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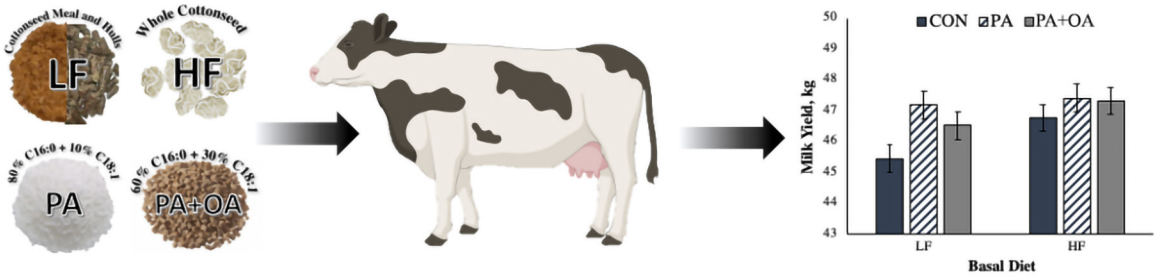
Milk production responses of dairy cows to fatty acid supplements with different ratios of palmitic and oleic acids in low- and high-fat basal diets

A. M. Bales,[1] J. de Souza,[2] and A. L. Lock[1]*

Graphical Abstract



Summary

As cows increase milk production, their nutrient and energy demands become greater. Oilseeds and fatty acid (FA) supplements are common ingredients used to increase the energy density of diets. High-producing, mid-lactation Holstein dairy cows were divided into 2 basal treatment diets to evaluate basal fat content, and then received a treatment of control (no fat supplementation), a C16:0-enriched supplement, or a Ca-salt of C16:0 and *cis*-9 C18:1 supplement. Basal fat content and FA supplementation interacted for milk yield, with FA supplementation increasing milk yield in both basal diets compared with control, and the profile of the supplement differing in milk yield response for the low-fat basal diet.

Highlights

- Interactions were observed between basal fat level and FA supplementation for milk yield and milk FA sources.
- The high-fat basal diet increased yields of milk components.
- Fatty acid supplementation increased milk production performance.
- Differences between FA supplements were minimal, with the C16:0 and *cis*-9 C18:1 supplement decreasing dry matter intake and milk protein yield.

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Milk production responses of dairy cows to fatty acid supplements with different ratios of palmitic and oleic acids in low- and high-fat basal diets

A. M. Bales,[1] J. de Souza,[2] and A. L. Lock[1]*

Abstract: We evaluated the effects of fatty acid (FA) supplements with different ratios of palmitic acid (C16:0) and oleic acid (*cis*-9 C18:1) in low- and high-FA basal diets on production responses of lactating dairy cows. Thirty-six multiparous Holstein cows (50.2 ± 5.8 kg/d of milk; 160 ± 36 d in milk) were used in a split-plot Latin square design balanced for carryover effects. Cows were blocked by milk yield and allocated to a main plot receiving either a low-FA (LF; 1.93% FA content) basal diet (n = 18) containing cottonseed meal and cottonseed hulls or a high-FA (HF; 3.15% FA content) basal diet (n = 18) containing whole cottonseed. Within each plot, a 3 × 3 Latin square arrangement of treatments was used in 3 consecutive 21-d periods. Treatments were (1) control (CON; no FA supplementation), (2) FA supplement containing 80% C16:0 + 10% C18:1 (PA), and (3) FA supplement containing 60% C16:0 + 30% *cis*-9 C18:1 (PA+OA). The FA supplements were fed at 1.5% of dry matter and replaced soyhulls in CON. Preplanned contrasts were (1) overall effect of FA supplementation {CON vs. the average of the FA treatments [1/2 (PA + PA+OA)]}, and (2) the effect of the PA treatment versus the PA+OA treatment (PA vs. PA+OA). Treatment by basal diet interactions were observed for yields of milk and lactose, where FA treatments increased yields of milk and milk lactose in LF but not in HF. Basal diet had no effect on dry matter intake (DMI) or milk yield. Compared with LF, HF increased milk fat yield and 3.5% fat-corrected milk (FCM) and tended to increase milk fat content and energy-corrected milk (ECM) yield. The FA treatments decreased DMI but increased the yields of milk fat, 3.5% FCM, and ECM, compared with CON, due to increases in mixed and preformed milk FA yields. The PA+OA treatment decreased DMI and milk protein yield compared with PA. In conclusion, a high-fat basal diet increased milk fat production, and the addition of FA supplements to a low-fat basal diet increased milk lactose yield and tended to increase milk yield. Additionally, regardless of basal diet fat level, FA supplements increased production responses compared with the non-FA-supplemented control diet.

