Short Communication: Diurnal Profiles of Conjugated Linoleic Acids and *Trans* Fatty Acids in Ruminal Fluid from Cows Fed a High Concentrate Diet Supplemented with Fish Oil, Linseed Oil, or Sunflower Oil

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ABSTRACT

Trans-18:1 and 18:2 isomer composition in ruminal fluid during the daily feeding cycle was examined in 3 cows fed a high concentrate diet (35:65) with 5% (DM basis) sunflower oil (SO), 5% linseed oil (LO), or 2.5% fish oil (FO) in a 3 × 3 Latin square with 3 4-wk periods. Grass hay and concentrate mixtures were fed at 0900, 1300, and 1700 h daily. Ruminal fluid was collected at 0900, 1100, 1300, 1500, 1700, 2000, and 0000 h. Feeding SO resulted in the greatest mean concentrations (% of total fatty acids) of *trans*10,*cis*12-18:2 and *cis*9,*trans*11-18:2. In particular, *trans*10,*cis*12-18:2 with SO was greater at 1500 (0.29%), 2000 (0.34%), and 0000 h (0.25%) relative to 0900 h (0.07%). *Cis*9,*trans*11-18:2 concentration increased from 0.47% at 0900 h to a peak of 2.06% at 1100 h; it remained greater than the percentage determined at 0900 h at 1300 (1.4%) through 0000 h (1.1%). Concentration of *trans*11,*cis*15-18:2 was greatest with LO, ranging from 3.3% (0900 h) to a peak of 11.4% at 2000 h. Mean *trans*10-18:1 concentration ranked by diet was SO > FO > LO. Peak *trans*10-18:1 with SO was observed at 1700 h (14.9%) compared with 0900 h (5.1%). *Trans*11-18:1 did not differ with diet or time. Stearic acid decreased over time with all diets reaching minimum concentrations at 1700 to 2000 h relative to 0900 h. Feeding FO, however, decreased mean 18:0 concentration 4-fold compared with LO or SO. The moderate effect on concentration of *trans*-18:1 coupled with accumulation of 18:2 intermediates and the decrease of 18:0 over time suggest that oils reduced the biohydrogenation of 18:2 isomers to *trans*-18:1.

(Key words: fish oil, linseed oil, sunflower oil, ruminal fatty acid)

Abbreviation key: CLA = conjugated linoleic acids, FO = fish oil, LO = linseed oil, SO = sunflower oil.

Vegetable or marine oils elevate the concentration and yield of conjugated linoleic acids (CLA) and *trans*-fatty acids in milk fat (Chilliard et al., 2001). Vaccenic acid, *trans*10-18:1, *cis*9,*trans*11-18:2, and *trans*10,*cis*12-18:2 are dramatically increased with high concentrate diets supplemented with polyunsaturated fatty acids (Bauman and Griinari, 2001) or mixed diets with fish oil (FO) (Chilliard et al., 2001). *Trans*10,*cis*12-18:2 and *trans*10-18:1 have been implicated in diet-induced milk fat depression (Bauman and Griinari, 2001).

Loor et al. (2002) presented the first detailed composition of *trans*-18:1, *cis*-18:1, nonconjugated 18:2, and some CLA in ruminal fluid from dairy cows fed canola or soybean oil. AbuGhazaleh et al. (2002) reported profiles of some 18:1 and 18:2 isomers in ruminal digesta from cows fed FO. Results from both studies corresponded to a single time point after feeding. AbuGhazaleh and Jenkins (2004ab) recently examined aspects of biohydrogenation of pure 20:5 n -3 and 22:6 n -3 administered as a single dose to batch cultures during 24-h incubations. Identification of key *trans*-18:1, non-conjugated 18:2, and CLA isomers during the daily feeding cycle could be used to evaluate the production of intermediates from different polyunsaturated oils with less risk of bias (e.g., acid accumulation) than in vitro. To examine this premise, we took advantage of a study that was primarily designed to assess ruminal digestion and production responses in cows fed a high concentrate diet plus FO, linseed oil (LO), or sunflower oil (SO) (Ueda et al., 2003). We identified 5 *cis*-18:1, *cis*9-through *cis*15-; 10 *trans*-18:1, *trans*4-through *trans*16-; 7 nonconjugated 18:2 isomers; 8 CLA isomers; and 4 18:3 isomers in ruminal fluid. All of these isomers were identified in duodenal contents, and most were increased by high dietary concentrate:forage (*cis*- and *trans*-18:1 primarily), LO (most 18:1, 18:2, and 18:3 isomers), or their interaction (Loor et al., 2004).

