AG[2]				*P*-value		
Item[3]	CON	MIX	PA	SEM	Elite	NA	JE	SEM	FAS	AG	FAS × AG
Milk yield, kg/d	20.5	20.6	20.2	0.29	21.5^{B}	22.3^{A}	17.5^{C}	0.29	0.65	<0.01	0.15
ECM yield, kg/d	25.7	25.9	25.7	0.33	26.5^{A}	25.8^{A}	24.9^{B}	0.33	0.94	<0.01	0.41
3.5% FCM yield, kg/d	25.1	25.3	25.3	0.33	26.1^{A}	24.4^{B}	25.3^{AB}	0.34	0.90	<0.01	0.54
Fat, %	4.97	5.00	5.13	0.06	4.83^{B}	4.35^{C}	5.92^{A}	0.06	0.11	<0.01	0.42
De novo, g/100g of fat	27.1^{a}	26.5^{b}	26.3^{b}	0.13	26.3^{B}	25.9^{C}	27.7^{A}	0.13	<0.01	<0.01	0.14
Mixed, g/100g of fat	34.8	34.4	34.5	0.15	33.9^{B}	33.4^{C}	36.3^{A}	0.15	0.17	<0.01	0.25
Preformed, g/100g of fat	37.6^{b}	38.4^{a}	38.7^{a}	0.22	39.1^{B}	40.2^{A}	35.5^{C}	0.22	<0.01	<0.01	0.03
Protein, %	4.05	4.06	4.10	0.03	3.93^{B}	3.73^{C}	4.55^{A}	0.03	0.46	<0.01	0.64
Lactose, %	4.82^{a}	4.77^{b}	4.79^{ab}	0.01	4.77^{B}	4.81^{A}	4.81^{A}	0.01	<0.01	<0.01	0.48
Fat yield, kg/d	1.00	1.01	1.02	0.01	1.03^{A}	0.97^{B}	1.04^{A}	0.01	0.58	<0.01	0.76
Protein yield, kg/d	0.82	0.83	0.82	0.01	0.84^{A}	0.83^{A}	0.80^{B}	0.01	0.83	<0.01	0.19
Lactose yield, kg/d	0.99	0.98	0.97	0.01	1.03^{B}	1.07^{A}	0.84^{C}	0.01	0.73	<0.01	0.15
Milk solids yield, kg/d	1.83	1.84	1.84	0.02	1.88^{A}	1.80^{B}	1.84^{AB}	0.02	0.90	0.04	0.44

[a,b]Means within row with different lowercase superscripts refer to a difference for FAS ($P < 0.05$).

[A–C]Means within row with different uppercase superscripts refer to a difference for AG ($P < 0.05$).

[1]CON = pasture + 3.6 kg DM concentrate supplement; MIX = pasture + 3.6 kg DM concentrate supplement containing a calcium salt of fatty acids with a fatty acid composition of 58% palmitic acid and 28% oleic acid; PA = pasture + 3.6 kg DM concentrate supplement containing a fat prill of fatty acids with a fatty acid composition of 97% palmitic acid.

[2]Elite = high EBI Holstein Friesian; NA = national average EBI Holstein Friesian and JE = Purebred Jersey.

[3]ECM = estimated according to Tyrrell and Reid, (1965); 3.5% FCM = [(0.4324 × kg of milk) + (16.216 × kg of milk fat)]; de novo = fatty acids C4 to C14; mixed = fatty acids C16, C16:1; preformed = fatty acids greater than or equal to C17; milk solids = kg of fat + CP.

cows fed CON and PA ($P = 0.03$), whereas the preformed FA concentrations of NA and JE cows did not differ by dietary treatment.