As cows increase milk production, their nutrient and energy demands become greater. One way to increase the energy density of the diet is the addition of fatty acid (**FA**) supplements, which has become a common practice in dairy cattle nutrition to help support milk production and milk fat yield (Rabiee et al., 2012; dos Santos Neto et al., 2021a,b). Palmitic (**C16:0**) and oleic (***cis*-9 C18:1**) acids are common FA found in commercially available fat supplements. Recent studies have reported positive effects of these 2 FA when supplemented to lactating dairy cows. de Souza et al. (2018) reported that, compared with a non-FA-supplemented control diet, a FA blend containing ~80% C16:0 increased energy partitioning toward milk, whereas a FA blend containing 45% C16:0 and 35% *cis*-9 C18:1 increased energy partitioning toward body tissues. Additionally, when comparing ratios of C16:0 + *cis*-9 C18:1, de Souza et al. (2019) and Western et al. (2020) observed that increasing the proportion of *cis*-9 C18:1 in a FA blend increased milk production of higher-producing cows, whereas increasing the proportion of C16:0 increased milk production of lower-producing cows.

Considering the FA content of the basal diets, Banks et al. (1976) observed that low-fat diets could limit the yields of milk and milk fat, but the addition of different oils and oilseeds to increase supply of FA, increased these yields. Decreased milk fat yield could be due to the low supply of preformed FA available for milk fat synthesis (Palmquist, 2006). Whole cottonseed (**WCS**) is a common byproduct ingredient added to dairy cow diets. It is a unique ingredient due to its high content of FA and moderate levels of fiber and CP (Coppock et al., 1987; Moreira et al., 2004). Dietary inclusion of WCS has increased yields of milk fat and 3.5% FCM compared with other ingredients such as soybean hulls, alfalfa hay, and concentrate mixtures with cottonseed meal (**CSM**; Smith et al., 1981; de Souza et al., 2018). Increases in milk fat yield when feeding WCS could be attributed to the amount of long-chain FA in WCS and the potential for greater incorporation of preformed FA into milk fat (Harrison et al., 1995; Rico et al., 2017).

When considering FA supplementation and the interactions with basal diets differing in FA content, Rico et al. (2017) and de Souza et al. (2018) compared a WCS basal diet with a soybean hull basal diet. Both studies observed an increase in milk fat yield with the WCS basal diet but found contrasting results for production responses for interactions between FA supplementation and basal diets (WCS vs. soybean hull basal diets). Rico et al. (2017) and de Souza et al. (2018) used WCS and soybean hulls to alter basal FA content, resulting in these diets differing in the amount and fermentability of other nutrients. Additionally, de Souza et al. (2018) blended multiple commercial FA supplements to achieve their desired FA ratios. We designed the diets in our study to have similar ingredients to keep rumen fermentation comparable, as well as using commercially available supplements with different ratios of C16:0 + *cis*-9 C18:1. Therefore, the objective of our study was to evaluate low- and high-fat basal diets and interactions with

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Table 1. Ingredient and nutrient composition of treatment diets

	Basal diet					
	Low FA (LF)			High FA (HF)		
Item, % of DM	CON	PA	PA+OA	CON	PA	PA+OA
Ingredient						
Alfalfa silage	13.3	13.3	13.3	13.4	13.4	13.4
Corn silage	33.6	33.6	33.6	33.8	33.8	33.8
Ground corn	12.2	12.2	12.2	12.1	12.1	12.1
High-moisture corn	9.63	9.63	9.64	9.20	9.20	9.19
Soybean meal	8.46	8.46	8.47	8.34	8.34	8.33
Soyhulls	5.36	3.73	3.44	5.36	3.71	3.50
Vitamin and mineral mix[1]	2.08	2.08	2.08	2.10	2.10	2.10
Lactation supplement[2]	6.72	6.72	6.72	6.72	6.73	6.72
Cottonseed hulls	4.50	4.50	4.50	—	—	—
Cottonseed meal	4.07	4.07	4.07	—	—	—
Cottonseed, whole	—	—	—	8.98	8.98	8.97
C16:0-enriched FA[3]	—	1.62	—	—	1.64	—
Ca-salt of palm FA[4]	—	—	1.92	—	—	1.88
Nutrient composition[5]						
NDF	27.0	26.3	26.2	28.8	27.7	27.6
Forage NDF	18.3	18.3	18.3	18.3	18.3	18.3
Starch	31.2	31.2	31.2	30.9	30.9	30.9
CP	17.9	17.7	17.7	17.5	17.3	17.2
FA	1.93	3.43	3.41	3.15	4.66	4.65
16:0	0.33	1.56	1.24	0.64	1.87	1.55
18:0	0.06	0.08	0.13	0.09	0.11	0.16
cis-9 18:1	0.34	0.54	0.75	0.54	0.74	0.95
cis-9,*cis*-12 18:2	0.94	0.97	1.00	1.68	1.72	1.76
cis-9,*cis*-12,*cis*-15 18:3	0.09	0.09	0.09	0.10	0.10	0.10

