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Effects of dietary palmitic acid and oleic acid ratio on milk production, nutrient digestibility, blood metabolites, and milk fatty acid profile of lactating dairy cows

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ABSTRACT

Adequate energy supply is a crucial factor for maintaining the production performance in cows during the early lactation period. Adding fatty acids (FA) to diets can improve energy supply, and the effect could be related to the chain length and degree of saturation of those FA. This study was conducted to evaluate the effect of different ratios of palmitic acid (C16:0) to oleic acid (*cis*-9 C18:1) on the production performance, nutrient digestibility, blood metabolites, and milk FA profile in early lactation dairy cows. Seventy-two multiparous Holstein cows (63.5 ± 2.61 days in milk) blocked by parity (2.39 ± 0.20), body weight (668.3 ± 20.1 kg), body condition score (3.29 ± 0.06), and milk yield (47.9 ± 1.63 kg) were used in a completely randomized design. Cows were divided into 3 groups with 24 cows in each group. Cows in the 3 treatment groups were provided iso-energy and iso-nitrogen diets, but the C16:0 to *cis*-9 C18:1 ratios were different: (1) 90.9% C16:0 + 9.1% *cis*-9 C18:1 (90.9:9.1); (2) 79.5% C16:0 + 20.5% *cis*-9 C18:1 (79.5:20.5); and (3) 72.7% C16:0 + 27.3% *cis*-9 C18:1 (72.7:27.3). Fatty acids were added at 1.3% on a dry matter basis. Although the dry matter intake fat-corrected milk yield and energy-corrected milk yield were not affected, the milk yield, milk protein yield, and feed efficiency increased linearly with increasing *cis*-9 C18:1 ratio. The milk protein percentage and milk fat yield did not differ among treatments, whereas the milk fat percentage tended to decrease linearly with the increasing *cis*-9 C18:1 ratio. The lactose yield increased linearly and lactose percentage tended to increase linearly with increasing *cis*-9 C18:1 ratio, but the percentage of milk total solids and somatic cell count decreased linearly. Although body condition scores were not affected by treatments, body weight loss decreased linearly with increasing *cis*-9 C18:1 ratio. The effect of treatment on nutrient digestibility was limited, except for a linear increase in ether extract and neutral detergent fiber digestibility with increasing *cis*-9 C18:1 ratio. There was a linear increase in the concentration of plasma glucose, but the triglyceride and nonesterified FA concentrations decreased linearly with increasing *cis*-9 C18:1 ratio. As the *cis*-9 C18:1 ratio increased, the concentration of de novo FA decreased quadratically, but the mixed and preformed fatty acids increased linearly. In conclusion, increasing *cis*-9 C18:1 ratio could increase production performance and decrease body weight loss by increasing nutrient digestibility, and the ratio that had the most powerful beneficial effect on early lactation cows was 72.7:27.3 (C16:0 to *cis*-9 C18:1).
Key words: palmitic acid, oleic acid, milk production

INTRODUCTION

During the early-lactation period, dairy cows (especially high-yield dairy cows) always suffer from negative energy balance (**NEB**) due to the rapid increase of milk production and the relatively slow increase of DMI (Grummer, 1993). The NEB could increase the mobilization of body fat, result in BW loss, decrease milk production, and increase the incidence of metabolic disorders like fatty liver and ketosis (Overton et al., 2017). Increasing dietary energy was reported to be a feasible strategy during NEB, but because incidences of ruminal acidosis can be increased by increasing starch content, fat supplementation should be the optimal strategy (Greco et al., 2015; Albornoz and Allen, 2018).

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The list of standard abbreviations for JDS is available at adsa.org/jds-abbreviations-24. Nonstandard abbreviations are available in the Notes.

However, the effects of fat supplementation are related to the chain length and degree of saturation of fatty acids (**FA**; Sun et al., 2023). In dairy cows, palmitic acid (C16:0) and oleic acid (*cis*-9 C18:1) are 2 important FA, and are widely used in dairy cows. Previous studies reported that feeding a C16:0-enriched supplement postpartum increased the yield of milk fat and ECM, but did not affect DMI, which resulted in greater BW loss and more severe NEB in early-lactation dairy cows (de Souza et al., 2019). Prom and Lock (2021) found that increasing dietary *cis*-9 C18:1 increased digestibility of DM, reduced BCS loss, and improved the digestibility of total FA in lactating cows. However, He et al. (2012) found that increased dietary *cis*-9 C18:1 ratio decreased milk fat percentage and milk fat yield, and decreased the proportion and yield of de novo milk FA in milk. de Souza et al. (2021b) found that feeding FA supplements containing C16:0 and *cis*-9 C18:1 during the postpartum period increased nutrient digestibility, energy intake, and milk energy distribution. However, to our knowledge, the appropriate ratio of C16:0 to *cis*-9 C18:1 is still unclear for early-lactation cows.

Additionally, previous studies have found that the FA composition of milk affects its flavor. And Eknæs et al. (2009) found that n-3 UFA have beneficial effects on the neurological and cardiovascular systems for people (Trautwein, 2001; Mozaffarian and Wu, 2012). The milk FA profile could be changed by NEB (Bauman and Griinari, 2003) and dietary FA composition (Samková et al., 2012). Inglingstad et al. (2017) found that substituting rapeseed oil for palm oil as the lipid source in dairy goat feed was advantageous, and could improve the flavor and increase the ratio of unsaturated FA in dairy goat milk in mid lactation. However, the effects of different dietary ratios of C16:0 to *cis*-9 C18:1 on the milk FA profile remains unclear.

