Interaction of unsaturated fat or coconut oil with monensin in lactating dairy cows fed 12 times daily. II. Fatty acid flow to the omasum and milk fatty acid profile¹

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ABSTRACT

Feeding animal-vegetable (AV) fat or medium-chain fatty acids (FA) to dairy cows can decrease ruminal protozoal counts. However, combining moderate to large amounts of AV fat with monensin (tradename: Rumensin, R) could increase the risk for milk fat depression (MFD), whereas it is not known if diets supplemented with coconut oil (CNO; rich in medium-chain FA) with R would cause MFD. In a 6 × 6 Latin square design with a 2 × 3 factorial arrangement of treatments, 6 rumen-cannulated cows were fed diets without or with R (12 g/909 kg) and either control (no fat), 5% AV fat, or 5% CNO. Diets were balanced to have 21.5% forage neutral detergent fiber, 16.8% crude protein, and 42% nonfiber carbohydrates. Omasal flows of FA were characterized by an increased percentage of *trans* 18:1 for AV fat and CNO diets compared with the control, a higher percentage of 12:0 and 14:0 for CNO, and higher *cis* 18:1 for AV fat. Milk FA composition reflected the changes observed for omasal FA digesta flow. The de novo FA synthesis in the mammary gland was decreased by the main effects of R compared without R (averaged over fat treatments) and for added fat (AV fat and CNO) versus control (averaged over R). The percentages of 6:0, 8:0, and 10:0 in milk fat were lower for R and for AV fat and CNO compared with the control. The percentage of *trans* 18:1 FA in milk fat also higher for AV fat and CNO compared with the control. Against our hypotheses, the feeding of CNO

did not prevent MFD, and few interactions between R and fat source were detected. The feeding of CNO did compromise ruminal biohydrogenation, with accumulation of *trans* 18:1 in the rumen and in milk fat.

Key words: omasal fatty acid, milk fatty acid, milk fat depression

INTRODUCTION

Feeding PUFA often decreases protozoal numbers in the rumen (Hristov and Jouany, 2005). Animal-vegetable (AV) fat, a byproduct of the restaurant industry, is readily available to provide PUFA in dairy diets. However, the response to AV fat supplementation on protozoal numbers is not consistent, possibly due to biohydrogenation (BH) of PUFA in the rumen, partially alleviating the inhibition of specific populations of lipid-metabolizing bacteria. Although protozoa are inhibited by PUFA, they can be a vehicle for passage of PUFA or other intermediates of BH that do not promote milk fat depression (MFD; Devillard et al., 2006) or can influence bacterial populations involved in BH (Firkins et al., 2008; Hristov et al., 2009). Supplementation of AV fat in combination with monensin (Rumensin, R), an ionophore often fed to improve feed efficiency, occasionally spontaneously decreases milk fat yield and percentage (Dubuc et al., 2009). This MFD is likely due to the partial BH of PUFA, which favors FA intermediates that are inhibitory to milk fat synthesis (Shingfield et al., 2010). Medium-chain FA (MCFA) have been researched to decrease protozoal population (Hristov et al., 2004), and lauric acid was identified as a potent protozoal inhibitor (Hristov et al., 2009). Because CNO has much fewer PUFA than does AV fat, we hypothesized that diets supplemented with CNO in combination with R would not cause MFD as would AV fat diets combined with R. Our objectives were to determine the effects of feeding AV fat or CNO in combination with R on FA flow to the omasum, apparent total-tract digestibility of FA, and milk FA profile.

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MATERIALS AND METHODS

Feed and Fecal Analyses

Animals, treatments, and the experimental design were described in the companion article (Reveneau et al., 2012). Feed, omasal, and fecal samples were collected and analyzed as described previously, except for FA analysis, which was conducted with one of the procedures reviewed by Palmquist and Jenkins (2003) as modified by Mathew et al. (2011). The FA were first methylated with 5 mL of 10% methanolic HCl (2 h at 90°C). Nonadecanoic acid (19:0) was used as internal standard. After addition of 1 mL of hexane and 10 mL of 6% K₂CO₃, the samples were centrifuged for 5 min at 500 × *g* to separate solvent layers. The organic layer was transferred to a 13- × 100-mm culture tube containing 1 g each of sodium sulfate and charcoal, capped with a Teflon-lined screw cap, and centrifuged under the same conditions. The samples were then transferred into 1-mL GLC autosampler vials, capped, and stored at -20°C until GLC analysis. Retention times and response factors were determined with methyl ester standards purchased from Nu-Chek Prep (Elysian, MN; GLC-60, UC-50M) and Matreya Inc. (Pleasant Gap, PA; FIM-FAME-7).