[1]Vitamin and mineral mix contained 22% ground corn, 21% MIN AD (calcium magnesium carbonate, Min Ad Inc.), 20% calcium carbonate, 19% calcium phosphate, 10% white salt, 5% sodium sesquinate, 2% selenium, <1% tallow, Micro 5 (Alltech), vitamin A, vitamin E, and vitamin D.

[2]Lactation supplement contained 39% Amino Plus (Ag Processing Inc.), 18% CFE pass (Papillon), 16% sodium sesquinate, 13% calcium carbonate, 10% ground corn, 3% urea, and 1% Smartamine (Adisseo).

[3]Spectrum Fusion (Perdue Agribusiness), total fatty acid (FA) content = 92.6% of DM, and containing (g/100 g) 0.78% C14:0, 81.1% C16:0, 1.32% C18:0, 13.0% *cis*-9 C18:1, 2.92% *cis*-9,*cis*-12 18:2, and 0.10% *cis*-9,*cis*-12,*cis*-15 18:3.

[4]Spectrum Distinct (Perdue Agribusiness), total FA content = 79.1% of DM, and containing (g/100 g) 1.02% C14:0, 61.6% C16:0, 4.59% C18:0, 26.7% *cis*-9 C18:1, 4.71% *cis*-9,*cis*-12 18:2, and 0.03% *cis*-9,*cis*-12,*cis*-15 18:3.

[5]Expressed as a percent as fed.

fat supplements containing 80% C16:0 + 10% *cis*-9 C18:1 and 60% C16:0 + 30% *cis*-9 C18:1 on production responses of lactating dairy cows. To keep the basal diets similar, we used WCS or a combination of cottonseed hulls (**CSH**) and CSM to manipulate FA content while keeping the rest of the ingredients the same. Our hypothesis was that basal FA content will alter the magnitude of production responses to FA supplementation.

All experimental procedures were approved by the Institutional Animal Care and Use Committee at Michigan State University (East Lansing). Thirty-six mid-lactation, multiparous Holstein cows from the Michigan State University Dairy Cattle Teaching and Research Center were randomly assigned to a treatment sequence in a replicated split-plot 3 × 3 Latin square design balanced for carryover effects in three 21-d periods. All animals received a common diet with no fat supplementation during a 7-d preliminary period to obtain baseline values.

Cows were assigned to a main plot, with 18 cows (mean ± SD; 50.6 ± 5.8 kg/d of milk, 146 ± 36 DIM, and 3.32 ± 0.25 BCS) receiving a low-fat basal diet (**LF**; average 1.93% FA content) containing CSH and CSM, and 18 cows (49.8 ± 5.9 kg of milk, 174 ± 30 DIM, and 3.36 ± 0.22 BCS) receiving a high-fat basal diet (**HF**; average 3.15% FA content) containing WCS throughout the study. Within each basal diet split-plot, replicated 3 × 3 Latin squares were used to assign FA treatments so that each animal received each of the FA treatments but only 1 basal diet. The FA treatments consisted of (1) control (**CON**; diet with no supplemental FA), (2) FA supplement containing 80% C16:0 + 10% *cis*-9 C18:1 (**PA**), and (3) FA supplement containing 60% C16:0 + 30% *cis*-9 C18:1 (**PA+OA**). Both FA supplements are commercially available and fed at 1.5% FA (DM basis) of the diet, and supplements replaced soyhulls from CON. All experimental diets were formulated to meet the nutrient requirements of the average cow (Table 1; NRC, 2001). The DM concentration of forages was determined twice weekly, and diets adjusted accordingly. Base diets were mixed in a wagon daily, with forages mixed in one base mix that was then split to make the LF and HF bases. Then, soyhulls, FA supplements, and respective base diet were mixed in a tumble-mixer for each treatment diet. Cows were fed 115% of expected intake and feed access was blocked for 2 h to collect orts and offer new feed. Cows were milked twice a day and housed in individual, sawdust bedded tiestalls throughout the experiment with water available ad libitum in each stall.