Effect of FAS and AG on DMI

The effect of FAS and AG on animal intake during the experimental and carryover periods are presented in Table 6. During the experimental period, there was no effect of FAS on DMI or OM intake. The NDF intake of cows fed CON was greatest, cows fed PA was intermediate, and cows fed MIX was lowest ($P < 0.01$). The FA intake of cows fed PA was greatest (690 g/d), cows fed MIX was intermediate (640 g/d), and cows fed CON was lowest (510 g/d; $P < 0.01$). The intake of C16:0 was greatest for cows fed PA with the intake of C18:1 greatest for cows fed MIX ($P < 0.01$). During the carryover period, there was no effect of FAS on any animal intake outcome (Table 6).

During the experimental period, there was an effect of AG on all animal intake outcomes except for FA intake when expressed relative to total DMI (Table 6). Elite cows had greater intakes of DM, OM, and NDF compared with NA and JE cows ($P < 0.01$). During the carryover period, there was no effect of AG on animal intake, except for DMI when expressed relative to BW, with JE cows being greater than elite and NA cows ($P < 0.01$). During both the experimental and carryover periods, there was no interaction between FAS and AG on animal intake (Table 6).

Effect of FAS and AG on BW, BCS, Feeding Behavior, and Plasma Metabolites

The effect of FAS and AG on BW, BCS, and feeding behavior outcomes are presented in Table 7. During the experimental period, there was no effect of FAS on BW, BCS, and feeding behavior except for BW change. Cows fed PA had greater BW gain when compared with cows fed CON, but were similar to cows fed MIX ($P = 0.01$). During the carryover period, cows fed MIX had lower BW and lower BW gain when compared with cows fed CON and PA (Table 7). In addition, cows fed MIX had greater rumination time when compared with cows fed PA, but were similar to cows fed CON.

During the experimental period, JE cows had lower BW, BW gain, and rumination time when compared with elite and NA cows ($P < 0.01$). During the carryover period, the BW of NA cows were greatest, elite cows were intermediate, and JE cows were lowest ($P < 0.01$). Jersey cows also had lower rumination time when compared with elite cows, but were similar to NA cows ($P < 0.01$). During both the experimental and carryover periods, there was no FAS by AG interaction for BW or BCS outcomes.

The effect of FAS and AG on plasma metabolites are presented in Table 8. During the experimental period, the PUN concentration of cows fed MIX was greatest, cows fed CON was intermediate, and cows fed PA was lowest ($P < 0.01$). Cows fed PA had greater BHB concentrations when compared with cows fed MIX, but

Table 6. Effect of concentrate fatty acid supplementation (FAS) and animal genetic group (AG) on the intake of DM, OM, NDF, and fatty acids during the 11-wk experimental period and 6-wk carryover period

Intake,[3] kg/d (unless otherwise stated)	FAS[1]				AG[2]				*P*-value		
	CON	MIX	PA	SEM	Elite	NA	JE	SEM	FAS	AG	FAS × AG
Experimental period											
Total DM	18.8	18.3	18.8	0.17	19.2[A]	18.6[B]	18.1[B]	0.18	0.09	<0.01	0.36
DMI, % of BW	3.9	3.8	3.9	0.04	3.7[B]	3.5[C]	4.5[A]	0.04	0.12	<0.01	0.97
Pasture DM	15.2	14.7	15.2	0.17	15.6[A]	15.0[B]	14.5[B]	0.18	0.09	<0.01	0.36
OM	17.2	16.9	17.2	0.16	17.6[A]	17.1[B]	16.7[B]	0.16	0.24	<0.01	0.37
NDF	6.7[a]	6.2[c]	6.5[b]	0.06	6.7[A]	6.5[B]	6.3[C]	0.06	<0.01	<0.01	0.39
FA	0.51[c]	0.64[b]	0.69[a]	0.01	0.63[A]	0.62[A]	0.60[B]	0.01	<0.01	<0.01	0.42
C16:0	0.09[c]	0.18[b]	0.26[a]	0.001	0.18[A]	0.18[A]	0.17[B]	0.001	<0.01	<0.01	0.31
C18:1	0.04[b]	0.07[a]	0.04[c]	0.001	0.05[A]	0.05[A]	0.05[B]	0.001	<0.01	<0.01	0.41
FA intake, % DMI	2.71[c]	3.51[b]	3.71[a]	0.01	3.30	3.31	3.32	0.01	<0.01	0.42	0.18
Carryover period											
Total DM	17.2	17.0	17.4	0.23	17.4	17.3	16.8	0.23	0.32	0.11	0.27
DMI, % of BW	3.4	3.5	3.5	0.05	3.2[B]	3.1[B]	4.0[A]	0.05	0.53	<0.01	0.76
Pasture DM	16.3	16.1	16.5	0.23	16.5	16.4	15.9	0.23	0.32	0.12	0.27
OM	15.7	15.7	16.1	0.21	16.0	16.0	15.5	0.21	0.24	0.14	0.19
NDF	6.8	7.0	6.7	0.15	6.8	7.0	6.7	0.15	0.36	0.28	0.04