Methyl esters of FA were separated using an HP 5890 Series II GLC (Hewlett Packard Co., Palo Alto, CA) equipped with a fused silica capillary column (SP-2380; 100-m length, 0.25-mm i.d., and 0.2-mm film thickness; Supelco Inc., Bellefonte, PA) and using helium as the carrier gas. The FA were then quantified with the ChemStation software (Agilent Technologies Inc., Wilmington, DE). Injector and flame ionization detector temperatures were set at 250 and 250°C, respectively, and the split ratio was set at 80:1. The oven temperature was set for 70°C, held for 2 min, increased by 8°C/min to 110°C, held for 4 min, increased by 5°C/min to 166°C, held for 45 min, increased by 4°C/min to 240°C, and held for 15 min.

Omasal and Milk FA Profile

Milk was sampled from 4 consecutive milkings during d 17 to 19 and composited per period, with one aliquot being preserved and used for milk composition (Reveneau et al., 2012) and the other aliquot frozen and stored at -20°C for FA analysis. Whole omasal samples and the frozen aliquot of milk were analyzed for FA profile by GLC. For milk, the frozen samples were rapidly thawed; about 20 mL was centrifuged at 12,000 × *g* for 20 min at 4°C, and then 300 to 350 mg of fat cake was removed. A 0.5-g sample of lyophilized

omasal digesta was used for methylation. First, the FA were methylated in 2 steps with 2 mL of 0.5 *M* sodium methoxide (10 min at 50°C), followed by 3 mL of 5% methanolic HCl (10 min at 80°C) as described by Kramer et al. (1997). Then, the samples were processed as described for feed and fecal samples.

Statistical Analysis

Omasal flow of FA, milk FA profile, and FA digestibility data were analyzed as a 6 × 6 Latin square using PROC MIXED of SAS (v9.1; SAS Institute Inc., Cary, NC) as described in the companion article (Reveneau et al., 2012). Treatment means were compared using 5 preplanned orthogonal contrasts, with 6 coefficients shown in brackets respectively for control, AV fat, CNO, control + R, AV fat + R, and CNO + R: 1) the main effect of R [1 1 1 -1 -1 -1], 2) the main effect of control versus fat (i.e., the average of AV fat and CNO) [-2 1 1 -2 1 1], 3) the main effect of the fat source (i.e., AV fat vs. CNO) [0 -1 1 0 -1 1], 4) the interaction between R and fat [-2 1 1 2 -1 -1], and 5) the interaction between R and the fat source [0 -1 1 0 1 -1].

RESULTS

Diet Composition

The control, AV fat, and CNO diets averaged 2.9, 7.5, and 7.4% FA (Table 1), which are close to expectations from diet formulation. The AV fat diet was rich in *cis*-9,*cis*-12 18:2 (33.0%) and *cis*-9 18:1 (28.1%), whereas diets with CNO contained 29.7% 12:0 and 19.8% *cis*-9,*cis*-12 18:2. The high proportion of *cis*-9,*cis*-12 18:2 (48.2%) and other PUFA in the control diet was expected. Only the AV fat diet contained *trans* 18:1 isomers from rendered animal fat and commercial BH of vegetable oil.

Intake and Omasal Flow of FA

The FA intake for the control diets was lower than the FA intake for fat-supplemented diets (*P* < 0.01), as by design, but FA intake was greater (*P* < 0.01) for AV fat than for CNO diets because of decreased DMI for CNO (Table 2). Rumensin did not affect the intake or omasal flow of total FA, but an R × source interaction (*P* = 0.07) was observed in which FA intake decreased for cows fed R with CNO but not with AV fat. The omasal flow of FA followed the same responses for fat supplementation, except that no R × source interaction was observed. For cows fed control and AV fat diets, the intake of MCFA often approached our sensitivity

Table 1. Fatty acid composition of fed diets without or with Rumensin (R) and without or with 5% fat from animal-vegetable (AV) fat or coconut oil (CNO)