The present study aimed to explore the effects of different dietary ratios of C16:0 to *cis*-9 C18:1 on the production performance, nutrient digestibility, plasma metabolites, and the milk FA profile, to determine the most beneficial C16:0 to *cis*-9 C18:1 ratio in early lactation dairy cows.

MATERIALS AND METHODS

This study was carried out at Shounong Dairy Farm in Xinxiang, China, from May to July in 2021, and the experimental protocol (GYX 1006) was approved by the Institutional Animal Care and Use Committee of Hebei Agricultural University (Baoding, China).

Cows, Diets, and Experiment Design

Seventy-two multiparous Holstein cows in early lactation (63.5 ± 2.61 DIM) were blocked by parity (2.39 ± 0.20), milk production (47.9 ± 1.6 kg/d), BW (668 ± 20 kg), and BCS (3.29 ± 0.06) and were randomly assigned to one of 3 groups (24 head/group). The FA blend was added at 1.3% (on a DM basis), and contained (1) 90.9% C16:0 + 9.1% *cis*-9 C18:1 (90.9:9.1); (2) 79.5% C16:0 + 20.5% *cis*-9 C18:1 (79.5:20.5); or (3) 72.7% C16:0 + 27.3% *cis*-9 C18:1 (72.7:27.3). The treatments were combinations of 3 commercially available fatty supplements (2 palmitic acid-enriched free fatty pills and 1 Ca salts of palmitic acid, Fengyi Oil Technology [Tianjin] Co. Ltd.), which differed in the ratios of C16:0 and *cis*-9 C18:1, and were added to the TMR as described.

The ingredient and nutrient composition of the diets fed as TMR were shown in Table 1. The diets were formulated to meet the nutrient requirements according to NRC (2001). The TMR was offered to individual cows twice per day, and the cows were milked 3 times daily at 0700, 1500, and 2300 h. The experiment lasted for 56 d after a prefeeding period of 7 d. All cows were housed in a mechanically ventilated tiestall barn, divided into 3 plots with 24 cows each, and had free access to water throughout the experimental period.

Sampling and Analysis

At d 0 and 56 of the experiment, all cows were weighed before morning feeding to calculate the BW changes. The scaled score (1, 2, 3, 4, or 5) was used to determine the BCS according to a standard procedure immediately after BW measurement. According to Wildman et al. (1982), a score of 1 was as follows: the area below the tailhead and between the pin bones was severely depressed, causing the bone structure of the area to appear extremely sharp. A score of 5 indicated the bone structure of the vertebral column, spinous processes, hooks, and pin bone regions was not visually apparent, evidence of subcutaneous fat deposition was prominent, and the tail head appeared to be buried in fatty tissue.

Feed offered and refused for each cow was recorded daily during the experiment. The TMR, feed ingredients, and feed refusal were collected daily. The DMI was calculated as the difference between DM offered and DM refused. Eleven cows from each group were randomly selected for fecal sample collection. The fecal samples (approximately 500 g on a wet weight basis) were collected from the rectum at 0900 h on d 0, 28, and 56, pooled for each cow. The feed and fecal samples were dried at 65°C for 48 h, and ground to pass through a 1-mm screen (stand model 4 Wiley Mill, Arthur H. Thomas, Philadelphia, PA) for chemical analysis.

The feed and fecal samples were used to analyze content of DM (method 930.15), CP (method 996.11), ash (method 942.05), ADF (method 973.18), and ether extract (**EE**, method 920.39) according to the standard

Table 1. Ingredients and compositions of experimental diets (on a DM basis)

Item	Treatment[1]		
	90.9:9.1	79.5:20.5	72.7:27.3
Ingredient, % of DM			
Whole corn silage	27.18	26.81	27.04
Alfalfa silage	4.66	4.67	4.66
Brewers grains	2.90	2.78	3.76
Alfalfa hay	5.57	5.57	5.57
Oat hay	1.94	1.94	1.94
Corn meal	16.20	16.73	16.08
Flaking corn	9.16	8.77	9.16
Soybean meal	13.22	12.72	13.37
Rapeseed meal	0.77	1.68	0.49
Soybean hull	1.55	1.55	1.55
Beet pulp	1.18	1.57	1.18
Yeast culture	1.95	1.95	1.95
Whole cottonseed	6.70	6.21	6.21
Sugarcane molasses	1.08	1.08	1.08
Premix[2]	4.68	4.68	4.68
Fat powder 1	0.96	0	0
Fat powder 2	0	1.28	0.85
Fatty acid calcium	0.26	0	0.43
Nutrient level[3]			
NE_L, MJ/kg	7.28	7.28	7.28
CP, % DM	16.31	16.27	16.36
NDF, % DM	30.64	29.37	30.26
ADF, % DM	16.16	16.38	16.18
Ether extract, % DM	4.14	4.43	4.26
Ca, % DM	0.95	1.02	1.03
P, % DM	0.36	0.37	0.35
Fatty acid composition, % of total fatty acids			
C16:0	30.31	27.78	26.42
C18:0	4.71	3.02	2.94
cis-9 C18:1	16.56	17.87	18.90
cis-9,*cis*-12 C18:2	37.76	40.77	41.08
cis-9,*cis*-12,*cis*-15 C18:3	4.37	4.23	4.53
Others	6.29	6.33	6.13

[1]Treatments were 90.9:9.1 = 1.25% of fatty acid supplement blend to provide 90.9% C16:0 and 9.1% *cis*-9 C18:1; 79.5:20.5 = 1.28% of fatty acid supplement blend to provide 79.5% C16:0 + 20.5% *cis*-9 C18:1; and 72.7:27.3 = 1.28% of fatty acid supplement blend to provide 72.7% C16:0 + 27.3% *cis*-9 C18:1.