[a–c]Means within row with different lowercase superscripts refer to a difference for FAS ($P < 0.05$).

[A–C]Means within row with different uppercase superscripts refer to a difference for AG ($P < 0.05$).

[1]CON = pasture + 3.6 kg DM concentrate supplement; MIX = pasture + 3.6 kg DM concentrate supplement containing a calcium salt of fatty acids with a fatty acid composition of 58% palmitic acid and 28% oleic acid; PA = pasture + 3.6 kg DM concentrate supplement containing a fat prill of fatty acids with a fatty acid composition of 97% palmitic acid.

[2]Elite = high EBI Holstein Friesian; NA = national average EBI Holstein Friesian; JE = purebred Jersey.

[3]Estimated using the n-alkane technique during wk 4–5, 9–10, and 15–16.

were similar to cows fed CON. During the carryover period, cows fed MIX had greater PUN concentrations when compared with cows fed CON and MIX ($P < 0.01$). Cows fed CON had greater NEFA concentrations and lower BHB concentrations when compared with cows fed MIX and PA ($P < 0.01$).

During the experimental period, there was an effect of AG on plasma metabolites with JE cows having greater PUN and BHB concentrations when compared with NA cows, but were similar to elite cows (Table 8). During the carryover period, JE cows had greater PUN concentrations when compared with elite and NA cows ($P < 0.01$).

DISCUSSION

There is an opportunity to increase the economic and environmental sustainability of pasture-based systems through mitigation of the seasonal reduction in milk fat concentration observed during the late spring to early summer period. Carty et al. (2017) reported the greatest prevalence of a reduction in milk fat concentration during April and May for both spring and autumn calving Irish herds, indicating that the reduction occurs regardless of the stage of lactation and, therefore, it could be diet induced. Offering FA supplements to grazing dairy cows poses an opportunity to alleviate this reduction in milk fat concentration (Granados-Rivera et al., 2017). Fat supplementation to TMR-fed dairy cows has been demonstrated to increase milk fat concentration and milk fat yield with interesting interactions observed with factors such as stage of lactation, parity, milk production level, and dietary basal fat concentration (Rabiee et al., 2012; de Souza et al., 2019; Bales et al., 2024b). There has been renewed interest into the use of specific FA compositions with experiments suggesting greater milk fat concentration and milk fat yield responses to palmitic acid enriched supplements when compared with FA supplements containing high concentrations of oleic acid, stearic acid, or both (de Souza et al., 2018; Bales et al., 2024a). A similar understanding of the response by grazing dairy cows to palmitic acid enriched FA supplements is lacking with many crucial factors differing among grazing and indoor systems, such as dietary basal fat concentration and fat composition, as well as, animal genetics. Thus, the objective of our experiment was to investigate the effect of FA supplementation on milk fat production and animal performance in genetically diverse grazing cows during the high-risk period for reduced milk fat synthesis.