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Three peak lactation multiparous Holstein cows (71 ± 16 DIM) were fed a diet with 65% concentrate (based on ground wheat, soybean meal, and rapeseed meal) and 35% forage (long-cut grass hay) supplemented with FO (2.5% of DM), SO (5%), or LO (5%) (Huilerie Van de Putte, Mouscron, Belgium) in a 3×3 Latin square design during 3 4-wk periods. The level of FO was chosen to avoid detrimental effects on DMI (Chilliard et al., 2001). Grass hay and concentrate mixtures were fed separately in 3 meals at 0900, 1300, and 1700 h daily. Ruminal fluid (300 mL) was collected during wk 4 by suction from the ventral sac via the ruminal cannula at 0900, 1100, 1300, 1500, 1700, 2000, and 0000 h. Ruminal pH was recorded using these samples, but no diet (6.42 ± 0.05) or diet by time effects were observed ($P > 0.39$). However, pH decreased ($P < 0.05$) between 1100 through 0000 h relative to 0900 h regardless of diet. Dry matter intake and milk yield did not differ because of treatments (17.9 ± 2.4 and 26.0 ± 6.8 kg/d). Milk fat percentage also was not affected ($P > 0.50$) by diet (2.64 ± 0.36). Methylation and identification of fatty acids was conducted as described by Looor et al. (2004). Methyl esters were separated on a 100-m $\times$ 0.25-mm i.d. fused-silica capillary column (CP-Sil 88; Chrompack, Middelburg, The Netherlands) using a Varian CP-3800 GC (Varian, Les Ulis, France). Data were analyzed as a Latin square with repeated measures using the MIXED procedure of SAS (SAS Inst. Inc., Cary, NC). Differences caused by diet, time, diet by time interactions, linear contrasts, and differences between means at 1100, 1300, 1500, 1700, 2000, and 0000 h relative to 0900 h were considered to be significant when $P \leq 0.05$.

The test oils changed concentrations of *trans*-18:1 and 18:2 (conjugated and nonconjugated) isomers without ($P > 0.05$) affecting total fatty acid concentration (34.5 ± 3.3 mg/g fluid DM). *Cis*9,*trans*11-18:2 was the most predominant CLA regardless of diet, and mean concentration was greater ($P < 0.05$) with SO (1.46 vs. 0.72 mg/g) compared with FO or LO (Figure 1A). The concentration of this isomer was 4.5-fold greater ($P < 0.05$) at 1100 h and 4.3-fold greater ($P < 0.05$) at 1500 h compared with 0900 h, suggesting active biohydrogenation of substrate after the 0900- and 1300-h feeding or a lag time effect. The overall correlation ($n = 63$) between 18:2 n -6 and *cis*9,*trans*11-18:2 was 0.54, and that between *cis*9,*trans*11-18:2 and *trans*11-18:1 was 0.26. The low correlation between this CLA and *trans*11-18:1 is likely due to the fact that *trans*11-18:1 could originate from *cis*9,*trans*11-18:2, *trans*11,*cis*15-18:2, and(or) other 18:1 isomers (Mosley et al., 2002; Proell et al., 2002). The 18:2 isomers accumulated to different extents depending on diet fed (Figure 1, A and B).

Figure 1. Diurnal pattern of *cis*(c)9,*trans*(t)11-18:2 (SEM = 0.41) and *t*10,*c*12 to 18:2 (SEM = 0.06) (Panel A) or *t*11,*c*15-18:2 (SEM = 1.7) (Panel B) in mixed ruminal fluid. Feeding was at 0900, 1300, and 1700 h daily. FO = fish oil, LO = linseed oil, and SO = sunflower oil.

Concentration of *trans*10,*cis*12-18:2 was nearly undetectable at 0900 h regardless of diet (Figure 1A). However, it increased ($P < 0.05$) steadily with SO through 2000 h after which it declined slightly. This resulted in greater ($P < 0.05$) concentrations at 1100 (0.21%), 1300 (0.22%), 1500 (0.29%), 2000 (0.34%), and 0000 h (0.25%) relative to 0900 h. Feeding SO led to greater ($P < 0.05$) mean *trans*10,*cis*12-18:2 concentration (0.23 vs. 0.07 mg/g DM) compared with other diets, but this isomer remained a minor one. The correlation between 18:2 n -6 and *trans*10,*cis*12-18:2 with SO was 0.72, which may suggest that a portion of dietary 18:2 n -6 was isomerized to this CLA, as proposed by Bauman and Griinari (2001).

There was a significant ($P = 0.05$) diet by time interaction for the concentration of *trans*11,*cis*15-18:2 (Figure 1B), resulting from the gradual increase in production of this isomer primarily with LO and to a smaller extent with FO. Feeding FO resulted in an increase ($P < 0.05$) in *trans*11,*cis*15-18:2 that followed an overall linear

trend, although concentrations at 1100 through 0000 h relative to 0900 h were not statistically significant ($P > 0.20$). With LO, concentrations at 1100 through 0000 h were 2.8- to 3.5-fold greater ($P < 0.05$) (Figure 1B). Mean concentration was markedly greater ($P < 0.05$) with LO (accounted for 68% of total nonconjugated 18:2) (8.96 mg/g) but also increased ($P < 0.05$) with FO (2.93 mg/g) compared with SO (0.95 mg/g). This isomer is the major nonconjugated 18:2 produced during hydrogenation of 18:3 n -3. We found an increase in the duodenal flow of this fatty acid in cows fed linseed oil compared with controls (Loor et al., 2004). A similar increase also was observed in omasal contents from cows fed FO (Shingfield et al., 2003). We hypothesize that FO resulted in incomplete biohydrogenation of 18:3 n -3 derived from the basal diet.