[2]Premix contained (per kg of diet): vitamin A, 800,000 IU; vitamin D_3, 280,000 IU; vitamin E, 12,000 IU, Cu, 1,800 mg; Fe, 1,960 mg; Zn, 8,000 mg; Mn, 4,500 mg; I, 126 mg; Se, 50 mg; and Co, 66 mg.

[3]NE_L was calculated as the sum of the NE_L of each raw material multiplied by their respective proportion in the TMR; the rest of the nutrient levels were the measured values.

procedures of AOAC International (2005). The content of NDF in feed and feces was measured using heat-stable α-amylase and sodium sulfite (Van Soest et al., 1991). Calcium and phosphorus content were determined by atomic absorption spectroscopy (method 985.35) and spectrophotometry (method 986.24) according to the standard procedures of the AOAC (1990), respectively. Acid detergent insoluble ash in TMR, feed refused, and feces were used as an internal marker for apparent total-tract digestibility calculation (Van Keulen and Young, 1977).

Milk samples were collected on d 0, 28, and 56 from each cow at every milking time using a continuous milk sampler (DeLaval Fat Sampler MM25–27 BC; DeLaval, Tumba, Sweden), pooled by cow, and the milk yield at each milking time (Manzocchi et al., 2023). Two 40-mL representative milk samples were transferred into two 50-mL centrifuge tubes for further analysis. One milk sample was used to determine milk fat percentage, milk protein percentage, milk lactose percentage, TS, SCC, and MUN using a FOSS Milko Scan (FOSS Food Technology Corp., Eden Prairie, MN). The other milk sample was used to determine the FA profiles, as described by Wang et al. (2022a).

The blood samples were collected on d 0, 28, and 56 through jugular vein puncture into evacuated tubes before morning feeding. Then the samples were centrifuged at 3,500 × *g* for 15 min at 4°C to get the plasma and stored at −20°C until analysis.

Plasma concentrations of total protein (no. A045-4-2), albumin (**ALB**; no. A028-2-1), BUN (no. C013-2-1), and total cholesterol (no. A111-1-1) in plasma were measured using commercial colorimetric assay kits (Nanjing Jiancheng Bioengineering Research Institute Co. Ltd., Nanjing, China) as described by Zhang et al. (2023b), and the plasma concentration of globulin (**GLB**) was calculated as the difference between total protein and ALB. Plasma glucose (**GLU**; no. F006–1-1) concentrations were determined using a colorimetric assay kit (Nanjing Jiancheng Bioengineering Institute) as described by Sun et al. (2023), and triglyceride (**TG**; no. A110–1-1) were determined by using the corresponding colorimetric assay kit (Nanjing Jiancheng Bioengineering Research Institute Co. Ltd.) as described by Wang et al. (2022b).

Plasma concentrations of alanine aminotransferase (**ALT**; no. C009-2-1), aspartate aminotransferase (**AST**; no. C010-2-1), malondialdehyde (no. A003-1-2), as well as the total antioxidant capacity (no. A015-3-1) were measured using commercial colorimetric assay kits (Nanjing Jiancheng Bioengineering Research Institute Co. Ltd., Nanjing, China) according to the method described by Zou et al. (2012). Plasma concentrations of BHB (no. E030-1-1) and nonesterified FA (**NEFA**; no. A045-4-2) were analyzed using a spectrophotometer (model 722, Gaomi Caihong Analytical Instrument Co.) with kits supported by Jiancheng Bioengineering. Institute as described by Williamson et al. (1962) and Johnson and Peters (1993), respectively.

Plasma was analyzed using commercial ELISA kits (Beijing Huayuechang Biotechnology Co., Ltd., Beijing, China) for acetyl-coenzyme A carboxylase (**ACC**; DRE98147), triacylglycerol esterase (**TGL**; DRE98562), carnitine palmitoyltransferase-1 (**CPT-1**; DRE98564), leptin (**LEP**; DRE98028), and insulin (**INS**; DRE98105). The detection ranges of the kits were 0.6 to 20 ng/mL, 20 to 1,500 pg/mL, 100 to 3,000 pg/mL, 0.2 to 8 μg/L, and 0.8 to 30 mU/L, respectively, for plasma ACC, TGL, CPT-1, LEP, and INS. The recoveries of the kits for plasma ACC, TGL, CPT-1, LEP, and INS were 95%, 91%, 93%, 94%, and 90%, respectively. The inter- and intraassay coefficients of variation for the plasma ACC, TGL, CPT-1, LEP, and INS assays averaged 8.4% and 13.8%, 8.8% and 13.5%, 8.7% and 14.6%, 8.4% and 14.8%, and 8.2% and 13.9%, respectively. The linearities of the dilution of kits were 0.9996, 0.9995, 0.9996, 0.9991, and 0.9995, respectively.

