



The effects of feeding time on milk production, total-tract digestibility, and daily rhythms of feeding behavior and plasma metabolites and hormones in dairy cows

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ABSTRACT

The timing of feed intake entrains circadian rhythms regulated by internal clocks in many mammals. The objective of this study was to determine if the timing of feeding entrains daily rhythms in dairy cows. Nine Holstein cows were used in a replicated 3 × 3 Latin square design with 14-d periods. An automated system recorded the timing of feed intake over the last 7 d of each period. Treatments were feeding 1×/d at 0830 h (AM) or 2030 h (PM) and feeding 2×/d in equal amounts at 0830 and 2030 h. All treatments were fed at 110% of daily intake. Cows were milked 2×/d at 0500 and 1700 h. Milk yield and composition were not changed by treatment. Daily intake did not differ, but twice-daily feeding tended to decrease total-tract digestibility of organic matter and neutral detergent fiber (NDF). A treatment by time of day interaction was observed for feeding behavior. The amount of feed consumed in the first 2 h after feeding was 70% greater for PM compared with AM feeding. A low rate of intake overnight (2400 to 0500 h; $2.2 \pm 0.74\%$ daily intake/h, mean $\pm$ SD) and a moderate rate of intake in the afternoon (1200 to 1700 h; $4.8 \pm 1.1\%$ daily intake/h) was noted for all treatments, although PM slightly reduced the rate during the afternoon period compared with AM. A treatment by time of day interaction was seen for fecal NDF and indigestible NDF (iNDF) concentration, blood urea nitrogen, plasma glucose and insulin concentrations, body temperature, and lying behavior. Specifically, insulin increased and glucose decreased more after evening feeding than after morning feeding. A cosine function within a 24-h period was used to characterize daily rhythms using a random regression. Rate of feed intake during spontaneous feeding, fecal NDF and iNDF concentration, plasma glucose, insulin, NEFA, body temperature, and lying behavior fit

a cosine function within a 24-h period that was modified by treatment. In conclusion, feeding time can reset the daily rhythms of feeding and lying behavior, core body temperature, fecal NDF and iNDF concentration, and plasma blood urea nitrogen, glucose, and insulin concentration of dairy cows, but has no effect on daily DMI and milk production.

Key words: circadian rhythm, feed intake, dairy cow

INTRODUCTION

Circadian rhythms are approximately 24-h repeating cycles followed by most physiological functions in mammals (reviewed by Bass, 2012). Daily rhythmicity is well recognized in dairy cows, and Giannetto and Picciano (2009b) reported that 12 of 25 variables investigated followed a circadian pattern. Briefly, rhythmic changes in plasma insulin, BUN, growth hormone concentration (Lefcourt et al., 1995), body temperature (Lefcourt et al., 1999), and locomotor activities (Giannetto and Picciano, 2009b) have been reported.

Circadian rhythms are entrained by numerous environmental cues, but the light-dark cycle and feed availability are dominant factors (reviewed by Asher and Schibler, 2011). Circadian rhythms also persist in the absence of environmental cues, as they are the product of time-keeping mechanisms present in the central nervous system and most peripheral tissues, although this is difficult to test in lactating cows. Circadian rhythms are adaptive, as they coordinate physiological processes with rhythmic changes occurring in the environment and asynchrony of rhythms have been reported to be detrimental to health and well-being (reviewed by Bass, 2012).

Feed intake is an important consideration in dairy cows, as milk production creates a high energy demand and energy intake can limit milk production. The daily pattern of feed intake is classically described with the majority of feed consumed between sunrise and sunset (e.g., Ray and Roubicek, 1971). Recent work using automated feed-observation systems have reported an active feeding period during the day and a less-active feeding period at night (e.g., DeVries et al., 2003; DeVries and

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von Keyserlingk, 2005). Additionally, the daily patterns of peripheral blood hormones and metabolites change in response to diet (e.g., Oba and Allen, 2003), and the concentration of ruminal AA, ammonia nitrogen, and VFA are responsive to change in feeding time (Robinson et al., 1997, 2002). More recently, Nikkhah et al. (2008) reported that offering feed once a day at 2100 h altered the daily patterns of plasma metabolites compared with feeding cows at 0900 h.