Table 2. Dry matter intake, production responses, and milk fatty acid (FA) sources of cows fed treatment diets (n = 36)

	Treatment (Trt)				Basal diet (BD)			*P*-value[1]			Contrast[2]	
Variable	CON	PA	PA+OA	SEM[3]	LF	HF	SEM[3]	Trt	BD	Trt × BD	CON vs. FAT	PA vs. PA+OA
DMI, kg/d	27.7	27.7	27.2	0.39	27.3	27.7	0.39	0.01	0.60	0.39	0.16	0.01
Yield, kg/d												
Milk[4]	46.1	47.3	46.9	0.89	46.4	47.2	1.25	<0.01	0.66	0.14	<0.01	0.17
3.5% FCM[5]	47.4	48.9	49.0	0.82	46.7	50.2	1.16	<0.01	0.04	0.35	<0.01	0.72
ECM[6]	47.8	49.0	48.9	0.82	47.1	50.0	1.15	<0.01	0.07	0.44	<0.01	0.50
Fat	1.69	1.76	1.77	0.04	1.65	1.83	0.05	<0.01	0.02	0.34	<0.01	0.35
Protein	1.52	1.52	1.50	0.03	1.49	1.54	0.04	0.08	0.43	0.83	0.55	0.03
Lactose[7]	2.17	2.23	2.21	0.05	2.25	2.17	0.07	<0.01	0.44	0.01	<0.01	0.10
Composition, %												
Fat, %	3.71	3.76	3.81	0.09	3.61	3.91	0.13	<0.01	0.09	0.34	<0.01	0.08
Protein, %	3.30	3.24	3.21	0.03	3.23	3.26	0.04	<0.01	0.65	0.31	<0.01	0.03
Lactose, %	4.73	4.73	4.72	0.07	4.90	4.60	0.10	0.21	0.09	0.20	0.10	0.49
Yield, g/d												
De novo FA	451	423	413	12.0	411	447	16.8	<0.01	0.14	0.35	<0.01	0.03
Mixed FA	590	668	650	17.6	606	666	24.8	<0.01	0.10	0.45	<0.01	<0.01
Preformed FA	546	556	597	9.80	525	608	13.5	<0.01	<0.01	0.54	<0.01	<0.01
3.5% FCM/DMI	1.42	1.48	1.53	0.02	1.44	1.51	0.05	<0.01	0.05	0.41	<0.01	<0.01
BW, kg	758	760	760	11.0	761	758	21.5	0.78	0.92	0.36	0.48	0.95
BW change, kg	0.42	0.36	0.18	0.10	0.29	0.40	0.08	0.23	0.88	0.48	0.24	0.24
BCS	3.45	3.42	3.44	0.05	3.43	3.43	0.06	0.16	0.83	0.77	0.14	0.22
BCS change	0.04	0.06	0.07	0.03	0.06	0.05	0.03	0.27	0.90	0.85	0.16	0.41

[1]*P*-values refer to the ANOVA results for the fixed effects of treatment, basal diet, and the interaction of treatment and basal diet.

[2]CON versus FAT and PA versus PA+OA contrasts tested the overall effect of FA supplementation and the difference between the FA supplements.

[3]Greatest SEM.

[4]Trt × BD: LF (45.5, 47.2, and 46.5 kg/d); CON versus FAT ($P < 0.01$) and PA versus PA+OA ($P = 0.08$). HF (46.8, 47.4, and 47.3 kg/d); CON versus FAT ($P = 0.07$) and PA versus PA+OA ($P = 0.82$).

[5]3.5% FCM = [(0.4324 × kg of milk) + (16.216 × kg of milk fat)].

[6]ECM = [(0.327 × kg of milk) + (12.95 × kg of milk fat) + (7.20 × kg of milk protein)].

