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Ruminant Nutrition

RUMINANT NUTRITION

Effect of stearic or oleic acid on milk performance and energy partitioning when fed in diets with low and high rumen-active unsaturated fatty acids in early lactation

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Abstract

This experiment was conducted to determine the effects of stearic acid (**SA**; C18:0) or rumen-protected oleic acid (**OA**; C18:1 *cis*-9) on milk performance and energy partitioning of early lactation cows when supplemented in diets with low and high level of rumen unsaturated fatty acids (**RUFA**). In low RUFA experiment (**LRUFA**), FA supplement rich in either SA or calcium salts OA was added to a basal diet with a low concentration of RUFA (0.75% vs. 1.4%, LRUFA-SA vs. LRUFA-OA). In high RUFA experiment (**HRUFA**), 2% soybean oil was added to the diet fed in the LRUFA experiment. In each experiment, 30 multiparous cows were blocked by parity and predicted transmitting ability for milk yield and were randomly fed 1 of 2 treatment diets from 2 to 13 wk postpartum. In the LRUFA experiment, LRUFA-SA had 2.4 kg/d more dry matter intake (**DMI**) ($P < 0.01$), 3.8 kg/d more energy-corrected milk ($P < 0.01$), and 0.3% units more milk fat percentage ($P < 0.01$) and 0.2 kg/d more milk fat yield ($P < 0.01$). Dietary treatments did not affect body weight, energy balance, and energy intake partitioning into milk, maintenance, and body tissues ($P > 0.1$). In the HRUFA experiment, HRUFA-SA had 1.4 kg/d more DMI ($P = 0.03$) but similar milk and milk components yields ($P > 0.1$). HRUFA-SA had a tendency to gain more body weight ($P = 0.07$) and had more positive energy balance ($P = 0.01$) and decreased gross feed efficiency (milk yield/DMI) ($P = 0.01$). Consistently, HRUFA-SA increased intake energy partitioning into body tissues ($P = 0.02$) and decreased energy partitioning into milk ($P = 0.01$). In summary, SA supplementation had more DMI relative to OA, but the effects on milk and milk fat production were different and affected by the level of RUFA in the basal diet. In application, SA supplementation was more effective to improve milk production when included in the basal diet with the low RUFA.

Key words: dairy cows, fatty acids, milk fat, ruminal biohydrogenation

Introduction

Dairy cows in early lactation have a significant energy deficit between intake and demands for physiological activity and lactation (Bauman and Griinari, 2003). Fatty acid (**FA**) supplementation increases energy intake, which is demonstrated to benefit milk, metabolic and reproductive performance of cows, especially in early lactation (Palmquist et al., 1986; Staples et al., 1998; Jenkins and Harvatine, 2014). However, there are challenges to the feeding strategy, for example a large amount of unsaturated FA supplementation may impair rumen

fermentation (Chalupa et al., 1986); FA intermediates derived from diet or ruminal biohydrogenation, such as C18:1 *trans*-10 and C18:2 *trans*-10 *cis*-12, could also induce milk fat depression (Bauman and Griinari, 2001); an excessive FA supplementation may also suppress feed intake, immuno- and endocrine systems (Grummer and Carroll, 1991; Overton and Waldron, 2004). Understanding the effects of individual FA on milk performance of lactating cows is necessary.

In the small intestine, adipose tissue, and milk fat, stearic acid (**SA**; C18:0) and oleic acid (**OA**; C18:1 *cis*-9) are dominant FA. In addition, the FAs are also major components of commercial FA supplements, including hydrogenated and calcium salts FA. Wu et al. (1993) fed enriched SA and calcium salts OA in mid-lactation cows and did not observe differences in milk performance. Similar results were also observed by Enjalbert et al. (2000) when infused pure OA and SA in the small intestine in mid-lactation cows. Recently, De Souza et al. (2018) found that SA supplementation increased feed intake but decreased the nutrient digestibility relative to OA in mid-lactation cows, resulting in similar milk production. To our knowledge, the comparison between SA and OA, either as pure or enriched form, is limited in early lactation cows. Because of the significant difference in FA absorption and utilization between early and mid-lactation cows (Bauman and Currie, 1980; Baldwin et al., 1987a, 1987b, 1987c), it is also of particular interest to determine whether SA and OA supplementations have different effects on milk production and energy partitioning in early lactation.

The effects of FA supplementation on milk production is variable (Rabiee et al., 2012; Guiling et al., 2017). Aside from lactation stage, the variation can be partially attributed to the complexity of dietary nutrients, including rumen-active unsaturated FA (**RUFA**) (Mannai et al., 2016; Guiling et al., 2017). In practice, cows are fed a wide range of fat sources, causing RUFA variation. In addition, a large inclusion of corn silage and oil seeds also substantially increases RUFA concentration in diets (Jenkins and McGuire, 2006). Although early lactation cows require unsaturated FA, such as conjugated linoleic acid, to support reproductive and immune systems (Lessard et al., 2004; Bilby et al., 2006), excessive RUFA in the basal diets may also suppress the rumen fermentation and induce milk fat depression (Harvatine and Allen, 2006; Glasser et al., 2008; Meignan et al., 2017). Studying SA and OA supplementation in the basal diet with varied level of RUFA could be an approach to understand the milk performance differences due to individual FA.

Corn silage is a common source of RUFA but sole effects on the rumen are confounded by the additional fiber or soluble carbohydrate. Comparatively, soybean oil supplementation not only substantially increases dietary RUFA but also has a minimum effect on other nutrient components (Abel-Caines et al., 1998; Bu et al., 2007; Alzahal et al., 2008; Barletta et al., 2016). Dry matter intake (DMI) is not reduced when soybean oil inclusion is less than 2 of diet DM (Bateman II and Jenkins, 1998; Dhiman et al., 2000; Fatahnia et al., 2008; Ye et al., 2009). Thus, we chose soybean oil to increase RUFA in the basal diet in order to better reveal the role of RUFA on milk yield and composition when supplementing SA or OA.

The objective of this study was to evaluate the effects of SA and rumen-protected OA enriched supplements on milk production and energy partitioning of early lactation cows when included to either low or high RUFA basal diet. As SA and OA have different digestion and utilization in milk synthesis, we hypothesized that SA and OA supplementations may differentially affect feed intake, milk fat composition, and yield in early lactation cows, and effects may be affected differently at low and high RUFA levels in the basal diet.

Materials and Methods

Animals in experiments were cared and handled according to the guidelines of Washington State University Animal Care and Use Committee (IACUC ASAF# 04823).