Item	Diet ¹					
	-R			+R		
	Control	AV fat	CNO	Control	AV fat	CNO
Fatty acids (% DM)	2.81	7.33	7.37	2.90	7.65	7.46
FA profile ² (% FA)						
8:0	0.05	0.15	5.41	0.06	0.12	5.40
10:0	0.01	0.06	3.86	0.04	0.03	3.86
12:0	0.47	0.47	29.70	0.73	0.29	29.61
14:0	0.24	0.81	11.40	0.46	0.75	11.39
16:0	16.9	19.8	12.0	17.2	19.8	11.9
<i>cis</i> -9 16:1	—	1.49	—	—	1.50	—
18:0	3.41	6.19	2.47	3.42	6.59	2.68
<i>cis</i> -9 18:1	20.2	28.2	11.8	19.6	28.0	11.5
Other <i>cis</i> -18:1	0.52	2.47	0.18	0.69	2.31	0.18
Total <i>trans</i> -18:1	—	2.14	—	—	2.24	—
<i>cis</i> -9, <i>cis</i> -12 18:2	48.7	33.0	19.8	47.7	33.0	19.7
18:3 n-3	7.27	3.95	2.62	8.07	3.98	2.90
VLCFA ³	1.86	1.12	0.72	1.92	1.14	0.76

¹Rumensin supplementation (12 g/909 kg of DM); AV fat: 5.0% added AV blend; CNO: 5.0% added CNO.

²Unresolved FA are not reported but contributed to the total FA.

³Very long-chain fatty acid are >C18.

limit for detection of FA (approximately 0.01% of total FA); their difference (i.e., ruminal disappearance) when calculated as a percentage of such low intakes were not physiologically meaningful, so we assumed that statistical evaluation would be biased. When only considering CNO diets, R increased the disappearance of 8:0 in omasal digesta. The percentage disappearance of unsaturated C18 FA was lower for CNO than for AV fat (main effect of source, $P < 0.05$). The ruminal total FA disappearance for AV fat was positive (averaging 14.9%) but lower than that for CNO diets (averaging 29.9%; main effect of source). The $R \times$ fat interaction ($P < 0.10$) resulted from a more negative total FA disappearance (i.e., more microbial FA synthesis) with R with control but not with fat diets. Biohydrogenation decreased with AV fat and especially CNO (main effects of fat and source).

The 12:0 and 14:0 percentages of fat in omasal contents were increased when CNO was fed, explaining both the main effects of fat and source (Table 2). In contrast, the 16:0 and especially 18:0 concentrations decreased for CNO. Cows had a lower percentage of 18:0 in omasal FA when R was supplemented. The *trans*-10 and *trans*-11 18:1 isomers could not be distinguished using our FA analysis method. The percentage of these isomers increased with fat supplementation but was not different among fat sources. The main effects of both fat and source for other *trans* 18:1 isomers resulted from a higher percentage for AV fat (5.39%) than for CNO (2.74%). *Cis*-9 18:1 (oleic acid) was increased by AV fat

but not by CNO, explaining both the fat and source main effects. The $R \times$ source interaction resulted when R increased *cis*-9 18:1 for the control and AV fat but decreased it for CNO. *Cis*-11 18:1 decreased with AV fat and even more for CNO, but *cis*-12 18:1 decreased only with CNO (fat and source effects). The percentages of other *cis* 18:1 isomers were all lower for CNO than for AV fat diets. Percentages of *cis*-9,*cis*-12 18:2 decreased when fat was fed, but other 18:2 isomers were increased with CNO. The percentage of 18:3n3 in omasal FA decreased when cows were fed either fat source. The percentage of *cis*-9,*trans*-11 18:2 in omasal fatty acids was lower with CNO diets (explaining both the fat and source responses), but the percentage of *trans*-10,*cis*-12 18:2 was higher with either fat source ($P < 0.01$ for fat without any effect from source). Concentrations of other conjugated linoleic acid (CLA) isomers in omasal contents were increased for AV fat but decreased for CNO compared with the control, explaining the source effect combined with a lack of fat effect. The amount of very long-chain FA was decreased when either fat was supplemented.

Milk FA Composition

The supplementation of R decreased ($P < 0.05$) the percentages of 6:0, 8:0, and 10:0 but tended to increase ($P = 0.07$) the percentages of *trans*-10 and *trans*-11 18:1 isomers in milk fat (Table 3). Rumensin tended to decrease *cis*-9,*cis*-12 18:2 (main effect of R, $P < 0.10$);

Table 2. Least squares means for fatty acid composition of omasal digesta for cows fed diets without or with Rumensin (R) and without or with 5% fat from animal-vegetable (AV) fat or coconut oil (CNO)