[7]Trt × BD: LF (2.19, 2.29, and 2.25 kg/d); CON versus FAT ($P < 0.01$) and PA versus PA+OA ($P = 0.06$). HF (2.15, 2.17, and 2.17 kg/d); CON versus FAT ($P = 0.39$) and PA versus PA+OA ($P = 0.69$).

Samples of milk, dietary ingredients, orts, BW, and BCS were collected according to Burch et al. (2021). Milk component and FA analysis were performed as described by Lock et al. (2013). Yields of individual FA (g/d) in milk fat and nutrient composition of feed ingredient samples were determined according to Boerman et al. (2017).

All data were analyzed using the PROC GLIMMIX model procedure of SAS (version 9.4, SAS Institute Inc.). The model included the random effect of cow nested in basal diet, the fixed effects of basal diet, period, and treatment, and the interactions of basal diet and treatment, period and treatment, and basal diet and period. The 3-way interaction of basal diet, treatment, and period was not significant and was removed from the model. Main effects were declared significant at $P \leq 0.05$ and tendencies at $P \leq 0.10$; interactions were declared significant at $P \leq 0.10$ and tendencies at $P \leq 0.15$. Two orthogonal contrasts were evaluated: (1) the overall effect of FA supplements: CON versus the average of the FA treatments (**FAT**) [1/2 (PA + PA+OA)], and (2) the effect of PA treatment versus PA+OA treatment (PA vs. PA+OA). These contrasts were used to test the main effect of FA treatments and to test the effect of FA treatments within basal level when the interaction of treatment and basal diet was significant.

We observed interactions between basal diet and FA treatments for yield of milk ($P = 0.14$) and milk lactose ($P = 0.01$). Compared with CON, FAT increased milk yield in LF ($P < 0.01$) and tended to increase milk yield in HF ($P = 0.07$) due to the magnitude of difference being greater in LF (Table 2; Figure 1). The PA treatment tended to increase milk yield in LF ($P = 0.08$) compared with PA+OA, but there was no difference in HF ($P = 0.82$). Compared with CON, FAT increased milk lactose yield in LF ($P < 0.01$; Table 2) but there were no differences in HF. The PA treatment tended to increase milk lactose yield in LF ($P = 0.06$) compared with PA+OA, but there was no difference in HF ($P > 0.10$).

Considering the main effect of basal diet, HF increased milk fat and 3.5% FCM yield (both $P \leq 0.04$; Table 2), tended to increase milk fat content and ECM yield (both $P \leq 0.09$), and tended to decrease lactose content ($P = 0.09$) compared with LF. There was no effect of basal diet on DMI, the yields of milk, protein, and lactose, protein content, feed efficiency (3.5% FCM/DMI), BW, BW change, or BCS (all $P \geq 0.16$).

Overall, compared with CON, FAT increased the yields of milk, fat, lactose, 3.5% FCM, and ECM, milk fat content, and 3.5% FCM/DMI (all $P < 0.01$; Table 2), decreased milk protein content ($P < 0.01$), tended to decrease milk lactose content ($P = 0.10$), and did not affect milk protein yield ($P = 0.55$). There was no effect of FAT on DMI, BW, BW change, BCS, or BCS change (all $P \geq 0.14$). Compared with PA+OA, PA increased DMI ($P = 0.01$) and milk protein content and yield (both $P = 0.03$), tended to increase milk lactose yield ($P = 0.10$), decreased 3.5% FCM/DMI ($P < 0.01$), and tended to decrease milk fat content ($P = 0.08$). There was no dif-