Design and Treatments

Because we used a long-term experimental design, it is not applicable to enroll sufficient number of cows to evaluate effects of low and high RUFA at same time at university herd, we compared SA and OA under low or high RUFA basal diet in 2 separate studies. Two experiments were conducted sequentially. In the first experiment, 30 Holstein cows were blocked by parity [1.6 ± 1.1 vs. 1.8 ± 0.9 (mean ± SD)] and predicted transmitting ability for milk production (**PTA**) [963 ± 226 vs. 965 ± 216 (mean ± SD)] and then randomly assigned to 1 of 2 dietary treatments from 2 to 13 wk postpartum. Treatments consisted of FA supplementations either rich in SA (C18:0) (**SA**) or calcium salts OA (C18:1 *cis*-9) (**OA**) in a low RUFA basal diet (**LRUFA experiment**). In the second experiment, the same experimental design was used except for the RUFA (**HRUFA experiment**) level in the basal diet [cow parity: 1.8 ± 1.1 vs. 1.8 ± 0.8; PTA, 966 ± 217; 968 ± 212 (mean ± SD)]. Soybean oil was chosen to increase RUFA because it is less affected by fiber and nonfermentable carbohydrates than corn silage. Approximate 2% of barely grain in the basal diet in the LRUFA experiment was replaced with soybean oil (Table 1). Fatty acid supplement rich in either SA or OA was added to the HRUFA basal diet (**HRUFA-SA** vs. **HRUFA-OA**) to determine the effects of SA and OA supplementations with high RUFA. Diets primarily included 26.1% corn silage, 15.9% corn grain (rolled), 34.4% alfalfa hay, 5.3% soybean meal, and 3.2% dried distillers' grain (Table 1).

A hydrogenated FA supplement (99% FA of DM), Energy Booster 100 (Milk Specialties Global, Eden Prairies, MN), was used to deliver SA, including approximate 35% C16:0, 54% C18:0, and 10% C18:1 *cis*-9 (% DM). Oleic acid was delivered as calcium salts FA supplement (85% FA of DM), Megalac (Church & Dwight Co. Inc., Ewing, NJ), including approximate 44% C16:0, 6% C18:0, and 35% C18:1 *cis*-9 (% DM). Besides with the differences of SA (~48%) and OA (~35%), the SA supplement also had approximately 9% more C16:0 than the OA supplement. Because total FA concentration is different between the FA supplements (99% vs. 85% FA on DM), supplementing 1.2% of Energy Booster 100 and 1.4% of Megalac on DM maintains the similar concentration of total FA between diets.

Diets were mixed and delivered by a Calan Super Data Ranger (American Calan, Northwood, NH). Dry matter concentration in the total mixed ration (**TMR**) was determined weekly, and diets were adjusted when necessary. Cows were housed in a free-stall barn with free access to water and fed individually through a Calan head gate system (American Calan, Northwood, NH) once a day. Intake and orts were recorded daily, and amount offered to cows was 115% of expected daily intake. Cows were milked twice daily at 0010 and 0022 h.

Sample Collection and Measurement

The TMR was sampled each week and analyzed for DM in a forced air oven at 55 °C. Dried samples were ground through 1 mm screen of Wiley mill (Arthur H. Thomas, Philadelphia, PA).

Table 1. Ingredient composition of stearic acid (SA) and oleic acid (OA) diets fed in low and high rumen-active unsaturated fatty acids (RUFA) experiments

	LRUFA experiment[1]		HRUFA experiment[1]	
Item, % DM	LRUFA-SA[2]	LRUFA-OA[2]	HRUFA-SA[2]	HRUFA-OA[2]
Corn silage	26.1	26.1	26.1	26.1
Corn grain	15.9	15.9	15.9	15.9
Alfalfa hay	34.4	34.4	34.4	34.4
Dried distiller grain	3.2	3.2	3.2	3.2
Soybean meal	5.3	5.3	5.3	5.3
Barley grain	11	11	9	9
Soybean oil[3]	0	0	2	2
Megalac[4]	0	1.4	0	1.4
Energy booster[5]	1.2	0	1.2	0
Vitamin and mineral grain mix[6]	1.3	1.3	1.3	1.3
Sodium bicarbonate[4]	0.73	0.52	0.73	0.51
Limestone[7]	0.47	0.24	0.47	0.24
DCAD plus[3]	0.39	0.55	0.39	0.55

[1]LRUFA represents the diets contain low content of RUFA; HAUFA represents the diets contain high content of RUFA.
[2]SA dietary treatment contained approximately 0.4% more C18:0 than OA dietary treatment on DM basis; OA dietary treatment contained approximately 0.3% more C18:1 *cis*-9 than SA dietary treatment on DM basis.
[3]TPI A Nutra Blend Brand, Hubbard, OR.
[4]Church and Dwight Co. Inc., Princeton, NJ.
[5]Milk Specialties Global Eden Prairie, Mountain Lake, MN.
[6]Vitamin and mineral grain mix (% DM) contains 0.5% salt livestock, 0.7% magnesium oxide, 0.6% calcium phosphate monobasic, 0.4% rice hull (Frontier AG, West Sacramento, CA), 0.3% Meta Smart (Adisseo, Alpharetta, GA), 0.2% yeast culture (Diamond V Mills, Inc., Cedar Rapids, IA), 0.1% mineral oil (Brenntag Pacific, Inc., Santa Fe Springs, CA), 0.6% MP 75 (Provimi North America, Inc., Brookville, OH), and <0.1% of each of the following: bioplex Zn, Mn (Alltech Inc., Nicholasville, KY), Mn sulfate (Isky Chemicals Co., Ltd., Hunan, China), Cu sulfate, Bioplex Cu (Alltech Inc., Nicholasville, KY), Selplex 2700 (Alltech Inc., Nicholasville, KY), selenium, TPI EDDI premix (Minerals, L.P., Quincy, IL), cobalt 7.5% (TPi, Madera, CA), vitamin A (Adisseo, Antony Cedex, France), vitamin D3 (TPi, Madera, CA), vitamin E (BASF, Florham, NJ).
[7]Blue Mountain Minerals, Columbia, CA.