Item ¹	Diet ²						SEM ³	P-value ⁴				
	–R			+R				R	Fat	Source	R × fat	R × source
	Control	AV fat	CNO	Control	AV fat	CNO						
FA intake (g)	575	1,491	1,171	556	1,506	1,104	37	0.20	<0.01	<0.01	NS	0.07
FA omasal flow (g)	577	1,309	853	648	1,225	783	59	NS	<0.01	<0.01	0.18	NS
FA disappearance (% intake)												
8:0	NA ⁵	NA	86.9	NA	NA	90.5	0.7	0.05	NA	NA	NA	NA
10:0	NA	NA	88.1	NA	NA	79.8	3.8	NS	NA	NA	NA	NA
12:0	NA	NA	48.2	NA	NA	48.3	3.3	NS	NA	NA	NA	NA
14:0	NA	NA	25.9	NA	NA	24.9	4.5	NS	NA	NA	NA	NA
MCFA	NA	NA	50.4	NA	NA	49.9	3.0	NS	NA	NA	NA	NA
Unsaturated C18	67.8	64.2	54.2	60.1	62.7	56.3	3.4	NS	0.16	0.04	NS	NS
Total FA	–1.1	11.8	29.7	–14.8	18.0	30.0	5.4	NS	<0.01	0.02	0.10	NS
Biohydrogenation ⁶	75.5	70.8	65.3	74.3	67.2	65.4	1.3	0.16	<0.01	0.02	NS	0.18
Omasal FA composition (g/100 g of FA)												
12:0	0.7	0.2	22.2	0.7	0.7	23.3	0.5	NS	<0.01	<0.01	NS	NS
14:0	1.8	1.0	12.2	1.7	1.3	12.7	0.3	NS	<0.01	<0.01	NS	NS
16:0	16.6	19.4	15.2	17.1	19.5	15.2	0.3	NS	NS	<0.01	NS	NS
18:0	47.2	46.7	21.5	45.7	42.3	20.5	1.5	0.05	<0.01	<0.01	NS	NS
<i>trans</i> -10 + <i>trans</i> -11 18:1	4.78	7.20	8.45	4.82	7.70	8.94	0.76	NS	<0.01	NS	NS	NS
Other <i>trans</i> 18:1	1.65	4.53	2.70	2.25	6.22	2.77	0.49	NS	<0.01	<0.01	NS	NS
<i>cis</i> -9 18:1	5.92	8.21	5.01	6.34	9.14	4.48	0.34	NS	0.04	<0.01	NS	0.03
<i>cis</i> -11 18:1	1.55	0.66	0.38	1.62	0.88	0.20	0.13	NS	<0.01	<0.01	NS	NS
<i>cis</i> -12 18:1	0.610	0.745	0.299	0.661	0.715	0.253	0.052	NS	<0.01	<0.01	NS	NS
Other <i>cis</i> -18:1	0.710	1.045	0.644	0.892	1.021	0.654	0.058	NS	NS	<0.01	NS	NS
<i>cis</i> -9, <i>cis</i> -12 18:2	7.46	4.24	3.60	7.72	4.66	3.41	0.47	NS	<0.01	NS	NS	NS
Other 18:2	0.29	0.25	0.60	0.25	0.28	0.85	0.06	NS	<0.01	<0.01	NS	NS
18:3 n-3	1.21	0.56	0.47	1.10	0.61	0.52	0.08	NS	<0.01	NS	NS	NS
<i>cis</i> -9, <i>trans</i> -11 18:2	0.475	0.580	0.071	0.513	0.377	0.028	0.082	NS	<0.01	<0.01	NS	NS
<i>trans</i> -10, <i>cis</i> -12 18:2	ND ⁷	0.055	0.078	ND	0.103	0.119	0.022	NS	<0.01	NS	NS	NS
Other CLA	0.008	0.132	ND	0.041	0.084	0.004	0.023	NS	NS	<0.01	NS	NS
VLCFA	1.05	0.52	0.56	1.17	0.40	0.50	0.12	NS	<0.01	NS	NS	NS
Summation												
<i>trans</i> 18:1	6.4	11.7	11.2	7.1	13.9	11.7	0.9	NS	<0.01	NS	NS	NS
<i>cis</i> 18:1	8.8	10.7	6.3	9.5	11.8	5.6	0.4	NS	NS	<0.01	NS	0.04
CLA	0.48	0.77	0.15	0.55	0.56	0.15	0.08	NS	NS	<0.01	NS	NS
SMCFA	2.50	1.19	36.18	2.40	1.95	37.68	0.74	NS	<0.01	<0.01	NS	NS
SFA	66.2	67.3	73.4	65.2	63.8	73.4	1.4	NS	<0.01	<0.01	NS	NS

¹MCFA = medium-chain FA (C8 to C14); CLA = conjugated linoleic acid; VLCFA = very long-chain FA (>C18); SMCFA = short- and medium-chain FA (C14 and shorter). Unresolved FA are not reported but contributed to total FA.