Entrainment of circadian rhythms by the timing of feed availability is well established in other mammals (reviewed by Asher and Schibler, 2011), but has not been well described in dairy cows. We hypothesized changing feeding time would shift the daily pattern of feeding behavior, which will modify rumen fermentation and shift the daily rhythms of plasma metabolites and hormones. Additionally, we hypothesized that the daily rhythm of body temperature, which is highly regulated by lighting through the central master clock, and lying behavior would not be as responsive to changes in feeding time. The objectives of the current study were to determine the effect of feeding time on milk production and daily behavioral and physiological rhythms. Additionally, total-tract digestibility, milk *trans* FA profile, and the daily rhythm of fecal NDF and iNDF concentration were used to indirectly observe the effect of treatments on rumen function.

MATERIALS AND METHODS

Experimental Design and Treatments

The experiment was conducted from February to March of 2012 at the Pennsylvania State University Dairy Production Research and Teaching Center. Nine multiparous Holstein cows (109 ± 31 DIM; mean $\pm$ SD) were housed in single-rail tiestalls with mattresses and sawdust bedding and randomly assigned to treatment sequences in a replicated 3×3 Latin square design with 14-d periods. Circadian rhythms normally entrain within 7 d, allowing the use of short observation periods. Lights were out from 2300 to 0500 h each day and a data-logging device (HOBO Pendant Temp/Light; Onset Computer Corp., Bourne, MA) verified that the lighting schedule was maintained and observed that the facility averaged 11.7°C during the experiment. Cows were individually fed the same diet, which contained 29.7% NDF and 17.9% CP (Table 1). Treatments were feeding $1 \times /d$ at 0830 h (AM) or 2030 h (PM) or feeding $2 \times /d$ in equal amounts at 0830 and 2030 h (AMPM). All treatments were fed ad libitum at 110% of previous daily intakes and the TMR was mixed immediately before each feeding. Refused feed was removed and weighed before feed delivery in the

Table 1. Ingredient and chemical composition of the experimental diet

Item	Value ¹
Ingredient (% of DM)	
Corn silage ²	41.4
Alfalfa haylage ³	13.8
Canola meal	9.5
Roasted soybeans	9.3
Ground corn	7.7
Grass hay ⁴	4.9
Cookie meal	4.3
Molasses	3.9
Mineral and vitamin mix ⁵	2.8
Cottonseed hulls	2.2
NPN supplement ⁶	0.4
Chemical composition ⁷ (% of DM)	
CP	17.9
NDF	29.7
ADF	20.1
Starch	25.0
Ash	6.3

¹All cows were fed the same diet.

²Contained 38% DM and 8.4% CP, 34.1% NDF, and 38% starch on a DM basis.

³Contained 48.3% DM and 23.4% CP, 38.5% NDF, and 1.3% starch on a DM basis.

⁴Contained 88.8% DM and 10.3% CP, 66.4% NDF, and 3.8% starch on a DM basis.

⁵Vitamin and mineral mix contained (as-fed basis): 45.8% dried corn distillers grains with solubles; 35.8% limestone (38% Ca); 8.3% magnesium oxide (54% Mg); 6.4% salt; 1.73% vitamin ADE premix; 1.09% selenium premix (0.06% selenium); and 0.88% trace mineral mix. Composition (DM basis): 11% CP; 18% NDF; 5.2% fat; 14.9% Ca; 0.35% P; 4.58% Mg; 0.41% K; 0.31% S; 357 mg/kg of Cu; 1,085 mg/kg of Zn; 181 mg/kg of Fe; 6.67 mg/kg of Se; 125,875 IU/kg of vitamin A (retinyl acetate); 31,418 IU/kg of vitamin D (Activated 7-dehydrocholesterol); and 946 IU/kg of vitamin E (DL- α tocopheryl acetate).

⁶Controlled-release urea (Optigen; Alltech Inc., Lexington, KY; 278% CP on DM basis).

⁷ $n = 3$.

morning for AM and AMPM treatments and at night for the PM treatment.

Data and Sample Collection and Analysis

Cows were housed in a feed intake observation system similar to Dado and Allen (1993). Briefly, hanging feed tubes were suspended from an electronic load cell wired to a data acquisition system (RLWS 920i; Rice Lake Weighing System, Rice Lake, WI). Feed weight was recorded every 8 s from d 8 to 14 of each period. The number, size, and intermeal interval of meals were determined by an algorithm developed in IGOR (WaveMetrics Inc., Lake Oswego, OR; algorithm provided by M.S. Allen, Michigan State University, East Lansing) according to Harvatine and Allen (2006). Feed intake across the day was calculated over 30-min and 2-h intervals. Raw data were smoothed by averaging over 180 s and the rate of feed intake (percent of daily intake

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