Samples were analyzed by Cumberland Valley Analytical Service (**CVAS**, Hagerstown, MD), including crude fat (method 954.02; AOAC, 1990), crude protein (**CP**; method 984.13), acid detergent fiber (**ADF**; method 973.18), neutral detergent fiber (Van Soest et al., 1991), lignin (Goering and Van Soest, 1970), and minerals (AOAC, 2000). Fatty acid composition was also analyzed by CVAS according to the description by Sukhija and Palmquist (1988) and Ulberth and Henninger (1992). Briefly, 1 mL of C19:0 solution, 1 mL toluene, and 3 mL freshly made 5% methanolic HCl were added grounded feeds. After mixing, feeds were heated in 70 °C water bath for 2 h. After that, 6% K_2CO_3 and 2 mL toluene were added in mixture following with centrifugation at 1,100 × *g* for 5 min. The organic layer was transferred to Pyrex tube and dried with Na_2SO_4. Chloroform and methanol were used to extract FA, and the solvent was further removed by N_2. In gas chromatography (**GC**; Shimadzu GC-14A, Columbia, MD) analysis, individual FA were separated with a 25 m × 0.32 mm fused silica column coated with 0.2 μm Sil 88. Samples were injected at a column temperature of 140 °C, and temperature was raised to 180 °C at 3 °C/min. The detector was set up to 230 °C.

Milk samples were taken each week and composited by a.m. and p.m. milking according to the relative weights. Milk samples were analyzed for true protein, fat, lactose, solids nonfat, and milk urea nitrogen (**MUN**) by Fossomatic 4000 Combi infrared analyzer in Utah DHIA lab (Logan, UT) (method 972.160; AOAC, 1990). Milk samples (~50 mL) collected at 3, 6, 9, 12 wk postpartum were frozen at –20 °C until for milk FA analyses. Milk FA composition was analyzed at Clemson University as previously described (Jenkins, 2000; Abughazaleh et al., 2005; Jenkins, 2010). Briefly, milk was centrifugated at 21,000 × *g* for 30 min at 4 °C, and the top fat layer was removed. Fat layer was methylated in 0.5 M sodium methoxide in methanol followed by a second methylation in acetyl chloride:methanol (1:10, v/v) to prevent epimerization and isomerization of conjugated acids. The FA methyl esters in milk were analyzed by GC to determine FA profile. The GC was equipped with a flame ionization detector and a 100 m × 0.25 mm × 0.2 μm film thickness column coated with CP-Sil88 (Chrompack, Raritan, NJ). The column oven was programmed for 140 °C for 3 min followed with 2 °C/min to 220 °C and held at 220 °C for 2 min. Temperatures of the injector and detector ovens were 250 °C. In the separation, helium was used as the carrier gas as 33 cm/s. Blood samples were collected into vacuum tubes from the coccygeal vein at 4, 6, 8, 10, 12 wk postpartum. Samples were immediately centrifuged at 1,000 × *g* for 30 min at 4 °C and supernatant serum was collected for analyses. Serum collected in the LRUFA experiment was analyzed for concentration of glucose by Sigma Glucose Assay kit (Saint Louis, MO), nonesterified FA (**NEFA**) by WAKO NEFA-HR kit (Wako, Richmond, VA), urea N by QuantiChrom Urea Assay DIUR-500 kit (Hayward, CA), and β-hydroxybutyrate (**BHBA**) by β-HB K632-100 kit (Milpitas, CA). In the HRUFA experiment, only the blood concentration of BHBA was analyzed with the same protocol.

Body weight was recorded weekly, and body condition score (**BCS**) was also scored weekly by 2 trained individuals. Body condition score estimation utilized a 5-point scoring system with 0.25 point increment according to the guidelines of Edmonson et al. (1989).

Net energy balance (NE_l) was estimated according to the guidelines of Dairy NRC (2001). NE_l intake was calculated for individual cows in each treatment from NE_l of diet × DMI; Milk energy and maintenance outputs were calculated according to NRC (2001) as

$$\text{Milk energy (Mcal/d)} = [\,9.29 \times \text{fat (kg)} + 5.63 \times \text{true protein (kg)} + 3.95 \times \text{lactose (kg)}\,];$$

$$\text{Maintenance energy (Mcal/d)} = 0.08 \times \text{body weight}^{0.75}\text{(kg)};$$

NE_l balance was calculated as NE_l intake – (Milk energy output + Maintenance energy output).

Statistical Analysis

In each experiment, data were analyzed as a randomized complete block design with repeated measurements using PROC MIXED procedure of SAS version 9.4 according to the following model:

$$Y_{ijkm} = \mu + \alpha_i + \beta_j + \gamma_k + \delta_m(\gamma_k) + (\alpha\beta)_{ij} + \varepsilon_{ijkm}$$

where *Y* is the observation of the *k*th cow at the *j*th sampling time given the *i*th treatment; μ is the overall mean; α_i is the fixed effect of dietary treatment i (SA diet and OA diet); β_j is the fixed effect of sampling time (week); $(\alpha\beta)_{ij}$ is the fixed effect of

interaction between treatment i and sampling time j; γ_k is the random factor of cow; δ_m is the random factor of block. $\delta_m(\gamma_k)$ is the random effect of kth cow nested within the mth block. ε_{ijkm} is the residual term. Estimation of parameters was used the residual maximum likelihood. The AR(1) covariance structure was used in the model. The Kenward–Roger option of the MODEL statement was used to adjust the degrees of freedom. Comparison was performed with TUKEY methods. SLICE option was used in LSMEANS statement to compare 2 treatments within week. Standard error of the mean was reported. $P \leq 0.05$ was considered as a significant difference between treatments. $0.05 < P \leq 0.1$ was considered as a trend.

Results and Discussion

Dietary Chemical and FA Composition

In the LRUFA experiment, LRUFA-SA and LRUFA-OA diets contained similar concentrations of DM, crude fat, total FA, CP, NDF, ADF, and minerals ($P > 0.1$) (Table 2). For individual FA, LRUFA-SA increased C18:0 from 0.11% to 0.55% DM ($P < 0.001$), but decreased C16:0 from 0.91% to 0.79% DM ($P = 0.04$) and C18:1 *cis*-9 from 0.65% to 0.35% DM ($P = 0.002$) when compared to LRUFA-OA. Other individual FA were similar between LRUFA-SA and LRUFA-OA diets.