²Rumensin supplementation (12 g/909 kg of DM); AV fat: 5.0% added AV blend; CNO: 5.0% added CNO.

³Standard error of the mean for n = 6.

⁴Probability of a treatment response; NS: $P > 0.20$. Contrasts compared the main effect of R (diets without vs. with R averaged over fat treatments), the main effect of fat (Fat = control vs. the average of the 2 fat diets averaged over R), the main effect of source of fat (source = AV fat vs. CNO averaged over R), the interaction between R and fat (R × fat), and the interaction between R and source of fat (R × source). See Materials and Methods section for contrasts.

⁵NA = not applicable because MCFA were not detected above baseline for control and AV fat diets.

⁶According to the equation of Tice et al. (1994) that accounts for numbers of double bonds per FA.

⁷Not detected.

R decreased *cis*-9,*trans*-11 CLA only when AV fat was fed ($R \times \text{source}$, $P = 0.09$); and R decreased ($P < 0.05$) percentages of other CLA isomers.

The main effect of fat was highly significant for numerous FA, but the response sometimes also was affected by the main effect of source (Table 3). The 6:0 to 16:0 even-chain FA were consistently lower, and *trans*-10 and *trans*-11 18:1 and all *cis* 18:1 isomers consistently higher, in milk fat when AV fat was supplemented compared with the control. Similarly, 18:2 isomers other than linoleic acid, the *cis*-9,*trans*-11 CLA, and other CLA isomers were higher for the main effect means for the average of fat treatments versus control. However, compared with the AV fat treatment, 12:0 and 14:0 were much higher in milk fat from cows fed CNO, but 18:0; *cis*-9 18:1; *cis*-11 18:1; *cis*-12 18:1; and *cis*-9,*trans*-11 CLA and other CLA (but not *trans*-10,*cis*-12 CLA) were lower for CNO than AV fat. Other *cis* 18:1 isomers were higher for CNO than AV fat. When fat was fed, 18:2n6 and 18:3n3 were decreased, especially for CNO versus AV fat (both main effects of fat and source, $P < 0.05$). Probably because the *trans*-10,*cis*-12 isomers were consistently low, no treatment effects occurred for this isomer.

When FA were summed, R decreased CLA, particularly when AV fat was fed (R effect, $P < 0.05$; $R \times \text{source}$ interaction, $P = 0.08$). *Trans* MUFA were increased when both fat sources were fed (fat effect only, $P < 0.01$), but, compared with the control, *cis* MUFA were increased only when AV fat was fed (main effects of fat and source, $P < 0.01$). Similarly, CLA increased only when AV fat was fed (both fat and source main effects, $P < 0.01$). Both fat and source main effects ($P < 0.01$) were observed for short- and medium-chain FA (SMCFA) and SFA. Compared with the control, the percentages of SMCFA decreased when AV fat was fed but increased when CNO was fed; SFA, which include SMCFA, decreased only when AV fat was fed, but the control and CNO were more similar.

Apparent Total-Tract Digestibility

The supplementation of R did not affect the apparent digestibility for total FA or for the different chain lengths of FA except for 18-carbon FA, which tended to increase ($P = 0.07$) when R was supplemented (Table 4). Fat supplementation increased the apparent digestibilities of total FA, total C16 ($P < 0.01$), and total C18 ($P = 0.08$). With CNO supplementation, total FA and C18 digestibilities increased ($P < 0.05$) compared with when cows were fed AV fat diets. As with omasal disappearance values, the intakes of MCFA were so low that digestibilities as a percentage of such low intakes

were not physiologically meaningful, and statistics were not performed.

DISCUSSION

The companion paper (Reveneau et al., 2012) describes the composition of the diets, DMI, and milk fat production. With the depression of DMI and decrease in NDF digestibility when cows were fed CNO diets, energy supply for ruminal fermentation was limited. The companion paper discussed the potential limitation of intake by ruminal fill due to metabolic appetite control (Allen, 2000). In the current study, ruminal disappearance of FA with CNO (29.9%) was higher than with AV fat (14.9%). Disappearance of FA with chain lengths shorter than C14 is extensive (Wu et al., 1991). Indeed, absorption of the FA or elongation to longer chains could contribute to the disappearance of MCFA (Doreau and Ferlay, 1995). When combined with a 29.9% ruminal disappearance of total FA and much higher disappearances of 8:0 and 10:0 and of 12:0 to a lesser extent, the MCFA were likely absorbed through the rumen wall, entered the blood stream directly, and entered mitochondria faster than longer chain FA to depress DMI (Allen et al., 2009). In a study by Hristov et al. (2011), direct supplementation of lauric acid through the rumen cannula depressed DMI. Their bolus-dosing strategy could concentrate this response over a shorter period, whereas our 2-h feeding schedule represents a longer-term DMI suppression.