Statistical Analysis

All data were analyzed using the mixed model procedure of SAS (version 9.4, SAS Institute Inc., Cary, NC). In the mixed model, the treatments were the fixed effects and the cows were the random effects. Sampling day was considered as a repeated measurement for variables measured over time. Day of feeding was considered as a repeated measurement for DMI. The repeated measures statistical analysis of results was subjected to 5 covariance structures: autoregressive (AR), unstructured (UN), compound symmetry (CS), spatial power (SP), and variance components (VC). The covariance structure that yielded the smallest Schwarz Bayesian criterion was chosen to provide the most desirable and reliable analysis (Littell et al., 1998). The data of the corresponding indexes measured from the samples collected at d 0 were used as covariates for the analysis. The linear and quadratic response to increasing *cis*-9 C18:1 ratio was determined by orthogonal contrasts. Data were presented by the SEM and least squares means. Treatment effects were declared significant at $P \leq 0.05$, and trends were discussed at $0.05 < P \leq 0.10$.

RESULTS

Lactation Performance

Although the yield of 3.5% FCM and ECM was not affected, the milk yield and feed efficiency increased linearly with increasing of *cis*-9 C18:1 ratio ($P < 0.05$; Table 2). The milk protein percentage and milk fat yield did not differ among treatments, whereas the milk fat percentage tended to decrease linearly ($P < 0.05$), and the milk protein yield increased linearly with increasing *cis*-9 C18:1 ratio ($P < 0.05$). The lactose yield increased linearly and lactose percentage tended to increase linearly with increasing *cis*-9 C18:1 ratio ($P < 0.05$), whereas the percentage of milk TS and SCC decreased linearly ($P < 0.05$). Although the BCS changes were not affected by treatments, the BW loss of cows decreased linearly with increasing *cis*-9 C18:1 ratio ($P < 0.05$). The effect of treatment on nutrient digestibility was limited, except for a linear increase in EE and NDF digestibility with increasing of *cis*-9 C18:1 ratio.

Nutrient Digestibility

The digestibility of nutrients, including DM, OM, CP, and ADF, was not affected by treatments (Table 3). The digestibility of EE and NDF increased linearly ($P < 0.05$) with increasing *cis*-9 C18:1 ratio.

Key Enzymes of Fat Metabolism in Plasma

As shown in Table 4, the concentrations of CPT-1 and TGL were not affected by treatments, but the concentration of ACC increased linearly ($P = 0.04$) with increasing *cis*-9 C18:1 ratio.

Antioxidation Index and Blood Biochemistry

The total antioxidant capacity and concentrations of malondialdehyde, ALT, and AST among the groups were

Table 2. Effects of different ratios of dietary C16:0 and *cis*-9 C18:1 on lactation performance and feed efficiency in dairy cows

Item	Treatment[1]			SEM	*P*-value	
	90.9:9.1	79.5:20.5	72.7:27.3		Linear	Quadratic
DMI, kg	24.9	25.0	25.0	0.149	0.62	0.96
Milk yield, kg/d						
Milk	43.2	44.0	44.4	0.249	0.01	0.93
ECM	44.4	44.6	45.5	0.522	0.18	0.39
3.5% FCM	44.9	45.1	45.9	0.599	0.26	0.53
Milk composition						
Fat, kg/d	1.65	1.64	1.66	0.032	0.89	0.56
Fat, %	3.95	3.81	3.79	0.065	0.08	0.57
Protein, kg/d	1.29	1.31	1.35	0.017	0.02	0.36
Protein, %	3.10	3.06	3.06	0.023	0.15	0.55
Lactose, kg/d	2.17	2.26	2.30	0.023	0.01	0.80
Lactose, %	5.19	5.28	5.25	0.028	0.08	0.15
TS, %	13.6	13.1	12.9	0.129	0.01	0.46
MUN, mg/dL	15.4	15.2	15.1	0.193	0.35	0.37
SCC, $\times 10^3$/mL	112	101	92.0	1.95	0.01	0.60
BW, kg	657	664	647	18.9	0.77	0.58
BW loss, kg/d	−0.28	−0.19	−0.14	0.007	0.01	0.62
BCS	3.21	3.25	3.20	0.06	0.95	0.54
BCS loss	−0.10	−0.08	−0.04	0.05	0.42	0.83
Actual milk/DMI	1.73	1.76	1.78	0.011	0.01	0.94

[1]Treatments were 90.9:9.1 = 1.25% of fatty acid supplement blend to provide 90.9% C16:0 and 9.1% *cis*-9 C18:1; 79.5:20.5 = 1.28% of fatty acid supplement blend to provide 79.5% C16:0 + 20.5% *cis*-9 C18:1; and 72.7:27.3 = 1.28% of fatty acid supplement blend to provide 72.7% C16:0 + 27.3% *cis*-9 C18:1.

not affected by treatments (Table 5). Supplementation with different ratios of *cis*-9 C18:1 did not affect the plasma concentrations of total cholesterol, BHB, total protein, ALB, GLB, INS, or LEP in dairy cows (Table 5). The concentrations of GLU increased linearly ($P < 0.05$), whereas TG and NEFA concentrations decreased linearly ($P < 0.05$) with increasing *cis*-9 C18:1 ratio.