In the high RUFA experiment, the concentrations of DM, crude fat, total FA, CP, NDF, ADF, and minerals between the HRUFA-SA and HRUFA-OA diets were similar ($P > 0.1$) (Table 2). For individual FA, HRUFA-SA increased C18:0 from 0.23% to 0.72% DM ($P < 0.001$), but decreased C16:0 from 1.45% to 1.32% DM ($P = 0.03$) and C18:1 *cis*-9 from 1.19% to 0.83% DM ($P = 0.002$) when compared to HRUFA-OA. After diets in the HRUFA experiment were blended with soybean oil, the concentration of total FA in the diets was increased approximately 2.2% on DM basis relative to the similar treated diets in the LRUFA experiment. In addition, the concentrations of both saturated and unsaturated FA were all increased, especially for unsaturated FA. The concentration of total unsaturated FA in the HRUFA experiment, including C18:1 *cis*-9, C18:1 *cis*-9 *cis*-12, C18:3 *cis*-9 *cis*-12 *cis*-15, was increased approximately 76% compared with the diets in the LRUFA experiment (1.7% vs. 3% DM).

DMI and FA Intakes

Table 3 summarizes the intake of DM and FA. An interaction between diet and week of lactation was not observed ($P > 0.1$).

In the LRUFA experiment, LRUFA-SA had 2.4 kg/d more DMI than LRUFA-OA ($P < 0.01$) (Table 3; Fig. 1 panel A). In the HRUFA experiment, HRUFA-SA had 1.4 kg/d more DMI than HRUFA-OA ($P = 0.03$) (Fig. 1 panel B). Allen (2000) suggested that the unsaturated FA has a hypophagic effect, and the impact is more significant when unsaturated FA are present in the small intestine. In a meta-analysis, calcium salts OA supplementation is also found to have more negative impact on DMI than saturated FA supplementation (Rabiee et al., 2012). In this study, OA was delivered by calcium salts which would have had limited ruminal biohydrogenation, and most would be absorbed in the small intestine (Wu et al., 1991). Thus, the reduced DMI in the OA supplementation in both studies may be caused by the hypophagic effect of OA in the small intestine. Of note, Wu et al. (1991) compared supplementation of OA and SA in the

Table 2. Chemical and fatty acids (FA) composition of stearic acid (SA) and oleic acid (OA) dietary treatments fed in low and high rumen-active unsaturated fatty acids (RUFA) experiments

	LRUFA experiment[1]			*P*-value	HRUFA experiment[1]			*P*-value
Items, % of DM	LRUFA-SA[2]	LRUFA-OA[2]	SEM	Diets	HRUFA-SA[2]	HRUFA-OA[2]	SEM	Diets
DM	56.4	54.4	0.83	0.54	55.2	54.9	0.36	0.23
CP	17.1	16.7	0.05	0.28	17.8	17.6	0.19	0.63
Crude fat	3.2	3.3	0.20	0.32	5.3	5.6	0.16	0.26
Fatty acids	2.8	2.7	0.37	0.29	4.8	4.9	0.19	0.85
NDF	29.9	30.6	0.86	0.68	28.9	28.7	0.49	0.95
ADF	20.7	21.2	0.50	0.65	18.9	18.3	0.36	0.30
Ash	9.3	9.2	0.15	0.78	9.3	9.0	0.19	0.44
Ca	1.1	1.1	0.02	0.26	1.0	1.1	0.03	0.13
P	0.38	0.36	0.01	0.29	0.38	0.36	0.01	0.1
K	2.2	2.3	0.04	0.28	2.1	2.2	0.03	0.16
Mg	0.36	0.31	0.01	0.15	0.36	0.33	0.01	0.11
Na	0.43	0.34	0.02	0.12	0.53	0.39	0.02	0.15
Cl	0.47	0.42	0.01	0.14	0.42	0.38	0.01	0.17
S	0.29	0.27	0.01	0.35	0.27	0.26	0.01	0.12
DCAD[3], mEq/100 g DM	44.1	43.4	1.09	0.76	47.9	46.0	0.93	0.25
NE_l (Mcal/kg)	1.5	1.5	0.01	0.95	1.8	1.8	0.01	0.9
Fatty acids (% DM)								
C16:0	0.79	0.91	0.03	0.04	1.32	1.45	0.05	0.03
C18:0	0.55	0.11	0.10	<0.001	0.72	0.23	0.06	<0.001
cis-9 C18:1	0.35	0.65	0.08	0.002	0.83	1.19	0.06	0.002
cis-9, *cis*-12 C18:2	0.75	0.83	0.03	0.10	1.4	1.4	0.09	0.98
cis-9, *cis*-12, *cis*-15 C18:3	0.21	0.2	0.01	0.48	0.19	0.17	0.01	0.65
Unsaturated FA[4]	1.3	1.7	0.38	0.23	2.5	3.0	0.15	0.26

[1]LRUFA represents the diets contain low content of RUFA; HAUFA represents the diets contain high content of RUFA.
[2]SA dietary treatment contained approximately 0.4% more C18:0 than OA dietary treatment on DM basis; OA dietary treatment contained approximately 0.3% more C18:1 *cis*-9 than SA dietary treatment on DM basis.
[3]DCAD = [(% Na × 43.5 + % K × 25.6) – (% Cl × 28.2 + % S × 62.5)] (% on DM basis).
[4]Includes *cis*-9 C18:1, *cis*-9, *cis*-12 C18:2, and *cis*-9, *cis*-12, *cis*-15 C18:3.

Table 3. Dry matter intake (DMI) and fatty acid (FA) intake of cows fed stearic acid (SA) and oleic acid (OA) dietary treatments in low and high rumen-active unsaturated fatty acids (RUFA) experiments

	LRUFA experiment[1]						HRUFA experiment[1]					
	Treatment[2]			P-value			Treatment[2]			P-value		
Intake, kg/d	SA	OA	SEM	Trt	Wk	Trt × Wk	SA	OA	SEM	Trt	Wk	Trt × Wk
DMI	29.0	26.6	0.60	<0.01	<0.01	0.11	29.8	28.4	0.52	0.03	<0.001	0.77
C16:0	0.22	0.24	0.005	0.15	<0.001	0.11	0.39	0.41	0.008	0.12	<0.001	0.82
C18:0	0.16	0.03	0.002	<0.001	<0.001	0.21	0.21	0.07	0.003	<0.001	<0.001	0.23
cis-9 C18:1	0.10	0.17	0.003	<0.001	<0.001	0.17	0.25	0.34	0.006	<0.001	<0.001	0.88
cis-9, *cis*-12 C18:2	0.22	0.22	0.004	0.37	<0.001	0.10	0.42	0.4	0.008	0.16	<0.001	0.71
cis-9, *cis*-12, *cis*-15 C18:3	0.06	0.05	0.001	<0.001	<0.001	0.34	0.06	0.05	0.001	<0.001	<0.001	0.49
Unsaturated FA[3]	0.39	0.46	0.008	<0.001	<0.001	0.15	0.74	0.85	0.016	<0.001	<0.001	0.87
Total FA	0.80	0.72	0.015	<0.001	<0.001	0.12	1.42	1.39	0.029	0.35	<0.001	0.73

[1]LRUFA represents the diets contain low content of RUFA; HAUFA represents the diets contain high content of RUFA.
[2]SA dietary treatment contained approximately 0.4% more C18:0 than OA dietary treatment on DM basis; OA dietary treatment contained approximately 0.3% more C18:1 *cis*-9 than SA dietary treatment on DM basis.
[3]Includes *cis*-9 C18:1, *cis*-9, *cis*-12 C18:2, and *cis*-9, *cis*-12, *cis*-15 C18:3.