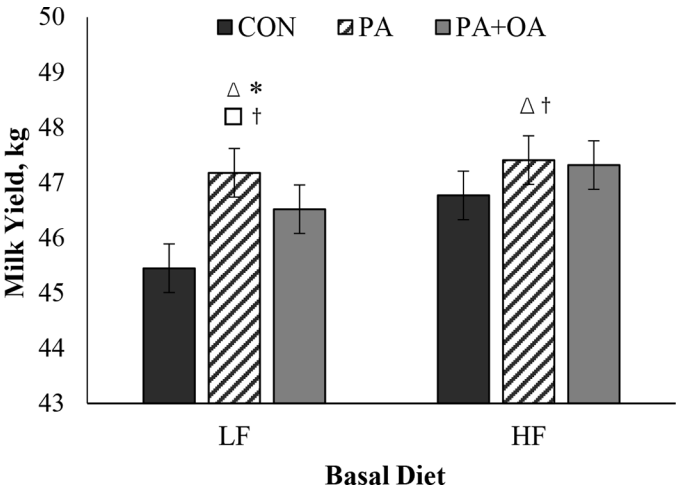

Figure 1. Effects of treatment on milk yield in low fatty acid (LF) and high fatty acid (HF) basal diets. CON = control diet; PA = 1.5% of fatty acid supplement to provide 80% C16:0 + 10% *cis*-9 C18:1; and PA+OA = 1.5% of fatty acid supplement to provide 60% C16:0 + 30% *cis*-9 C18:1. A tendency for an interaction between basal diet and fatty acid (FA) treatment was detected for milk yield ($P = 0.14$). Error bars represent SEM used for each individual treatment. CON versus FAT (Δ) and PA versus PA+OA (□) contrasts were to test the overall effect of FA supplementation and the difference between FA supplements within basal level. For contrast effect within basal diets, a single asterisk (*) denotes ($P < 0.05$) and a dagger (†) denotes ($P < 0.10$).

ference between PA and PA+OA for yields of milk, milk fat, 3.5% FCM, and ECM, milk lactose content, BW, BW change, BCS, and BCS change (all $P \geq 0.17$).

Milk FA are derived from de novo synthesis in the mammary gland and FA derived from extraction from plasma (preformed). Mixed FA originates from both sources. No interactions between basal diet and FA treatments were observed for the yields of de novo, mixed, or preformed milk FA (all $P \geq 0.35$; Table 2). We observed that HF increased the yield of preformed FA ($P < 0.01$) and tended to increase mixed FA ($P = 0.10$) compared with LF. There was no difference in the yield of de novo milk FA between basal diets ($P = 0.14$). Overall, compared with CON, FAT decreased the yield of de novo FA and increased the yields of mixed and preformed FA (all $P < 0.01$). Compared with PA+OA, PA increased the yields of de novo FA ($P = 0.03$) and mixed FA ($P < 0.01$) and decreased the yield of preformed FA ($P < 0.01$).

The primary objectives of our study were to evaluate if differing basal FA contents interacted with the feeding of FA supplements with different ratios of C16:0 and *cis*-9 C18:1. We used WCS for the HF basal diet and CSM and CSH for the LF basal diet to control rumen fermentation, which to our knowledge has not been done previously. The main interaction observed between basal diet and FA supplementation was for milk yield, as PA increased milk yield compared with PA+OA in LF, but not in HF. Additionally, FAT increased milk yield in both basal diets, although the magnitude of difference was greatest for LF. The overall results showed that production responses can be affected by basal FA content, supplementation of FA, as well as the FA profile of the supplement.

We did not observe interactions between basal diet and FA treatments for DMI. In contrast, de Souza et al. (2018) reported interactions between basal diet and FA supplementation with a 45% C16:0 + 35% *cis*-9 C18:1 FA blend decreasing DMI in a WCS basal diet but not in a soyhull basal diet compared with a non-FA-supplemented control. Potential differences in results could be attributed to the FA profile of FA treatments or rumen fermentation differences of the dietary ingredients. Altering the total FA content of the basal diet in our study had no effect on DMI, with the inclusion rate of WCS likely affecting our results. In the literature, experiments with similar WCS inclusion rates as ours (9.0% DM) reported no differences in DMI with addition of WCS (de Souza et al., 2018), but higher inclusion rates have contrasting results on DMI (Sklan et al., 1992; Rico et al., 2017). Dry matter intake is variable for cows fed supplemental FA due to the degree of saturation (Relling and Reynolds, 2007) and the type of supplement being fed (Rabiee et al., 2012). Calcium salts of palm oil, for example, have been reported to decrease DMI (dos Santos Neto et al., 2021a), which is similar to our results with the PA+OA treatment, most likely due to an increase in amount of UFA reaching the small intestine.

As mentioned previously, milk yield was influenced by interactions between basal FA content and FA supplementation. de Souza et al. (2018) observed that FA treatments increased milk yield in a soyhull basal diet but had no effect in a WCS basal diet. The basal FA level in our study had no effect on milk yield. Previous research using WCS to increase basal FA content has reported either a positive effect on milk yield (dos Santos Neto et al., 2021c), no difference (Smith et al., 1981; Rico et al., 2017), or a decrease (de Souza et al., 2018). Reasons for these differences need to be explored further.