Milk Fatty Acid Concentration and Yield

The concentration of de novo synthesis FA tended to decrease quadratically ($P = 0.03$; Table 6), the concentration of mixed-source FA decreased linearly ($P = 0.01$; Table 6), and the concentration of preformed FA increased linearly with increasing *cis*-9 C18:1 ratio ($P = 0.01$; Table 6). The concentration of C16:0 decreased linearly ($P = 0.01$; Supplemental Table S1; see Notes), several *trans* milk FA increased quadratically (all $P < 0.05$, Supplemental Table S1; see Notes), and *cis*-9 C18:1 increased linearly with increasing *cis*-9 C18:1 ratio ($P = 0.02$; Supplemental Table S1; see Notes). The concentration of total SFA decreased linearly ($P = 0.01$; Supplemental Table S1; see Notes), the concentration of MUFA increased linearly ($P = 0.01$; Supplemental Table S1; see Notes), and the concentration of PUFA tended to increase quadratically ($P = 0.06$; Supplemental Table S1; see Notes) with increasing *cis*-9 C18:1 ratio. The concentrations of n-3 and n-6 UFA decreased quadratically ($P < 0.05$; Supplemental Table S1; see Notes). On a yield basis, the de novo synthesis FA yield increased

Table 3. Effects of different ratios of dietary C16:0 and *cis*-9 C18:1 on nutrients' apparent digestibility in dairy cows

Item, %	Treatment[1]			SEM	*P*-value	
	90.9:9.1	79.5:20.5	72.7:27.3		Linear	Quadratic
DM	70.4	71.1	71.7	0.703	0.19	0.91
OM	71.3	71.8	72.3	0.626	0.30	0.90
CP	77.1	77.6	77.7	0.908	0.63	0.95
Ether extract	90.9	92.4	93.3	0.459	0.01	0.29
NDF	43.9	47.4	47.3	1.13	0.03	0.33
ADF	41.8	44.7	43.5	1.22	0.25	0.23

[1]Treatments were 90.9:9.1 = 1.25% of fatty acid supplement blend to provide 90.9% C16:0 and 9.1% *cis*-9 C18:1; 79.5:20.5 = 1.28% of fatty acid supplement blend to provide 79.5% C16:0 + 20.5% *cis*-9 C18:1; and 72.7:27.3 = 1.28% of fatty acid supplement blend to provide 72.7% C16:0 + 27.3% *cis*-9 C18:1.

Table 4. Effects of different ratios of dietary C16:0 and *cis*-9 C18:1 on blood lipid metabolism–related enzymes in dairy cows

	Treatment[1]				*P*-value	
Item[2]	90.9:9.1	79.5:20.5	72.7:27.3	SEM	Linear	Quadratic
ACC, ng/mL	15.3	17.0	17.9	0.899	0.04	0.93
CPT-1, pg/mL	2,722	2,673	2,616	137	0.59	0.92
TGL, pg/mL	1,414	1,394	1,367	71.5	0.66	0.92

[1]Treatments were 90.9:9.1 = 1.25% of fatty acid supplement blend to provide 90.9% C16:0 and 9.1% *cis*-9 C18:1; 79.5:20.5 = 1.28% of fatty acid supplement blend to provide 79.5% C16:0 + 20.5% *cis*-9 C18:1; and 72.7:27.3 = 1.28% of fatty acid supplement blend to provide 72.7% C16:0 + 27.3% *cis*-9 C18:1.

[2]ACC = acetyl-coenzyme A carboxylase; CPT-1 = palmitoyltransferase-1; and TGL = triacylglycerol esterase.

quadratically (P = 0.01; Supplemental Table S2; see Notes), and the concentration of C14:0 decreased quadratically with increasing *cis*-9 C18:1 ratio (P = 0.01; Supplemental Table S2; see Notes). The yield of mixed-source FA decreased linearly and that of preformed FA increased linearly with increasing *cis*-9 C18:1 ratio (P = 0.01; Table 6). The yield of total SFA decreased linearly (P = 0.01; Supplemental Table S2; see Notes), the yield of total MUFA increased linearly (P = 0.01; Supplemental Table S2; see Notes), and the yield of PUFA tended to increase quadratically (P = 0.09; Supplemental Table S2; see Notes) with increasing *cis*-9 C18:1 ratio. The yield of n-6 PUFA increased linearly with increasing *cis*-9 C18:1 ratio (P = 0.02; Supplemental Table S2; see Notes).

DISCUSSION

Production Performance

The effect of fat supplements on DMI is variable and usually depended on the chain length and degree of saturation of the FA (Rabiee et al., 2012). The results of this study showed that the different ratios of C16:0 to *cis*-9 C18:1 in diets had no significant effect on DMI, which was consistent with the study of de Souza et al. (2019). In other words, the ratios of C16:0 to *cis*-9 C18:1 in diets may have a limited effect on the DMI of dairy cows.