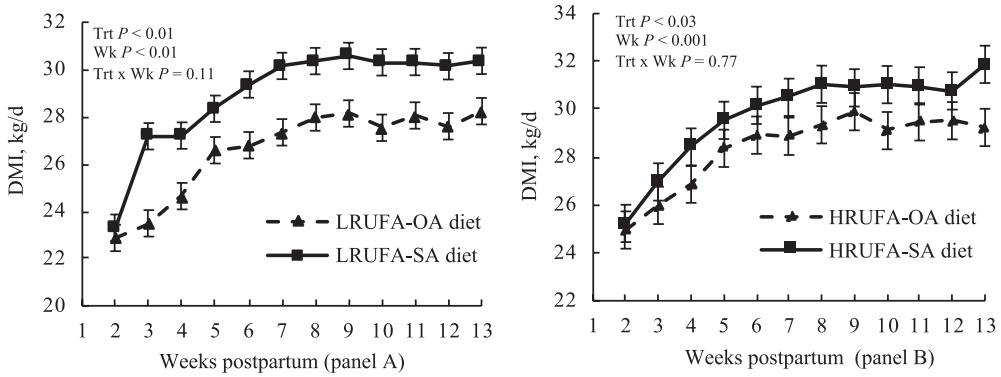

Figure 1. Dry matter intake of early lactating cows fed stearic acid (SA) and oleic acid (OA) diets in low (panel A) and high (panel B) rumen-active unsaturated fatty acids (RUFA) experiments. Data are presented as mean ± SEM.

mid-lactation cows and did not observe a difference on DMI, so the hypophagic effects of OA may be also dependent on the stage of lactation.

In both LRUFA and HRUFA experiments, the greater DMI in SA treatment resulted in the similar C16:0 intake between SA and OA treatments ($P > 0.1$) though SA diet contained ~2% more C16:0 than OA diet (% DM). In the LRUFA experiment, C18:0 intake was increased ~0.13 kg/d in LRUFA-SA ($P < 0.001$; 0.03 vs. 0.16 kg/d); C18:1 *cis*-9 intake was increased ~0.07 kg/d in LRUFA-OA ($P < 0.001$; 0.17 vs. 0.1 kg/d). In the HRUFA experiment, C18:0 intake was increased ~0.14 kg/d in HRUFA-SA ($P < 0.001$; 0.07 vs. 0.21 kg/d); C18:1 *cis*-9 intake was increased 0.09 kg/d in HRUFA-OA ($P < 0.001$; 0.34 vs. 0.25 kg/d). In both experiments, C18:3 *cis*-9 *cis*-12 *cis*-15 intake was also increased 0.01 kg/d in HRUFA-SA (0.05 vs. 0.06 kg/d, $P < 0.001$). Thus, SA and OA were major difference of FA intakes between dietary treatments in each study.

Milk Production

Data of BW, BCS, milk yield, and milk composition are summarized in Table 4. In the LRUFA experiment, the change of BW ($P = 0.69$) and BCS ($P = 0.43$) was similar between LRUFA-SA and LRUFA-OA, suggesting the treatments may not affect body fat mobilization and deposition (Table 4). Milk yield was also similar between treatments ($P = 0.2$), but LRUFA-SA had 0.3% units greater milk fat % ($P < 0.01$; 3.4% vs. 3.7%) and 0.2 kg/d more milk fat yield ($P < 0.01$; 1.7 vs. 1.9 kg/d). In early lactation, intake and body fat are 2 major sources to support milk fat production (Bauman and Currie, 1980; Baldwin et al., 1987a, 1987b, 1987c). Because body energy status was not affected by diets, the differences of milk fat percentage and yield would result from the FA intake. Although LRUFA-SA had ~0.08 kg/d more FA intake, SA usually has a lower digestibility than OA in small intestine (De Souza et al., 2018), causing less than 0.08 kg/d difference for postabsorptive FA. Intriguingly, LRUFA-SA profoundly increased milk fat yield (~0.2 kg/d) corresponded with milk fat % increase (0.3% units), suggesting the mammary gland might prefer to utilize SA for milk fat synthesis relative to OA. Consistently, Vargas-Bello-Pérez et al. (2019) treated bovine mammary gland epithelial cells with long-chain FA in vitro and observed that SA had a more potent capacity to increase milk triglycerides production relative to OA, further supporting our in vivo observation. Interestingly, milk protein yield was also increased approximate 0.1 kg/d in LRUFA-SA ($P = 0.02$; 1.4 vs. 1.5 kg/d). Currently, the FA effects on milk protein synthesis remain largely unknown, but FA intake not only provides energy for milk protein synthesis, but individual FA can be also integrated into the metabolic signaling in activating milk protein synthesis (Rhoads and Grudzien-Nogalska, 2007; Osorio et al., 2016). Thus, the increase of milk protein yield may be attributed to the additional FA intake and activation of SA on milk protein synthesis, but the

Table 4. Body weight (BW), body condition score (BCS), and milk production of cows fed stearic acid (SA) and oleic acid (OA) dietary treatments in low and high rumen-active unsaturated fatty acids (RUFA) experiments