Cows fed the CNO diet had a lower 18:2 percentage in the diet, but the percentages of *trans*-10 and *trans*-11 18:1 isomers and other intermediates of BH in omasal digesta were similar to those from cows fed AV fat diets. Oldick et al. (2000) also discussed that feeding 12 times daily, as in the current study, allowed extensive BH without a large buildup of BH intermediates. However, a greater inhibition of BH took place with CNO in the current study. Increasing the concentration of bioactive lipids can feed back to rate-limit BH (Maia et al., 2010), potentially explaining the accumulation of *trans*-18:1 in the rumen (Wu and Palmquist, 1991; Enjalbert et al., 2003). Thus, even though AV fat diets had more unsaturated 18C FA available for BH (Table 1) and higher omasal total *cis* 18:1 percentages (Table 2), cows fed both fat sources had similar total *trans* 18:1 concentrations in omasal digesta (Table 2). Ruminal conditions controlling kinetics of lipolysis and BH are still being worked out using models (Jenkins et al., 2008), and these models probably cannot be fully adapted to explain in vivo feeding conditions. *Butyrivibrio fibrisolvens* and other taxonomically related bacteria are responsible for both steps of BH, which are

Table 3. Least squares means for FA composition of milk fat for cows fed diets without or with Rumensin (R) and without or with 5% fat from animal-vegetable (AV) fat or coconut oil (CNO)

Item (g/100 g of milk fat) ¹	Diet ²						SEM ³	P-value ⁴				
	-R			+R				R	Fat	Source	R × fat	R × source
	Control	AV fat	CNO	Control	AV fat	CNO						
4:0	2.71	2.58	2.00	2.43	2.33	2.39	0.20	NS	0.15	NS	NS	0.11
6:0	1.89	1.25	1.01	1.68	1.02	1.03	0.10	0.05	<0.01	NS	NS	0.16
8:0	1.10	0.59	0.57	1.01	0.47	0.52	0.06	0.04	<0.01	NS	NS	NS
10:0	3.13	1.44	1.47	2.85	1.25	1.35	0.17	0.05	<0.01	NS	NS	NS
12:0	3.62	1.70	9.76	3.41	2.01	10.53	0.34	NS	<0.01	<0.01	0.18	NS
14:0	12.8	8.0	15.3	12.7	8.5	15.2	0.4	NS	<0.01	<0.01	NS	NS
16:0	30.2	26.2	26.1	30.0	28.0	26.2	0.8	NS	<0.01	NS	NS	NS
18:0	10.9	14.4	8.2	11.8	13.5	7.3	1.0	NS	NS	<0.01	NS	NS
<i>trans</i> -10 + <i>trans</i> -11 18:1	2.03	5.86	5.62	2.39	7.10	6.29	0.55	0.07	<0.01	NS	NS	NS
Other <i>trans</i> 18:1	0.653	1.367	0.827	0.707	0.950	0.985	0.250	NS	0.11	NS	NS	NS
<i>cis</i> -9 18:1	17.5	23.6	16.3	17.8	22.3	15.5	1.0	NS	0.02	<0.01	NS	NS
<i>cis</i> -11 18:1	0.869	1.149	1.033	0.859	1.114	0.986	0.060	NS	<0.01	0.02	NS	NS
<i>cis</i> -12 18:1	0.288	0.560	0.199	0.310	0.434	0.154	0.037	0.12	NS	<0.01	0.11	NS
Other <i>cis</i> -18:1	0.430	0.715	0.954	0.437	0.762	0.966	0.078	NS	<0.01	0.01	NS	NS
<i>cis</i> -9, <i>cis</i> -12 18:2	2.74	2.41	1.69	2.51	2.14	1.71	0.12	0.10	<0.01	<0.01	NS	NS
Other 18:2	0.314	0.437	0.414	0.328	0.453	0.412	0.051	NS	0.02	NS	NS	NS
18:3 n-3	0.360	0.254	0.176	0.332	0.227	0.170	0.016	0.15	<0.01	<0.01	NS	NS
<i>cis</i> -9, <i>trans</i> -11 18:2	0.555	0.931	0.459	0.577	0.744	0.471	0.066	NS	0.09	<0.01	NS	0.09
<i>trans</i> -10, <i>cis</i> -12 18:2	0.102	0.062	0.114	0.036	0.092	0.109	0.034	NS	NS	NS	0.20	NS
Other CLA	0.059	0.147	0.069	0.047	0.077	0.045	0.019	0.05	0.10	0.01	NS	NS
VLCFA	1.01	0.83	0.76	0.81	1.11	0.93	0.15	NS	NS	NS	0.13	NS
Summation												
<i>trans</i> MUFA	2.78	7.39	6.65	3.19	8.21	7.60	0.58	0.13	<0.01	NS	NS	NS
<i>cis</i> MUFA	22.0	28.6	22.4	22.4	27.2	21.3	1.2	NS	<0.01	<0.01	NS	NS
CLA	0.70	1.14	0.64	0.65	0.91	0.62	0.07	0.04	<0.01	<0.01	NS	0.08
SMCFA	28.4	17.7	33.2	27.4	17.7	33.8	1.0	NS	<0.01	<0.01	NS	NS
SFA	69.0	58.9	66.5	68.2	59.4	66.8	1.5	NS	<0.01	<0.01	NS	NS