Although the effect of C16:0 to *cis*-9 C18:1 ratio on DMI was limited, de Souza et al. (2019, 2021b) found

Table 5. Effects of different ratios of dietary C16:0 and *cis*-9 C18:1 on blood antioxidation index and biochemistry in dairy cows

	Treatment[1]				*P*-value	
Item[2]	90.9:9.1	79.5:20.5	72.7:27.3	SEM	Linear	Quadratic
T-AOC, mmol/L	0.33	0.34	0.35	0.009	0.23	0.84
MDA, nmol/mL	4.43	4.28	4.32	0.323	0.78	0.83
ALT, U/L	5.40	5.14	5.04	0.208	0.25	0.90
AST, U/L	9.51	9.16	9.50	0.626	0.93	0.65
GLU, mmol/L	2.01	2.30	2.40	0.087	0.01	0.19
TG, mmol/L	0.36	0.31	0.23	0.023	0.01	0.25
TCHO, mmol/L	6.88	6.53	6.51	0.305	0.37	0.76
NEFA, mmol/L	0.22	0.22	0.16	0.018	0.02	0.22
BHB, mmol/L	0.83	0.78	0.76	0.036	0.17	0.91
TP, g/L	70.6	72.7	72.9	1.32	0.28	0.37
ALB, g/L	33.7	33.3	32.2	0.603	0.10	0.46
GLB,[3] g/L	37.4	39.4	40.2	1.60	0.26	0.93
ALB/GLB	0.92	0.88	0.82	0.048	0.21	0.76
BUN, mmol/L	4.36	4.13	3.94	0.128	0.03	0.85
INS, mU/L	24.3	26.6	25.9	0.980	0.20	0.30
LEP, μg/L	6.60	6.60	6.61	0.339	0.99	0.99

[1]Treatments were 90.9:9.1 = 1.25% of fatty acid supplement blend to provide 90.9% C16:0 and 9.1% *cis*-9 C18:1; 79.5:20.5 = 1.28% of fatty acid supplement blend to provide 79.5% C16:0 + 20.5% *cis*-9 C18:1; and 72.7:27.3 = 1.28% of fatty acid supplement blend to provide 72.7% C16:0 + 27.3% *cis*-9 C18:1.

[2]T-AOC = total antioxidant capacity; MDA = malondialdehyde; ALT = alanine aminotransferase; AST = glutamic oxaloacetic transaminase; GLU = glucose; TG = triglyceride; TCHO = total cholesterol; NEFA = nonesterified fatty acid; TP = total protein; ALB = albumin; INS = insulin; LEP = leptin.

[3]Globulin (GLB) = TP − ALB.

Table 6. Effects of different ratios of dietary C16:0 and *cis*-9 C18:1 on the summation concentration and yield of milk fatty acid for dairy cows

Item[2]	Treatment[1]			SEM	*P*-value	
	90.9:9.1	79.5:20.5	72.7:27.3		Linear	Quadratic
Summation by source, g/100 g						
De novo	26.8	25.6	26.7	0.388	0.46	0.03
Mixed	35.0	34.0	33.1	0.358	0.01	0.76
Preformed	38.2	40.4	40.2	0.475	0.01	0.15
Summation by source, g/d						
De novo	443	421	444	6.3	0.78	0.01
Mixed	578	558	552	6.1	0.01	0.65
Preformed	628	663	671	8.0	0.01	0.41

[1]Treatments were 90.9:9.1 = 1.25% of fatty acid supplement blend to provide 90.9% C16:0 and 9.1% *cis*-9 C18:1; 79.5:20.5 = 1.28% of fatty acid supplement blend to provide 79.5% C16:0 + 20.5% *cis*-9 C18:1; and 72.7:27.3 = 1.28% of fatty acid supplement blend to provide 72.7% C16:0 + 27.3% *cis*-9 C18:1.

[2]De novo fatty acids (FA; <16 carbons) originate from mammary de novo synthesis; preformed FA (>16 carbons) are extracted from plasma; and mixed FA (*iso*-C16:0 + C16:0 + *trans*-C16:1 + *cis*-7 C16:1 + *cis*-9 C16:1) originate from both sources.

that increasing the ratio of *cis*-9 C18:1 could decrease BW and BCS losses and had a trend of increasing energy distribution to reservation of body fat. In this experiment, although the BCS was not affected, the decreased BW loss indicated that an increased *cis*-9 C18:1 ratio could effectively reduce the body fat mobilization in dairy cows. In dairy cows, the large mobilization of body fat is usually strongly associated with metabolic disorders, including fatty liver and ketosis, which result in numerous health issues (Esposito et al., 2014). Plasma concentrations of BHB, NEFA, and TG were usually used to help diagnose these metabolic disorders (Sordillo, 2016; Dervishi et al., 2021). In dairy cows NEFA always releases from body fat mobilization, especially during NEB, to provide energy (Xu et al., 2020). Nonesterified FA could be re-esterized into TG in the liver and could induce liver damage when accumulated in excess there, which could result in greater AST and ALT concentrations (Zhang et al., 2023a). In the present study, the concentration of blood AST and ALT was not affected by the different treatments, which suggested that the livers of the cows in different groups remained healthy during this experiment. And because BHB is mainly produced by incomplete oxidation in the liver (Zhang et al., 2023a), the hypothesis should be proven by the lack of a treatment effect on BHB concentration in the present study. Because the blood concentration of TG is strongly associated with TG accumulation in the liver, and although the blood concentrations of AST, ALT, and BHB were not affected by the treatments, the linearly decreased blood TG concentration illustrated that increasing the *cis*-9 C18:1 ratio may have a positive potential to maintain liver health. In the present study, although the incidence of metabolic disorders was not recorded, the linearly decreased BW loss, together with decreased plasma concentrations of BHB, NEFA, and TG, demonstrated that increasing the *cis*-9 C18:1 ratio could reduce the risk of metabolic disorders in early lactation dairy cows. The most optimal ratio of dietary FA should be 72.7:27.3 (C16:0 to *cis*-9 C18:1) for cows in early lactation.