In the current experiment, offering grazing dairy cows a FA supplement with 97% palmitic acid increased milk fat concentration, whereas offering a FA supplement with 58% palmitic acid and 28% oleic acid had no effect on milk fat concentration when compared with cows fed a nonfat supplemented control diet. These findings support our hypotheses and are in general agreement with indoor feeding system experiments investigating

Table 7. Effect of concentrate fatty acid supplementation (FAS) and animal genetic group (AG) on BW, BCS, feeding, resting and ruminating times during the 11-wk experimental period and 6-wk carryover period

	FAS[1]				AG[2]				*P*-value		
Item	CON	MIX	PA	SEM	Elite	NA	JE	SEM	FAS	AG	FAS × AG
Experimental period											
BW, kg	486	483	484	1.60	524[A]	526[A]	402[B]	1.60	0.44	<0.01	0.99
BW change, kg/wk	2.72[b]	3.31[ab]	3.81[a]	0.26	3.45[A]	3.79[A]	2.60[B]	0.26	0.01	<0.01	0.45
BCS	3.02	3.03	3.04	0.01	3.05	3.03	3.01	0.01	0.65	0.14	0.19
BCS change score/wk	0.004	0.003	0.002	0.003	0.003	0.004	0.002	0.003	0.92	0.92	0.86
Feeding time, min/d	602	602	597	6.33	590	604	607	6.32	0.78	0.13	0.08
Resting time, min/d	291	280	292	6.76	283	284	296	6.76	0.30	0.29	0.02
Ruminating time, min/d	454	462	446	6.77	467[A]	463[A]	433[B]	6.76	0.21	<0.01	0.27
Carryover period											
BW, kg	507[a]	492[b]	509[a]	1.98	542[B]	547[A]	418[C]	2.00	<0.01	<0.01	0.48
BW change, kg/wk	3.11[a]	0.50[b]	2.28[a]	0.33	2.06	2.12	1.71	0.33	<0.01	0.63	0.32
BCS	3.01	3.01	3.02	0.01	3.03[X]	3.02[XY]	2.99[Y]	0.01	0.92	0.09	0.85
BCS change score/wk	−0.002	−0.007	−0.004	0.004	−0.001	−0.008	−0.005	0.004	0.62	0.39	0.85
Feeding time, min/d	636	637	639	6.15	626[X]	643[Y]	642[XY]	6.08	0.94	0.08	0.01
Resting time, min/d	241	235	243	5.96	237	239	244	5.96	0.60	0.70	0.01
Ruminating time, min/d	477[ab]	481[a]	462[b]	5.95	486[A]	475[AB]	460[B]	5.95	0.05	<0.01	0.28

[a,b]Means within row with different lowercase superscripts refer to a difference for FAS ($P < 0.05$).

[A–C]Means within row with different uppercase superscripts refer to a difference for AG ($P < 0.05$).

[X,Y]Means within row with different uppercase superscripts refer to a tendency to differ for AG ($0.1 > P > 0.05$).

[1]CON = pasture + 3.6 kg DM concentrate supplement; MIX = pasture + 3.6 kg DM concentrate supplement containing a calcium salt of fatty acids with a fatty acid composition of 58% palmitic acid and 28% oleic acid; PA = pasture + 3.6 kg DM concentrate supplement containing a fat prill of fatty acids with a fatty acid composition of 97% palmitic acid.

[2]Elite = high EBI Holstein Friesian; NA = national average EBI Holstein Friesian; JE = purebred Jersey.

the effect of FA supplements containing palmitic and oleic acid (de Souza et al., 2019; Western et al., 2020b; de Souza et al., 2021). Rico et al. (2017) demonstrated a linear increase in milk fat concentration as supplementation of an enriched palmitic acid ingredient increased; however, as palmitic acid supplementation increased so did total fat supplementation. de Souza et al. (2019) and Western et al. (2020b) both reported, at equal FA intake, that cows supplemented with enriched palmitic acid ingredients increased milk fat concentration when compared with cows supplemented with a blend of palmitic and oleic acid. In contrast, Bales et al. (2024b) found similar milk fat concentrations regardless of the basal dietary fat composition. Our findings suggest that FA supplementation of grazing dairy cows can increase milk fat concentration but it is dependent on the FA composition of the ingredient.