¹CLA = conjugated linoleic acid; VLCFA = very long-chain FA (>C18), SMCFA = short- and medium-chain FA (C14 and shorter). Unresolved FA are not reported but contributed to total FA.

²Rumensin supplementation (12 g/909 kg of DM); AV fat: 5.0% added AV blend; CNO: 5.0% added CNO.

³Standard error of the mean for n = 6.

⁴Probability of a treatment response; NS: $P > 0.20$. Contrasts compared the main effect of R (diets without vs. with R averaged over fat treatments), the main effect of fat (Fat = control vs. the average of the 2 fat diets averaged over R), the main effect of source of fat (source = AV fat vs. CNO averaged over R), the interaction between R and fat (R × fat), and the interaction between R and source of fat (R × source). See Materials and Methods section for contrasts.

Table 4. Least squares means for apparent total-tract FA digestibility for cows fed diets without or with Rumensin (R) and without or with 5% fat from animal-vegetable (AV) fat or coconut oil (CNO)

Item	Diet ¹						SEM ²	<i>P</i> -value ³				
	−R			+R				R	Fat	Source	R × fat	R × source
	Control	AV fat	CNO	Control	AV fat	CNO						
Total FA	74.8	76.9	89.9	75.0	82.7	93.3	3.3	0.18	<0.01	<0.01	NS	NS
Total C12	NA ⁴	NA	98.1	NA	NA	98.3	0.3	NS	NA	NA	NA	NA
Total C14	NA	NA	93.2	NA	NA	94.5	1.0	NS	NA	NA	NA	NA
Total C16	70.5	81.7	81.9	68.8	85.2	85.6	3.0	NS	<0.01	NS	NS	NS
Total C18	78.3	76.3	83.6	78.9	82.8	90.5	3.5	0.07	0.08	0.02	NS	NS

¹Rumensin supplementation (12 g/909 kg of DM); AV fat: 5.0% added AV blend; CNO: 5.0% added CNO.

²Standard error of the mean for n = 6.

³Probability of a treatment response; NS: $P > 0.20$. Contrasts compared: the main effect of R (diets without vs. with R averaged over fat treatments), the main effect of fat (Fat = control vs. the average of the 2 fat diets averaged over R), the main effect of source of fat (Source = AV fat vs. CNO averaged over R), the interaction between R and fat (R × fat), and the interaction between R and source of fat (R × source). See Materials and Methods section for contrasts.

⁴Not applicable because of negligible FA concentrations in control and AV fat treatments.

formation of *trans* isomers and complete reduction of those isomers to 18:0, although some lipid-metabolizing bacteria are not yet known (Wallace et al., 1997; Jenkins et al., 2008). Moreover, more work is needed to quantify the importance of protozoal incorporation of PUFA and BH intermediates and resultant flow to the duodenum (Devillard et al., 2006; Firkins et al., 2008). Coconut oil profoundly inhibits protozoa (Hristov et al., 2009; Reveneau et al., 2012), which should provide a compensatory increase in substrate available to increase bacterial counts (Machmüller and Kreuzer, 1999). Moreover, the loss of protozoa could shift butyrylvibrios (Karnati et al., 2009). Thus, the CNO treatment likely increased accumulation of *trans*-10 and *trans*-11 18:1 by decreasing the flow of FA in protozoa and shifting the abundance or activity of bacteria.