	LRUFA experiment[1]						HRUFA experiment[1]					
	Treatment[2]			P-value			Treatment[2]			P-value		
Items	SA	OA	SEM	Trt	Wk	Trt × Wk	SA	OA	SEM	Trt	Wk	Trt × Wk
BW, kg	693	689	13.5	0.82	0.15	0.30	726	682	13.8	<0.001	0.54	0.70
BW gain, kg/d	0.9	0.7	0.43	0.69	0.22	0.42	0.5	−0.1	0.24	0.07	0.67	0.73
BCS	2.69	2.66	0.04	0.59	0.04	0.84	2.59	2.63	0.06	0.35	0.13	0.83
BCS change	−0.004	−0.002	0.001	0.43	0.92	0.88	−0.004	−0.001	0.01	0.82	0.02	0.6
Milk yield, kg/d	50.6	48.8	1.3	0.2	<0.01	0.93	54.4	55.2	1.11	0.58	<0.001	0.84
ECM, kg/d[3]	51.8	48.0	0.8	<0.01	0.02	0.90	51.6	52.1	0.93	0.69	<0.01	0.87
Milk fat, %	3.7	3.4	0.08	<0.01	<0.01	0.98	3.1	3.2	0.1	0.74	<0.001	0.48
Milk fat yield, kg/d	1.9	1.7	0.03	<0.01	0.01	0.96	1.7	1.7	0.05	0.65	0.61	0.64
Milk protein, %	2.9	2.9	0.05	0.5	<0.01	0.30	2.9	2.8	0.03	<0.001	<0.001	0.24
Milk protein yield, kg/d	1.5	1.4	0.02	0.02	0.07	0.63	1.6	1.5	0.02	0.52	<0.001	0.82
Milk lactose, %	5.0	4.9	0.04	0.07	0.77	0.83	4.8	4.8	0.03	0.67	0.1	0.36
Milk lactose yield, kg/d	2.5	2.4	0.06	0.09	<0.01	0.78	2.6	2.7	0.06	0.56	<0.001	0.87
Milk SNF, %[4]	8.8	8.7	0.08	0.10	0.01	0.44	8.7	8.6	0.06	0.15	0.01	0.49
Milk SNF yield, kg/d	4.5	4.2	0.09	0.05	0.05	0.7	4.7	4.7	0.09	0.96	<0.001	0.7
MUN, mg/dL	15.0	14.1	0.7	0.17	0.59	0.5	13.4	13.7	0.35	0.51	<0.001	0.67
ECM/DMI	1.8	1.8	0.05	0.18	0.47	0.44	1.8	1.9	0.03	0.01	<0.001	0.83
NE_l balance, Mcal/d	−3.0	−3.9	0.70	0.31	<0.01	0.99	7.2	4.7	0.71	0.01	<0.001	0.72

[1]LRUFA represents the diets contain low content of RUFA; HAUFA represents the diets contain high content of RUFA.
[2]SA dietary treatment contained approximately 0.4% more C18:0 than OA dietary treatment on DM basis; OA dietary treatment contained approximately 0.3% more C18:1 *cis*-9 than SA dietary treatment on DM basis.
[3]Energy-corrected milk = [(0.327 × kg of milk) + (12.95 × kg of milk fat) + (7.2 × kg of milk protein)].
[4]Solids nonfat.

exact mechanisms remain to be further explored. Due to the increases of milk fat and protein yields, LRUFA-SA had 3.8 kg/d more ECM than LRUFA-OA ($P < 0.01$; 51.8 vs. 48 kg/d) (Fig. 1 panel A).

In the HRUFA experiment, although BCS change was not affected by FA treatments ($P > 0.1$), HRUFA-SA gained more BW ($P = 0.07$; 0.5 vs. −0.1 kg/d) and had greater positive NE_l balance ($P = 0.01$; 7.2 vs. 4.7 Mcal/d), suggesting HRUFA-SA increased dietary energy deposition into body fat (Table 4). In mid-lactation, SA and OA supplementations were also observed to have different effects on energy status of cows, but OA increased BCS and BW more than SA (De Souza et al., 2018), suggesting the effect of SA and OA on energy deposition might be also dependent on the stage of lactation. Milk, milk components yields, and ECM (Fig. 2 panel B) were not affected by treatments ($P > 0.1$). Although HRUFA-SA had greater DMI, there was no effect on milk production, with HRUFA-SA mainly resulting in greater nutrient deposition in body fat, which limited the nutrient partitioning into milk, especially for milk fat. Because less dietary energy was partitioned into milk, HRUFA-SA also decreased gross feed efficiency (ECM/DMI) relative to HRUFA-OA ($P = 0.01$; 1.8 vs. 1.9).

In the LRUFA experiment, SA supplementation improved the milk production and milk synthesis without changing energy partitioning to milk. However, in the HRUFA experiment, high concentration of RUFA altered the energy partitioning of SA supplementation from milk to body weight gain, resulting in the lack of milk response relative to the LRUFA experiment. Although the reasons remain unclear, feeding high HRUFA potentially changes the ruminal biohydrogenation pathways and rumen fermentation, which may further contribute to the changes of energy and nutrient partitioning between body fat and mammary gland (Jenkins and Bridges Jr, 2007). Overall, our experiment provides an evidence that RUFA in the basal diet could be an important factor to mediate the evaluation of FA supplementation.

Milk FA Concentration

Dietary FA is the major source of preformed FA (carbon length > 16) in milk, resulting in the FA composition in milk easily manipulated by FA supplementation (Bauman and Griinari, 2002). In the LRUFA experiment, consistent with the greater FA intake, LRUFA-SA had greater concentration of C18:0 in milk ($P < 0.01$; 10.4% vs. 12.2% FA) (Table 5). Similarly, C18:1 *cis*-9 in milk was greater in LRUFA-OA than LRUFA-SA ($P = 0.05$; 23.8% vs. 22.4% FA). In the HRUFA experiment, HRUFA-SA had a trend for a greater concentration of C18:0 in milk ($P = 0.06$; 14.9% vs. 13.8%). HRUFA-OA diet did not change the concentration of C18:1 *cis*-9 in milk though the concentration was numerically increased approximately 1% units relative to HRUFA-SA ($P = 0.26$; 27.6% vs. 26.6% FA). Besides intake, C18:1 *cis*-9 in milk is also from desaturation of C18:0 in adipose tissue and mammary gland (Soyeurt et al., 2008). Beaulieu and Palmquist (1995) observed significant desaturation of dietary C18:0 to C18:1 *cis*-9 in the mammary gland that process was further activated by the increase of C18:0 intake. The greater C18:0 intake in HFRU-SA