In this study, increasing the ratio of *cis*-9 C18:1 in diet increased milk yield in the early lactation cows, which was consistent with the changes of lactose percentage and yield. Because lactose is the most important component to regulate osmotic pressure in the mammary gland (Vilotte, 2002; Wickramasinghe et al., 2012), the similar changes in milk yield and lactose production should be acceptable. Furthermore, the mammary gland can synthesize lactose by using approximately 70% GLU in the blood (Sunehag et al., 2003). Thus, the requirement of GLU in the mammary gland should be increased to maintain the increasing milk yield during the early lactation period (Scott et al., 1976). The concentration of GLU in the blood could be the main reason affecting lactose synthesis (Cant et al., 2002). In the present study, although the reason for increasing GLU concentration was not clear, it may be contributed by the increased NDF and EE digestibility with increasing *cis*-9 C18:1 ratio, which resulted in the improvement of nutrient digestion and absorption. Adeyemi et al. (2016) found that increasing the ratio of *cis*-9 C18:1 in the diet increased the molar ratio of propionic acid. Therefore, we speculate that increasing the ratio of *cis*-9 C18:1 in the diet of dairy cows can promote the decomposition of fiber substances in the rumen, produce a large amount of GLU precursor (propionic acid), increase synthesis of GLU in the liver, and further convert GLU into lactose in the mammary gland, which would result in a greater milk yield. In this study, the plasma concentration of GLU, milk lactose yield, and milk yield all peaked when the ratio of C16:0

to *cis*-9 C18:1 was 72.7:27.3, which suggested that the ratio 72.7:27.3 in early lactation cows will improve their production performance.

Increasing the ratio of *cis*-9 C18:1 in diet may overcome the normal rumen biohydrogenation ability and change the biohydrogenation pathway (de Souza et al., 2018, 2019), which could result in a decrease of milk fat synthesis (de Souza et al., 2021a). In our study, although increasing the ratio of *cis*-9 C18:1 did not cause milk fat depression, the milk fat percentage tended to decrease linearly and the concentrations of several *trans* FA in milk fat increased, manifesting that the milk fat synthesis could be slightly depressed by rumen biohydrogenation.

de Souza et al. (2021a) found that the milk protein percentage and yield did not differ among different FA supplementation treatments during postpartum in dairy cows. However, in our study, although the milk protein percentage did not differ among treatments, the milk protein yield increased linearly with increasing *cis*-9 C18:1 ratio. Another interesting finding in the present study was the linear decrease of BUN concentration with increasing *cis*-9 C18:1 ratio. In dairy cows, BUN could be easily excreted out as MUN or urine N. Because MUN and urine N could be hardly used by humans and dairy cows, which could result in N waste and environment pollution, BUN is widely used as an indicator to evaluate N efficiency in dairy cows (Nasrollahi et al., 2019). In the present study, with the increase in *cis*-9 C18:1 ratio, both BUN and MUN decreased linearly, which illustrated that the N efficiency should be increased and the linearly increased milk protein yield could be explained (Herremans et al., 2020). The BUN was usually transformed by the absorbed ruminal ammonia in the liver. Wu et al. (2016) found that *cis*-9 C18:1 could reduce ruminal ammonia concentration in vitro by inhibiting ruminal microbes and limiting the deamination of proteins and peptides. In the present study, the reason for decreased BUN concentration was unknown due to the lack of data on ruminal ammonia and ruminal microbes, but the linearly decreased BUN concentration, together with the linearly increased milk protein yield, suggested that increasing the *cis*-9 C18:1 ratio could improve N efficiency in early lactation dairy cows. According to the lowest BUN concentration and the greatest milk yield, the most beneficial ratio of C16:0 to *cis*-9 C18:1 was suggested to be 72.7:27.3 in early lactation dairy cows.

The SCC in milk is one indicator for evaluating the inflammatory response of dairy cows (Li et al., 2020), and the decrease in SCC indicated that the inflammatory response of the cows was attenuated. AbuGhazaleh et al. (2003) showed that supplementing with a high *cis*-9 C18:1 ratio decreased the milk SCC of cows, which was in accordance with our findings, indicating that increasing dietary *cis*-9 C18:1 may improve the immune function of the cows and inhibit inflammatory responses.

Nutrient Digestibility

de Souza et al. (2018) observed that feeding an FA mixture (45% C16:0 + 35% *cis*-9 C18:1) increased the NDF digestibility, which was consistent with our results. Brandao et al. (2018) found that adding palm oil could increase the NDF digestibility by increasing cellulose-degrading bacteria. Because NDF could be mainly digested by rumen bacteria, although the rumen bacteria composition was not assessed in the present study, the increased NDF digestibility could also be due to an increase of cellulose-degrading bacteria in the rumen. A previous study reported that increasing the *cis*-9 C18:1 ratio could linearly increase EE digestibility (Adeyemi et al., 2016), which was in consistent with this study. Freitas et al. (2018) reported that the supplementation of *cis*-9 C18:1 could promote the endogenous absorption of EE and resulted in greater EE digestibility.