In agreement with the review by Schroeder et al. (2004), supplementation of FA in our experiment led to a reduction in de novo milk FA concentrations when compared with the nonfat supplemented control; however, the effect on mixed and preformed milk FA concentrations was dependent on the FA composition of the supplement. When a blend of palmitic and oleic acid was offered, cows increased their preformed milk FA concentrations whereas when fed an enriched palmitic acid ingredient cows increased their mixed milk FA concentrations. The increases in mixed and preformed milk FA concentrations for each dietary treatment follow the greater intakes of palmitic and oleic acid demonstrating the transferability of feed FA into milk fat. Furthermore, the reduction in de novo milk FA concentrations when FA supplements were offered, regardless of the FA composition of the supplementary ingredient, suggests an inhibitory effect of these ingredients on mammary gland de novo FA synthesis as previously suggested by Rico et al. (2014b). The increase in mixed milk FA concentrations when cows were fed an enriched palmitic acid ingredient might have led to the increased milk fat concentration observed (Shepardson and Harvatine, 2021). Although, cows fed a blend of palmitic and oleic acid increased their preformed milk FA concentrations, a similar increase in milk fat concentration was not observed. de Souza et al. (2019) suggested that this might be related to the unsaturated oleic acid increasing ruminal unsaturated FA load and affecting rumen biohydrogenation capacity and changing biohydrogenation pathways. Further investigation to better understand the flow of FA from the rumen of a grazing dairy cows when offered FA supplements is required.

Cows fed tropical grasses typically exhibit large milk yield (>15%) and milk fat yield (>10%) responses when offered FA supplements that comprise of a blend of palmitic and oleic acid (Batistel et al., 2017; de Souza et al., 2017; dos Santos Neto et al., 2022). For cows offered temperate grasses, milk yield response is typically closer to 5% (Schroeder et al., 2004). In a meta-analysis

Table 8. Effect of concentrate fatty acid supplementation (FAS) and animal genetic group (AG) on PUN, NEFA, and BHB concentrations during the 11-wk experimental period and 6-wk carryover period

	FAS[1]				AG[2]				*P*-value		
Item[3]	CON	MIX	PA	SEM	Elite	NA	JE	SEM	FAS	AG	FAS × AG
Experimental period											
PUN, mg/dL	11.03^{b}	12.08^{a}	10.33^{c}	0.20	11.15^{AB}	10.69^{B}	11.59^{A}	0.20	<0.01	<0.01	0.01
NEFA, mmol/L	0.14	0.15	0.15	0.01	0.14	0.14	0.15	0.03	0.54	0.77	0.35
BHB, mmol/L	0.80^{ab}	0.74^{b}	0.87^{a}	0.01	0.80^{AB}	0.75^{B}	0.86^{A}	0.03	<0.01	<0.01	0.04
Carryover period											
PUN, mg/dL	10.63^{b}	13.00^{a}	10.21^{b}	0.30	11.04^{B}	10.64^{B}	12.15^{A}	0.30	<0.01	<0.01	0.04
NEFA, mmol/L	0.14^{a}	0.12^{b}	0.11^{b}	0.01	0.13	0.11	0.13	0.01	<0.01	0.13	0.82
BHB, mmol/L	0.71^{b}	0.86^{a}	0.87^{a}	0.04	0.80	0.79	0.84	0.04	<0.01	0.48	0.73

[a–c]Means within row with different lowercase superscripts refer to a difference for FAS ($P < 0.05$).

[A,B]Means within row with different uppercase superscripts refer to a difference for AG ($P < 0.05$).

[1]CON = pasture + 3.6 kg DM concentrate supplement; MIX = pasture + 3.6 kg DM concentrate supplement containing a calcium salt of fatty acids with a fatty acid composition of 58% palmitic acid and 28% oleic acid; PA = pasture + 3.6 kg DM concentrate supplement containing a fat prill of fatty acids with a fatty acid composition of 97% palmitic acid.

[2]Elite = high EBI Holstein Friesian; NA = national average EBI Holstein Friesian; JE = purebred Jersey.