Although we did not observe a milk yield response between basal diets, HF increased milk fat yield, 3.5% FCM, and ECM compared with LF, similar to results observed from Smith et al. (1981) and Harrison et al. (1995). The feeding of fat supplements has also been shown to increase milk component yields (dos Santos Neto et al., 2021a,b), and results from the current study are similar as FAT increased yields of milk, 3.5% FCM, and ECM. Previously, altering the ratios of C16:0 and *cis*-9 C18:1 in FA blends has been observed to increase milk production yields, but responses were dependent on production level (de Souza et al., 2019; Western et al., 2020). The cows at the start of our current study averaged 50 kg/d of milk yield and we observed minor production responses differences between PA and PA+OA, similar to de Souza et al. (2019) who observed no production differences between differing ratios of C16:0 + *cis*-9 C18:1 in cows averaging 53 kg/d of milk yield. This could explain our lack of differences between FA treatments, and therefore, it is likely we would have observed more production differences between PA and PA+OA if the cows in our study were producing >55 or <45 kg/d of milk as observed in de Souza et al. (2019) and Western et al. (2020). Also, there is potential that the PA+OA and PA treatments increased milk production responses to similar extents, but through different digestive and metabolic mechanisms. Our current study did not evaluate digestibility parameters, but previously we have reported that *cis*-9 C18:1 increases FA digestibility compared with other FA (Boerman et al., 2015a; Prom and Lock, 2021); thus, we expect the PA+OA treatment to have increased FA digestibility compared with PA, allowing greater flow of FA available for absorption and utilization by the mammary gland. Additionally, in adipose tissue of lactating cows, *cis*-9 C18:1 increased mitochondrion biogenesis

(Abou-Rjeileh et al., 2023), which supports milk biosynthesis (Favorit et al., 2021). Supplementation of C16:0 has been extensively researched, and consistently increases NDF digestibility and milk fat yields compared with no additional FA, and other FA supplements (dos Santos Neto et al., 2021a).

The increase in milk fat yield with the HF diet was due to an increase in preformed milk FA, which would be expected due to the greater intake of long-chain FA from the WCS. These results are similar to Rico et al. (2017) and de Souza et al. (2018), where a basal diet with WCS increased preformed milk FA yield compared with a basal diet containing soyhulls. Due to the current study using cottonseed products, rumen fermentation of basal diets should be comparable; thus, increases observed would be attributed to the FA content and not necessarily potential interactions with diet fermentation. The LF basal diet potentially limited milk fat synthesis due to the lower yields of preformed milk FA compared with HF, as there was no difference in de novo milk FA yield between LF and HF. Overall, FAT increased milk fat yield due to PA increasing mixed milk FA yield, whereas PA+OA increased preformed milk FA yield. These results are similar to those observed by de Souza et al. (2019). We observed a decrease in de novo milk FA yield for FAT, with a greater decrease for PA+OA than for PA, which could be attributed to a substitution effect, where de novo milk FA are compensated for by an increase in preformed milk FA, which often occurs when FA, especially 18-carbon FA, are supplemented in the diet (Glasser et al., 2008; He et al., 2012).

Our study was able to address previous study limitations, as Rico et al. (2017) and de Souza et al. (2018) used WCS and soyhulls to manipulate total FA content of their basal diets, which could have altered rumen fermentation and contributed to the observed production results. Our current study used cottonseed products in our basal diets to have similar rumen fermentations; thus, we decreased the variability between HF and LF. However, we used commercially available FA supplements with 2 specific ratios of C16:0 + *cis*-9 C18:1 that also differed in form of the supplement; thus, we are unable to evaluate if the form (i.e., prilled fat versus Ca-salt of palm FA) would influence production results compared with the FA profile. Although a recent experiment evaluating form versus profile of a FA supplement suggested differences between SFA prills and Ca-salt of palm FA with a similar FA profile (Shpirer et al., 2023), several diet differences between the treatments used could also have resulted in the observed production differences, so results should be interpreted with caution. Therefore, we are confident that FA profile is the most important aspect of a FA supplement when being added to dairy cow diets (de Souza et al., 2018; dos Santos Neto et al., 2021a).

Overall, a HF basal diet increased production compared with a low-FA basal diet, and addition of FA supplements to a LF basal diet increased milk yield. Additionally, both FA supplements (containing either 80% C16:0 + 10% *cis*-9 C18:1 or 60% C16:0 + 30% *cis*-9 C18:1) increased production responses compared with no FA supplementation, regardless of basal diet.

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Notes

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