A greater formation of *trans* 18:1 and CLA should inhibit milk fat synthesis in the bovine mammary gland and increase the risk for MFD (Baumgard et al., 2002; Peterson et al., 2003; Liu et al., 2006). In our samples, *trans*-10,*cis*-12 CLA percentage was very low. Shingfield et al. (2006) reported low percentage of this isomer in milk and subsequently suggested the use of other isomers as indicators of MFD (Shingfield et al., 2010). Overall, the changes in milk FA profile mirrored the profile of omasal flow of FA. The lower percentage of short-chain FA in milk fat reflects the inhibition of de novo FA synthesis with either fat source (main effect of fat). The inhibition of de novo FA synthesis by feeding unsaturated FA has been previously demonstrated (Pipero et al., 2004; Castañeda-Gutiérrez et al., 2005). The higher proportion of *trans* FA in omasal digesta and milk for both fat-supplemented diets was observed in the current study. AlZahal et al. (2008) detected an interaction between R and increasing the

amount of soybean oil supplementation increasing the percentage of *trans* 18:1 and decreasing SMCLA in milk fat. In contrast, no R interaction occurred with feeding flaxseed (da Silva-Kazama et al., 2011). In our companion study (Reveneau et al., 2012), we reported that these same cows fed AV fat decreased milk fat yield by 0.08 kg/d without R, but AV fat decreased milk fat more (0.18 kg/d) when combined with R; in contrast, CNO severely depressed milk fat (>0.30 kg/d) regardless of R (R × source interaction, $P < 0.10$). There was an R × fat interaction ($P < 0.05$) and a tendency ($P = 0.12$) for an R × source interaction for the ratio of *cis*-9,*trans*-11 CLA:*trans*-10,*cis*-12 CLA (data not shown). Feeding AV fat should increase substrate for BH pathways, and the shift from *trans*-11 to *trans*-10 pathways might be mediated by R. In contrast, with CNO, *cis*-9,*cis*-12 18:2 for substrate for BH should be lower compared with AV fat (Table 1) and we previously suggested that BH (of both pathways) was likely decreased for CNO; in addition, another important factor might be that CNO profoundly depressed the acetate:propionate ratio in our study, as reported in the companion article (Reveneau et al., 2012). Thus, the increased proportion of propionate for CNO might have been additive with respect to MFD, as reported in a recent article (Maxin et al., 2011). Lauric acid did not depress milk fat yield in one report (Hristov et al., 2009), but did so in another experiment conducted similarly (Hristov et al., 2011). Maintaining proper formulations for starch and forage NDF lessens risk of milk fat depression by R (Dubuc et al., 2009), and our diets had moderately high concentrations of NFC and high inclusion rates of dietary fat. Further work explaining variability in milk fat responses among dietary combinations of fat sources or among studies should

evaluate a role for protozoa and other microbial activities producing or using other BH intermediates besides just *trans*-10 18:1 or CLA isomers.

At 3.75% added CNO in the diet of lactating dairy cows, Mohammed et al. (1964) measured an increase in 12:0 in milk fat. When 5% of DMI was fed as 14:0 to dairy cows (Odongo et al., 2007), the percentage of 14:0 in milk fat was increased from 12.8% for control to 30.6% for 14:0 supplementation. Neither *trans*-10 nor *trans*-11 18:1 were affected by treatment. However, other *trans* and *cis* 18:1 isomers were decreased by 14:0 supplementation. In contrast, feeding lauric acid or CNO did increase *trans* 18:1 (Hristov et al., 2009, 2011).

The percentage of SFA in milk fat was significantly lower with fat supplementation, and feeding AV versus CNO diets decreased SFA by 7 percentage units. These responses were probably a result of the increased 12:0 and decreased linoleic acid in CNO than in other diets (Table 1). Rumensin decreased 6:0, 8:0, and 10:0, but few other effects or interactions with R were observed in our study, perhaps because of the frequent feeding schedule. With dietary fat supplementation, 12:0 and 14:0 increased with CNO, as would be expected from the high dietary concentrations and apparent total-tract digestibilities of these FA.

CONCLUSIONS

The feeding of R did not promote MFD except perhaps with the high PUFA intake from AV fat. The supplementation of AV fat and CNO increased the omasal flow and milk FA percentage of *trans* 18:1 isomers. The feeding of CNO increased apparent FA digestibility in the rumen, especially for MCFA, and also increased apparent digestibilities of total FA and 18C in the total tract, thus increasing risk for metabolic depression of DMI, at least at our high inclusion rates of CNO. The BH of 18C FA was decreased in CNO treatments, helping to explain why it promoted MFD. Thus, the current data do not support our hypothesis that CNO + R would decrease the risk of MFD under our conditions (2-h feeding intervals of 5% supplemental fat). Further characterization of CNO's effects on microbial populations and BH activity is warranted.

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