The correlation between *trans*10:*cis*12-18:2 and *trans*10-18:1 when SO was fed was 0.58. This positive relationship may indicate that a portion of *trans*10-18:1 was produced via the *trans*10:*cis*12-18:2 isomer (Loor and Herbein, 2001). Mean *trans*10-18:1 increased ($P < 0.05$) with FO and SO (8.30 vs. 3.1 mg/g) (Figure 2A), which was unexpected for FO based on previous data (Shingfield et al., 2003). However, those data were obtained in cows fed mixed diets (60:40 forage to concentrate) and may emphasize the importance of high dietary concentrate (Pipero et al., 2002; Loor et al., 2004) for ruminal accumulation of *trans*10-18:1. Concentration of *trans*10-18:1 with SO was 2.9-fold greater ($P = 0.05$) at 1700 than 0900 h, but with FO it was 2.2-fold lower ($P < 0.05$) (Figure 2A). Concentrations of 20:5 n -3, 22:6 n -3, *trans,trans*-CLA, *cis*8:*trans*10-CLA, and *trans*15-18:1 at 1700 h with FO were 3- to 13-fold greater ($P < 0.05$) relative to 0900 h (data not shown). This can, in part, explain the low concentrations of *trans*10-18:1 and *trans*11-18:1 with FO at 1700 h (Figure 2, A and B). Greater *trans*10-18:1 with FO (Figure 2A) was not associated with linoleic acid intake. The ratio (data not shown) of *trans*10-18:1 to *trans*11-18:1 with FO was 2.1 at 0900 h then decreased ($P = 0.05$) to 1.1-1.4 at 1100 h through 0000 h. It could be possible that as *trans*11-18:1 accumulated, a portion was converted to *trans*10-18:1. Several *trans*-18:1 were formed from ^{13}C -*trans*9-18:1 when incubated in vitro (Proell et al., 2002). Overall, diets did not ($P > 0.05$) affect *trans*11-18:1 concentration.

The pattern of 18:0 concentration over time (Figure 2C) indicated that all diets resulted in incomplete biohydrogenation during the feeding cycle. Concentration at 1500 through 0000 h was lower ($P < 0.05$) relative to 0900 h with all diets. Among treatments, however, feeding FO resulted in lower ($P < 0.05$) 18:0 at 0900 h and in lower ($P < 0.05$) overall mean 18:0 concentration (11.9 vs. 25.0 mg/g). There was a negative correlation

Figure 2. Diurnal pattern of *trans*10-18:1 (SEM = 2.3; Panel A), *trans*11-18:1 (SEM = 3.0; Panel B), and 18:0 (SEM = 4.4; Panel C) in mixed ruminal fluid. Feeding was at 0900, 1300, and 1700 h daily. FO = fish oil, LO = linseed oil, and SO = sunflower oil.

($r = -0.79$) between the concentration of 20:5 n -3 and 22:6 n -3 with FO and 18:0 in ruminal fluid, suggesting that these fatty acids at very low concentrations (2 to 3% of total fatty acids in fluid) depress ruminal biohydrogenation of unsaturated fatty acids in the basal diet.

Although speculative, it could be possible that the greater ($P < 0.05$) concentration of ruminal entodinium spp. that we found with FO (Ueda et al., 2003) may have, to some degree, caused the loss of bacteria carrying out the reduction of *trans*-18:1-18:0. Such response could account for the marked increase in milk *trans*-18:1 observed in cows fed mixed diets plus various levels of FO (Chilliard et al., 2001).

In summary, production of *cis*9,*trans*11-18:2, *trans*10,*cis*12-18:2, and *trans*10-18:1 was enhanced with SO containing high linoleic acid as a substrate for hydrogenation; whereas, LO enhanced production of *trans*11,*cis*15-18:2 from supplemental 18:3*n*-3. Fish oil resulted in a drastic reduction in 18:0 while increasing *trans*10-18:1. The pattern of 18:0 concentration over time coupled with a moderate effect on the concentration of *trans*10-18:1 and *trans*11-18:1 and the apparent accumulation of *cis*9,*trans*11-18:2 and *trans*11,*cis*15-18:2 suggest that the diets might have reduced the biohydrogenation of both 18:2 isomers to *trans*-18:1.

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