Blood Metabolites

In animals, ACC was reported to be involved in lipogenesis, whereas CPT-1 and TGL are involved in lipolysis in adipose tissue. Although the blood enzymes are not totally equal to the enzyme in the tissue, the blood concentration of enzymes was reported to be strongly associated with their activity in tissue (Hess, 2013). Thus, the blood concentrations of ACC, CPT-1, and TGL could be used to evaluate the effect of treatment on lipolysis and lipogenesis (Yang et al., 2019; Dirandeh et al., 2021). And in the present study, the linearly increased ACC concentration, together with the lack of difference in CPT-1 and TGL concentration in blood, indicated that the lipogenesis could be increased but the lipolysis was not affected. Because lipolysis and lipogenesis always exist and are balanced in adipose tissue, the increased lipogenesis and unaffected lipolysis illustrated that more fat should be synthesized and deposited in cows supplemented with more *cis*-9 C18:1. In the present study, with the increasing *cis*-9 C18:1 ratio, the linearly decreased BW loss could be partly explained by the increased lipogenesis. The increased lipogenesis resulting from increasing *cis*-9 C18:1 could be due to the effect of oleamide, a derivative of *cis*-9 C18:1, which was reported to act as a ligand for peroxisome proliferator-activated receptor γ, which could promote lipogenesis (Dionisi et al., 2012). Nonesterified FA was reported to be the precursor for lipogenesis, and in our study, although the concentration of oleamide and peroxisome proliferator-activated receptor γ were not determined, increased lipogenesis could be proven by the decreased NEFA concentration. The decreased BW loss could also the result of greater energy balance. In the present study, although the digestibility of DM and OM was not affected by different FA ratios, the digestibility of NDF and EE increased with the increase of *cis*-9 C18:1 ratio, and this result together with the similar DMI among treatment groups suggested that more energy should be absorbed in cows fed more *cis*-9 C18:1. Because the rumen fermentation and microbial composition were not determined, the reason for the greater energy balance resulting from increasing *cis*-9 C18:1 supplementation was not clear; the greater energy balance could be proven by the increased GLU and decreased NEFA and TG concentrations.

Milk Fatty Acids

Milk FA mainly come from 2 sources, one of which is the de novo synthesis FA from the mammary gland, mainly FA less than C16, and half of the C16 FA come from the de novo synthesis FA of the mammary gland, and half come from the blood circulation. Fatty acids in the blood circulation are derived from feed and body fat degradation. When body fat is mobilized, plasma NEFA bound to ALB can also be hydrolyzed by lipoprotein lipase to obtain long-chain FA that are used by the mammary gland to synthesize milk fat (Shi et al., 2016). There was a negative correlation between the *cis*-9 C18:1 content in the diet and the yield of de novo synthesis FA in milk (Dorea and Armentano, 2017). Previous studies have demonstrated that increasing *cis*-9 C18:1 FA reduced the concentration of FA in de novo synthesis and mixed milk FA and increased the concentration of premilk FA (de Souza et al., 2018, 2021a; Western et al., 2020). Similarly to studies mentioned above, this study reported that the mixed-source FA decreased linearly and the concentration of premilk FA increased. In addition, the concentration of C16:0 in milk decreased, whereas that of *trans*-6 C18:1, *trans*-9 C18:1, *cis*-6 C18:1, and *cis*-9 C18:1 increased.

There was a negative correlation between *trans*-oleic acid concentration and milk fat rate (Gaynor et al., 1995; Kadegowda et al., 2008). In the present study, the increase of *trans*-6 C18:1 and *trans*-9 C18:1 concentration may be one of the reasons for the decline in milk fat percentage. In this experiment, the decrease in SFA concentration and the increase of MUFA concentration was mainly due to the decrease in C16:0 ratio and the increase in *cis*-9 C18:1 ratio. With the increase in dietary long-chain FA concentration, the synthesis of short-chain FA was inhibited, the concentration of SFA in milk decreased, and that of MUFA increased (Lashkari et al., 2021).

The C16:0 increased cholesterol and low- and high-density lipoproteins (Amit et al., 2014), but *cis*-9 C18:1 as an UFA reduced blood cholesterol levels, thereby reducing the risk of cardiovascular disease. Omega-3 UFA plays an important role in maintaining the central nervous system, preventing chronic diseases such as cardiovascular diseases and cancer, regulating immune function, and inhibiting inflammatory response (Trautwein, 2001). However, Wu et al. (2010) found that excessive intake of n-6 UFA increased the risk of multiple chronic diseases. In the present study, increasing dietary *cis*-9 C18:1 ratio increased the concentrations of *cis*-9 C18:1 and n-3 and n-6 UFA, but decreased the n-6:n-3 ratio and C16:0 concentration. Therefore, we suggest that the ratio of dietary FA that had the most powerful beneficial effect on early lactation cows is 79.5:20.5 (C16:0 to *cis*-9 C18:1).

CONCLUSIONS

Although the effect of different C16:0 to *cis*-9 C18:1 ratios on DMI was limited, increasing the ratio of *cis*-9 C18:1 could increase milk yield by increasing nutrient digestibility. Additionally, increasing the ratio of *cis*-9 C18:1 could also decrease body fat mobilization and BW loss.

NOTES

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Abbreviations used: ACC = acetyl-coenzyme A carboxylase; ALB = albumin; ALT = alanine aminotransferase; AST = aspartate aminotransferase; CPT-1 = carnitine palmitoyltransferase-1; EE = ether extract; FA = fatty acid; GLB = globulin; GLU = glucose; INS = insulin; LEP = leptin; MDA = malondialdehyde; NEB = negative energy balance; NEFA = nonesterified fatty acid; T-AOC = total antioxidant capacity; TCHO = total cholesterol; TG = triglyceride; TGL = triacylglycerol esterase; TP = total protein.

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