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

focusing on indoor feeding systems, dos Santos Neto et al. (2021) reported increases of 1.53 kg/d of milk yield and 0.04 kg/d of milk fat yield when cows were offered calcium salts of palm FA. In the current experiment, offering grazing dairy cows a FA supplement with 58% palmitic acid and 28% oleic acid tended to increase milk yield (+ 0.9 kg/d) and milk fat yield (+ 0.04 kg/d) when compared with cows fed a nonfat supplemented control diet. These responses are slightly lower for milk yield but similar for milk fat yield when compared with those reported by Heffernan et al. (2025; +1.5 kg/d and +0.04 kg/d for milk yield and milk fat yield, respectively) and by Freeman and Kirkland (2015; +1.3 kg/d and +0.05 kg/d for milk yield and milk fat yield, respectively). The lower milk yield response in the current experiment might be related to pasture basal fat concentration. In Heffernan et al. (2025), the pasture fat concentration was 1.9% of DM, whereas in the current experiment, average pasture fat concentration was 3.1% of DM. When investigating the effect of FA supplementation, Bales et al. (2024b) reported a greater magnitude of increase in milk yield for cows fed low basal fat concentration diets (2% of DM) when compared with cows fed high basal fat concentration diets (3% of DM). This is unlikely the factor involved for the greater responses in milk yield when cows consume tropical pastures, as pasture fat concentrations can be high (3%–4% of DM; Batistel et al., 2017; de Souza et al., 2017; dos Santos Neto et al., 2022). Other factors such as pasture NDF concentration, pasture digestibility, and animal genetics should be considered.

Investigations involving the supplementation of enriched palmitic acid ingredients to grazing dairy cows are lacking in the literature. For indoor feeding systems, supplementation of enriched palmitic acid ingredients, when compared with a nonfat supplemented control diet, generally increases milk fat yield whereas the milk yield response is equivocal (Rico et al., 2017; de Souza and Lock, 2019; Shepardson and Harvatine, 2021; Bales et al., 2024b). In the current experiment, grazing dairy cows fed an enriched palmitic acid ingredient had similar milk yield and milk fat yield when compared with cows fed the nonfat supplemented control diet, which does not support our hypotheses. Several experiments have also reported no effect on milk yield when cows were fed an enriched palmitic acid ingredient; however, these experiments did observe greater milk fat yield due to milk fat concentration increases of greater magnitude (Rico et al., 2017; Shepardson and Harvatine, 2021). In contrast to the meta-analysis by dos Santos Neto et al. (2021), there was no effect of FAS on DMI in the current experiment, ruling out the involvement of this factor in the lack of a milk fat yield response. However, we did observe increased BW gain for cows fed an enriched palmitic acid ingredient when compared with cows fed the nonfat supplemented control diet. Typically, palmitic acid supplementation leads to energy partitioning toward milk production, whereas supplementing with a blend of palmitic and oleic acid increases energy partitioning toward BW (de Souza et al., 2018). This conflicting outcome in the current experiment is unclear but could be related to the many differing factors between pasture and indoor feeding systems previously outlined.

Notably, cows fed a blend of palmitic and oleic acid had reduced milk protein concentration when compared with cows fed the nonfat supplemented control diet, in agreement with the literature (Batistel et al., 2017; de Souza et al., 2017; dos Santos Neto et al., 2021). Some authors have suggested that this is a consequence of milk dilution, as FA supplementation typically increases milk yield but has no effect on milk protein yield. In agreement with the current experiment, Schroeder et al. (2004) reported the reduction in milk protein concentration was greater for unsaturated FA supplements when compared with saturated FA supplements. Future experiments should investigate the combined supplementation of FA and rumen-protected AA to potentially overcome nutrient limitations (Dineen et al., 2021b). Overall, the different milk production responses to FA supplementation in the current experiment highlights the importance of considering the FA composition of a supplement rather than just the additional energy supply. Furthermore, the concept of grazing dairy cows being primarily first limited by energy supply deserves more in-depth investigation.