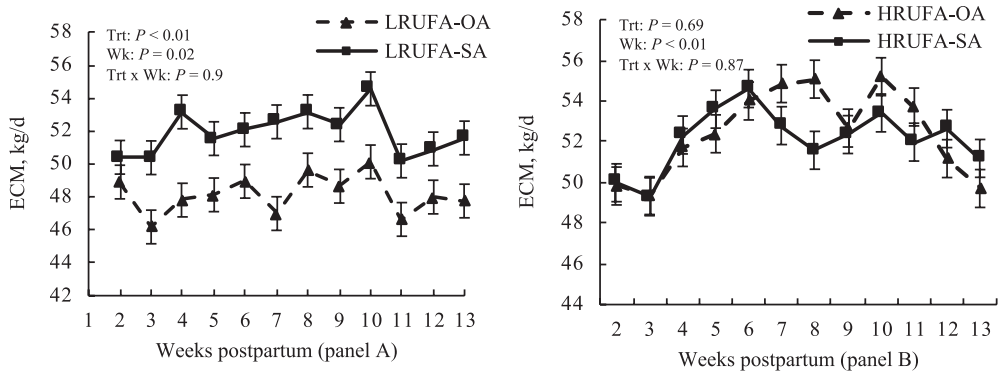

Figure 2. Energy-corrected milk (ECM) of cows fed stearic acid (SA) and oleic acid (OA) diets in low (panel A) and high (panel B) rumen-active unsaturated fatty acids (RUFA) experiments. Data are represented as mean ± SEM.

Table 5. Fatty acids (FA) concentration in milk of cows fed stearic acid (SA) and oleic acid (OA) dietary treatments in low and high rumen-active unsaturated fatty acids (RUFA) experiments

	LRUFA experiment[1]						HRUFA experiment[1]					
	Treatments[2]			P-value			Treatments[2]			P-value		
Items (% of total FA)	SA	OA	SEM	Trt	Wk	Trt × Wk	SA	OA	SEM	Trt	Wk	Trt × Wk
C6:0	0.76	0.84	0.07	0.21	0.31	0.33	0.8	0.78	0.03	0.51	<0.001	0.87
C8:0	0.81	0.83	0.05	0.72	0.10	0.45	0.69	0.65	0.03	0.32	<0.001	0.95
C10:0	2.5	2.5	0.13	0.59	<0.01	0.67	1.9	1.7	0.10	0.27	<0.001	0.91
C12:0	3.3	3.2	0.15	0.17	<0.01	0.71	2.2	2.1	0.11	0.30	0.02	0.90
C14:0	11.8	11.3	0.32	0.08	<0.01	0.70	8.9	8.5	0.28	0.37	<0.01	1.00
C15:0	0.78	0.78	0.04	0.91	<0.01	0.47	0.52	0.51	0.03	0.73	<0.001	0.70
C16:0	37.3	37.7	0.60	0.47	<0.01	0.80	29.9	30.2	0.33	0.59	<0.001	0.28
cis-9 C16:1	1.8	1.9	0.09	0.30	<0.01	0.24	1.1	1.1	0.10	0.50	<0.001	0.34
C18:0	12.2	10.4	0.30	<0.01	<0.01	0.50	14.9	13.8	0.43	0.06	<0.01	0.09
cis-9 C18:1	22.4	23.8	0.80	0.05	<0.01	0.56	26.6	27.6	0.61	0.26	0.42	0.89
trans-9 C18:1	0.13	0.14	0.01	0.33	0.56	0.37	0.54	0.60	0.03	0.17	<0.001	0.62
trans-10 C18:1	0.16	0.24	0.03	0.08	0.17	0.24	1.1	0.93	0.18	0.51	<0.001	0.33
trans-11 C18:1	0.32	0.28	0.06	0.51	0.23	0.48	1.4	1.9	0.10	<0.001	<0.001	0.01
cis-9 *trans*-11 C18:2	0.23	0.28	0.01	<0.01	0.58	0.86	0.44	0.55	0.03	<0.01	<0.001	0.17
trans-10 *cis*-12 C18:2	0.017	0.005	0.005	0.07	0.08	0.17	0.01	0.01	0.001	0.65	0.91	0.40
cis-9 *cis*-12 *cis*-15 C18:3	0.47	0.49	0.02	0.32	0.64	0.50	0.46	0.47	0.02	0.84	<0.001	0.50
C20:0	0.67	1.33	0.37	0.09	0.24	0.95	3.7	3.9	0.11	0.12	<0.001	0.06
C20:4	0.11	0.15	0.01	0.03	0.22	0.66	0.13	0.13	0.01	0.73	<0.001	0.30
C22:0	0.01	0.01	0.001	0.46	0.12	0.62	0.08	0.08	0.001	0.48	<0.001	0.85
C23:0	0.09	0.09	0.01	1.00	0.30	0.74	0.11	0.1	0.01	0.35	0.02	0.28
De novo FA[3]	21.1	20.4	0.70	0.18	<0.01	0.65	16.2	15.5	0.55	0.29	0.01	0.10
Mixed FA[3]	39.0	39.6	0.6	0.38	<0.01	0.84	30.9	31.1	0.33	0.65	<0.001	0.47
Preformed FA[3]	38.2	38.3	1.10	0.89	<0.01	0.62	52.8	53.4	0.72	0.53	<0.001	0.91

[1]LRUFA represents the diets contain low content of RUFA; HAUFA represents the diets contain high content of RUFA.
[2]SA dietary treatment contained approximately 0.4% more C18:0 than OA dietary treatment on DM basis; OA dietary treatment contained approximately 0.3% more C18:1 *cis*-9 than SA dietary treatment on DM basis.
[3]De novo FA are primarily synthesized in mammary gland (carbon length < 16), preformed FA are primarily absorbed from blood circulation (carbon length > 16), and mixed FA are from both sources (C16:0 + C16:1 *cis*-9).

may increase the activity of *cis*-9 desaturase in the mammary gland, which resulted in a similar concentration of C18:1 *cis*-9 in milk relative to HRUFA-OA ($P = 0.26$).

