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Short communication: Milk fat depression induced by conjugated linoleic acid and a high-oil and low-fiber diet occurs equally across the day in Holstein cows

L. Ma,* K. L. Cook,*[1] D. E. Bauman,† and K. J. Harvatine*[2]
*Department of Animal Science, Penn State University, University Park 16802
†Department of Animal Science, Cornell University, Ithaca, NY 14850

ABSTRACT

Recently a circadian rhythm of milk and milk component synthesis has been characterized that is partially dependent on the timing of feed intake. Our objective was to determine if inhibition of milk fat synthesis during diet-induced milk fat depression occurred to a higher degree during certain phases of the day. A retrospective analysis was conducted on 2 experiments that induced milk fat depression while milking cows 3 times per day at equal intervals. The response at each milking was analyzed using mixed model ANOVA analysis using repeated measures. In experiment 1, nine multiparous Holstein cows were arranged in a 3 × 3 Latin square design, and treatments were control, 3-d intravenous infusion of 10 g/d of *trans*-10,*cis*-12 conjugated linoleic acid (CLA), and a low-forage and high-fat diet for 10 d. In experiment 2, ten multiparous ruminally cannulated cows were arranged in a replicated design and milk samples were collected during a control period or after 5 d of abomasal infusion of 10 g/d of CLA. The daily pattern of milk fat concentration and yield did not differ between treatments in either experiment. In experiment 1, an effect was found of treatment and milking time on milk fat concentration and yield. Similarly, in experiment 2, main effects were found of treatment and milking time on milk fat concentration and an effect of treatment, but no effect of milking time on milk fat yield. Milk fat percent was increased from 3.41 to 4.06% and 3.25 to 3.48% from the morning to the afternoon milking in experiments 1 and 2, respectively. Additionally, milk fatty acid profile, including *trans* intermediates, was changed over the day in experiment 1, but the magnitude of the changes were small and the pattern did not differ among treatments. A daily rhythm of milk fat concentration and yield was observed in cows milked 3 times a day, but milk fat depression decreases milk fat yield equally over the day.
Key words: milk fat depression, circadian, diurnal, conjugated linoleic acid

Short Communication

Fat is the most variable component of milk and its synthesis is affected by many factors, with diet being a major factor. Diet-induced milk fat depression (**MFD**) is a specific reduction in milk fat synthesis caused by feeding diets with high levels of rapidly fermentable carbohydrates and unsaturated FA (reviewed by Bauman and Griinari, 2003; Harvatine et al., 2009). The reduction in milk fat synthesis is attributed to reduced mammary capacity for lipid synthesis caused by bioactive *trans* FA intermediates generated during altered rumen biohydrogenation of unsaturated FA by rumen microbes. Specifically, *trans*-10,*cis*-12 CLA is one of the bioactive isomers that has been extensively studied with its specific effects confirmed by intravenous and abomasal infusion experiments in dairy cows. Importantly, infusion of CLA replicates the phenotype and molecular events associated with MFD without other confounding factors such as changes in the amount and profile of absorbed VFA.

Most physiological processes follow a daily or circadian rhythm that is controlled by endogenous timekeeping mechanisms commonly referred to as biological clocks. Clocks enable the synchronization of behaviors and physiological processes with daily changes in the external environment. Clocks also permit the coordinated activities between organs and circadian rhythms have been well described for most physiological processes [reviewed by Dibner et al. (2010)]. In mammals, the dominant circadian pacemaker is located in the suprachiasmatic nucleus of the hypothalamus, which organizes the temporal activity of peripheral clocks located throughout different organs of the body by regulating a series of neural and hormonal signals. A daily rhythm of intake also creates a daily rhythm of rumen fermentation and nutrient absorption. For ex-

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[1]Current address: Lancaster, Pennsylvania.
[2]Corresponding author: kjh182@psu.edu

ample, Loor et al. (2004) reported a daily pattern of ruminal *trans* FA isomers in rumen fluid of cows fed high-oil diets.