In the current experiment, there was no effect of FAS during the carryover period on the majority of outcomes. dos Santos Neto et al. (2021) also reported no carryover effect of FA supplementation across a wide range of experiments; however, some experiments where cows were fed tropical pastures reported large carryover effects (de Souza et al., 2017; dos Santos Neto et al., 2022). The lack of a carryover effect in the current experiment is likely due to the modest differences reported during the experimental period. Notably, cows fed a blend of palmitic and oleic acid had reduced BW and reduced BW gain during the carryover period when compared with cows fed an enriched palmitic acid ingredient and with cows fed the nonfat supplemented control diet. It is important that future grazing experiments investigating the effect of FA supplementation undertake carryover measurements and include the measurement of circulating insulin concentrations (De Koster and Opsomer, 2013).

Several interactions, such as stage of lactation and milk production level, as well as genetic merit, have been reported when investigating the effect of FA supplementation (Palmquist et al., 1993; Schroeder et al., 2004; de Souza et al., 2019). For example, both de Souza et al. (2019) and Western et al. (2020b) observed that high-producing cows had greater milk production with greater concentrations of oleic acid, whereas lower-producing cows had greater milk production with greater concentrations of palmitic acid. Thus, it was important to investigate the effect of FA supplementation across genetically diverse groups of grazing dairy cows. Overall, there were no interactions among FAS and AG for the majority of milk production, animal intake, and BW and BCS outcomes. In relation to the main effect of AG, our results are in agreement with previous studies which reported greater milk fat concentration for Jersey cows when compared with Holstein Friesian cows (White et al., 2001; Palladino et al., 2010; Lahart et al., 2024), as well as increased milk fat concentration for cows of greater EBI (O'Sullivan et al., 2019). The greater milk fat concentration observed for JE cows could be related to increased de novo and mixed FA concentrations, combined with lower preformed FA concentrations, when compared with both groups of Holstein Friesian cows.

In the current experiment, FAS numerically increased milk solids yield by 0.055 kg/d and no carryover effect was observed. This modest increase in grazing dairy cow performance must be considered in combination with the likely increase in feed costs. Although the current experiment had a relatively low inclusion level of supplemental fat in the diet, the numerical increases observed in milk fat yield are in line with those observed for indoor feeding systems (dos Santos Neto et al., 2021). Furthermore, the practicality of achieving greater inclusion level of supplemental fat in pasture-based settings is challenging as concentrate pellet stability and palatability would likely deteriorate (Schroeder et al., 2004).

CONCLUSIONS

In this experiment, offering grazing cows a FA supplement with 97% palmitic acid increased milk fat concentration but did not affect milk fat yield when compared with cows fed a nonfat supplemented control diet. Offering grazing cows a FA supplement with 58% palmitic acid and 28% oleic acid tended to increase milk fat yield but reduced milk protein concentration when compared with cows fed a nonfat supplemented control diet. Milk fat production was affected by animal genetics; however, there were no major interactions observed between FA supplementation and AG. Fatty acid supplementation of grazing dairy cows can increase milk fat concentration during the high-risk period for reduced milk fat synthesis but it is dependent on the FA composition of the supplemental ingredient. Overall, careful consideration is required when determining the suitability of offering FA supplements to cows consuming high nutritive value pasture.

NOTES

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Nonstandard abbreviations used: AG = animal genetic group; CON = pasture supplemented with 3.6 kg of DM/cow per day of concentrate containing no supplemental fat; EBI = Economic Breeding Index; FA = fatty acid; FAS = FA supplementation; JE = purebred Jersey; MIX = P supplemented with 3.6 kg of DM/cow per day of concentrate containing a calcium salt of FA with a FA composition of 58% palmitic acid and 28% oleic acid; NA = national average EBI; NEFA = nonesterified FA; OMD = OM digestibility; PA = pasture supplemented with 3.6 kg of DM/cow per day of concentrate containing a fat prill of FA with a FA composition of 97% palmitic acid; PTA = predicted transmitting ability; PUN = plasma urea nitrogen.

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