In both experiments, the concentration of C18:2 *cis*-9 *trans*-11 ($P < 0.01$) in milk was greater in cows fed the OA versus SA diet (Table 5). In addition, cows fed the HRUFA-OA diet had the greater concentration of C18:1 *trans*-11 in milk than cows fed the HRUFA-SA diet ($P < 0.001$; 1.9% vs. 1.4% FA). C18:2 *cis*-9 *trans*-11 and C18:1 *trans*-11 in milk are mainly derived from the ruminal biohydrogenation of C18:2 *cis*-9 *cis*-12 (Jenkins, 2016). In the rumen, dietary C18:2 *cis*-9 *cis*-12 tends to be saturated to C18:0, but the metabolic process is limited by activity of enzymes, leading to C18:2 *cis*-9 *cis*-12 converted to C18:2 *cis*-9 *trans*-11 and C18:1 *trans*-11 (Harfoot, 1981; Jenkins, 2016). In the OA treatment, the FA supplement also contained a limited concentration of calcium salts C18:2 *cis*-9 *cis*-12. Harvatine and Allen (2006) found that calcium salts did not well protect C18:2 *cis*-9 *cis*-12 from ruminal biohydrogenation, although C18:1 *cis*-9 is well protected. Thus, the increased concentrations of C18:1 *trans*-11 and C18:2 *cis*-9 *trans*-11 in milk could be associated with

Table 6. Blood metabolites and energy partitioning to milk, maintenance, and body tissue of cows fed stearic acid (SA) and oleic acid (OA) dietary treatments in low and high rumen-active unsaturated fatty acids (RUFA) experiments

	LRUFA experiment[1]						HRUFA experiment[1]					
	Treatments[2]			P-value			Treatments[2]			P-value		
Item[3]	SA	OA	SEM	Trt	Wk	Trt × Wk	SA	OA	SEM	Trt	Wk	Trt × Wk
Glucose, mg/mL	63.9	63.8	0.97	0.92	<0.01	0.60	—	—	—	—	—	—
NEFA, mg/dL[4]	0.27	0.3	0.03	0.54	<0.01	0.24	—	—	—	—	—	—
Urea N, mg/dL	15.7	15.7	0.50	0.95	0.21	0.30	—	—	—	—	—	—
BHBA, mg/dL[5]	4.2	3.6	0.22	0.03	0.21	0.36	4.1	4.1	0.09	0.83	0.42	0.93
Energy, Mcal/d												
NE_l intake	42.7	39.2	0.86	<0.001	<0.001	0.11	53.6	51.1	0.94	0.03	<0.001	0.77
Milk	35.2	32.5	0.66	<0.001	0.01	0.96	34.8	35.2	0.66	0.70	0.01	0.92
Maintenance	10.8	10.8	0.17	0.85	0.01	0.45	11.2	10.6	0.16	<0.001	0.55	0.70
Body tissues[6]	−3	−3.9	0.74	0.37	<0.001	0.99	7.2	4.7	0.71	0.01	<0.001	0.72
Partitioning, % of energy intake												
Milk	82.9	84.1	1.58	0.56	<0.001	0.99	66.2	70.2	1.20	0.01	<0.001	0.84
Maintenance	25.7	27.8	0.66	0.24	<0.001	0.14	21.1	21.1	0.35	0.91	<0.001	0.97
Body tissues[6]	−8.3	−11.7	2.04	0.19	<0.001	0.98	12.7	8.5	1.32	0.02	<0.001	0.79

[1]LAUFA represents the basal diet contains low content of rumen-active unsaturated FA; HAUFA represents the basal diet contains high content of rumen-active unsaturated FA.
[2]SA dietary treatment contained approximately 0.4% more C18:0 than OA dietary treatment on DM basis; OA dietary treatment contained approximately 0.3% more C18:1 *cis*-9 than SA dietary treatment on DM basis.
[3]Glucose, NEFA, and urea N were only analyzed in LRUFA experiment.
[4]Nonesterified FA.
[5]β-Hydroxybutyrate.
[6]Negative value indicates cows were in the negative energy balance and gained NE_l from body tissue mobilization; positive value indicates cows were in the positive NE_l balance and body tissues gained energy from intake energy.

an incomplete ruminal biohydrogenation of C18:2 *cis*-9 *cis*-12 in the OA supplement.

Blood Metabolites and Energy Partition

Blood metabolites are summarized in Table 6. In the LRUFA experiment, the blood concentration of glucose, NEFA, and urea nitrogen were not affected by diet ($P > 0.1$), but the concentration of BHBA was greater in LRUFA-SA than LRUFA-OA ($P = 0.03$; 4.2 vs. 3.6 kg/d) (Table 6). In early lactation, due to a deficiency in energy intake, an abundance of NEFA is mobilized from body fat to support milk and milk fat syntheses (McNamara, 1991). However, excessive NEFA mobilization from body fat aggregates the metabolic burden in the liver that NEFA may not be sufficiently oxidized and translocated to peripheral tissues as high-density lipoprotein, causing hepatic metabolic disorders, such as fatty liver and ketosis (Grum et al., 1996). In an incomplete catabolic process, some NEFA is converted to BHBA (Adewuyi et al., 2005); thus, the blood concentration is often used to indicate the energy status and hepatic health of dairy cows (Roberts et al., 2012). Ospina et al. (2010) suggested that 10 mg/dL of BHBA and 0.57 mg/dL of NEFA in blood are as thresholds to indicate the hepatic health of cows. In the LRUFA experiment, LRUFA-SA had a greater concentration of BHBA than LRUFA-OA, but the concentration was less than the recommended threshold. Thus, we suggest that SA intake may shift FA metabolism in the liver to produce more BHBA through some alternative metabolic pathways. However, in HRUFA experiment, the concentration of BHBA was not affected by HRUFA-SA ($P = 0.21$), indicating that SA intake did not directly alter the concentration of BHBA in the LRUFA experiment (Table 6).

The effect of diet on energy partitioning is summarized in Table 6. In the LRUFA experiment, the energy intake and milk energy output were greater in LRUFA-SA than LRUFA-OA ($P < 0.001$), but the milk energy output as a proportion of energy intake was not affected ($P = 0.56$). In the HRUFA experiment, HRUFA-SA had the greater energy intake ($P = 0.03$) but the milk energy output was similar between diets ($P = 0.7$). Also, HRUFA-SA had greater energy outputs in maintenance ($P < 0.001$) and body weight gain ($P = 0.01$). As a result, HRUFA-OA had greater energy intake partitioning into milk ($P = 0.01$) but less energy intake partitioning into body tissues ($P = 0.02$) relative to HRUFA-SA.

In summary, SA and OA supplementation in the early lactation had different effects on milk production and energy partitioning, but the difference was dependent on the RUFA concentration in the basal diet. In both low and high RUFA diets, SA supplementation had greater DMI. In the LRUFA experiment, SA supplementation increased milk fat %, yield, and energy-corrected milk without changing the energy balance compared with OA supplementation. However, in the HRUFA experiment, SA supplementation did not change the milk production due to more energy intake partitioning into body tissues. In conclusion, the effect of FA supplementation on early lactation cows was dependent on the FA composition in the fat supplements, which is mediated by the dietary RUFA.

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