Dairy farmers commonly recognize that morning and evening milking differ in milk yield and composition. Gilbert et al. (1972) reported 0.65 kg higher milk yield at the morning milking, but 0.32 and 0.09 percentage unit higher milk fat and protein, respectively, at the evening milking in cows milked at 12-h intervals. More recently, Quist et al. (2008) conducted a survey of the milking-to-milking variation in milk yield and composition on 16 dairy farms. Milk yield and milk fat concentration showed a clear repeating daily pattern over the 5 d of observation in herds that milked 2 and 3 times per day. Morning milking was over 1 percentage unit lower in milk fat than the evening milking. We recently observed milk and milk component yield at each milking while milking every 6 h and feeding cows 1 time per day at 0800 h or in 4 equal feedings every 6 h and demonstrated that the daily rhythm of milk fat synthesis was dependent on the timing of nutrient intake (Rottman et al., 2014). Specifically, feeding cows 4 times per day in equal meals increased milk fat yield and milk fat concentration across the entire day and reduced the amplitude of the daily rhythm of milk fat synthesis. However, no daily rhythm of milk *trans* C18:1 FA occurred and only minor differences in concentrations of FA <16 carbons (de novo synthesized) and FA >16 carbons (preformed from plasma) were observed.

The daily rhythm of milk fat synthesis in the dairy cow may be due to the daily rumen dynamics including *trans* FA synthesis and passage to the intestine, an endogenously generated rhythm driving mammary metabolic capacity, or a within-day change in the responsiveness of the mammary gland to bioactive FA. To our knowledge, the daily pattern of milk fat during MFD has not been investigated. Therefore, the objective of the current study was to determine whether bioactive FA decreased milk fat synthesis equally across the day. To answer this question, the daily rhythm of milk synthesis from 2 previously conducted experiments that induced MFD by either feeding a low-fiber, high-oil diet or by intravenous or abomasal infusion of *trans*-10,*cis*-12 CLA was analyzed. Our hypothesis was that MFD occurs equally across the day during both diet- and CLA-induced MFD.

All animal procedures were approved by the Cornell University Institutional Animal Care and Use Committee. Briefly, 9 mid-lactation cows (193 ± 32 d postpartum; mean ± SD) were housed in tie-stalls with approximately a 20L:4D cycle and assigned randomly to a 3 × 3 Latin square design. Treatments were control (**CON1**), 3 d intravenous infusion of *trans*-10,*cis*-12 CLA (**iCLA**; 10 g/d in intralipid emulsion), and feeding a low-forage, high-oil diet for 10 d (**LF/HO**; 3.0% soybean oil and 1.5% fish oil). Cows were milked every 8 h (0800, 1600, and 2400 h) and milk yield and composition were determined at each milking on the last day of each experimental period (described in Harvatine and Bauman, 2006). The LF/HO treatment decreased daily milk fat yield 38% and intravenous CLA infusion decreased daily milk fat yield 24% (Harvatine and Bauman, 2006). To investigate the daily rhythm of milk synthesis, data from a single day for each cow-period were analyzed using the PROC MIXED procedure in SAS with the repeated statement (version 9.3, SAS Institute Inc., Cary, NC). The model included the fixed effect of treatment, milking time, and the interaction of treatment and milking time and the random effect of cow and period. Subject was cow by period, and the Kenward-Rogers denominator degree of freedom adjustment and the ARH1 covariance structure were used. Cosine analysis was not conducted because of the lower frequency of data collection.

No treatment by milking time interaction was found for milk fat yield and concentration when MFD was induced by intravenous CLA infusion and feeding the LF/HO diet (Table 1). The decreases in milk fat yield and concentration were similar at all milkings (24 to 25% for iCLA and 38 to 42% for LF/HO; Table 1). A main effect was found of time on fat yield and concentration, with milk fat yield in control cows ranging from 392 to 445 g/milking (Table 1; $P < 0.05$). A treatment by milking time interaction was found for milk and milk protein yield, supporting a daily rhythm of milk synthesis.

Milk FA profile was analyzed as described by Rico and Harvatine (2013) to characterize *trans* FA isomers and de novo synthesized FA that are known to change during MFD. An effect was observed of treatment and time, but no treatment by time interaction was observed for the proportion of de novo synthesized and preformed FA and the 16-carbon desaturase ratio (Table 2). De novo synthesized FA were lowest at the 1600 h milking and highest at the 2400 h milking, although the difference between milkings was less than a 5% change. Similarly, there was an effect of treatment and time and no interaction of treatment and milking time on the proportion of *trans*-10 C18:1, an isomer of the alternate biohydrogenation pathway associated with diet-induced MFD. *Trans*-10 C18:1 was lowest at 2400 h, although the difference between milkings was between a 6.5 to 13% change. Lastly, a treatment by milking time interaction was found for *trans*-11 C18:1, which peaked at 1600 h. *Trans*-11 C18:1 was almost 11% different between milkings in the LF/HO treatment, whereas little difference occurred over the day in control and CLA infusion. The complete FA profile is reported in Supplemental