Exposure to chronic light–dark phase shifts during the prepartum nonlactating period attenuates circadian rhythms, decreases blood glucose, and increases milk yield in the subsequent lactation

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ABSTRACT

Maintaining metabolic balance is a key factor in the health of dairy cattle during the transition from pregnancy to lactation. Little is known regarding the role of the circadian timing system in the regulation of physiological changes during the transition period. We hypothesized that disruption of the cow's circadian timing system by exposure to chronic light–dark phase shifts during the prepartum period would negatively affect the regulation of homeostasis and cause metabolic disturbances, leading to reduced milk production in the subsequent lactation. The objective was to determine the effect of exposure to chronic light–dark phase shift during the last 5 wk prepartum of the nonlactating dry period on core body temperature, melatonin, blood glucose, β -hydroxybutyric acid (BHB) and nonesterified fatty acid (NEFA) concentrations, and milk production. Multiparous cows were moved to tiestalls at 5 wk before expected calving and assigned to control (CTR; $n = 16$) or phase-shifted (PS; $n = 16$) treatments. Control cows were exposed to 16 h of light and 8 h of dark. Phase-shifted cows were exposed to the same photoperiod; however, the light–dark cycle was shifted 6 h every 3 d until parturition. Resting behavior and feed intake were recorded daily. Core body temperature was recorded vaginally for 48 h at 23 and 9 d before expected calving using calibrated data loggers. Blood concentrations of melatonin, glucose, BHB, and NEFA were measured during the pre- and postpartum periods. Milk yield and composition were measured through 60

DIM. Treatment did not affect feed intake or body condition. Cosine fit analysis of 24-h core body temperature and circulating melatonin indicated attenuation of circadian rhythms in the PS treatment compared with the CTR treatment. Phase-shifted cows had lower rest consolidation, as indicated by more total resting time, but shorter resting period durations. Phase-shifted cows had lower blood glucose concentration compared with CTR cows (4 mg/mL decrease), but BHB and NEFA concentrations were similar between PS and CTR cows. Milk yield and milk fat yield were greater in PS compared with CTR cows (2.8 kg/d increase). Thus, exposure to chronic light–dark phase shifts during the prepartum period attenuated circadian rhythms of core body temperature, melatonin, and rest–activity behavior and was associated with increased milk fat and milk yield in the postpartum period despite decreased blood glucose pre- and postpartum. Therefore, less variation in central circadian rhythms may create a more constant milieu that supports the onset of lactogenesis.

Key words: circadian rhythm, nonlactating period, nonesterified fatty acid, blood glucose, transition period

INTRODUCTION

During the transition period, the last 3 wk before calving and the first 3 wk postpartum, major hormonal shifts and metabolic adaptations occur in dairy cows to accommodate the increased energetic demands of fetal growth and the onset of milk production (Bauman and Currie, 1980; Bell and Bauman, 1997). Successful adaptation to both the onset of lactation and the resulting negative energy balance can provide for a healthy and productive lactation, whereas a poor adaptive response can lead to ketosis, milk fever, and reduced milk production (Duffield et al., 2009). Management and environ-

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mental conditions, such as photoperiod and heat stress, during the 45- to 60-d dry nonlactating period before parturition can affect the milk production capacity of the cow in the subsequent lactation (Renaudeau et al., 2001; Dahl, 2008; Mabjeesh et al., 2013). Understanding the biological mechanisms that regulate the changes that occur in dairy cattle during the transition period can help in developing farm management approaches that maximize milk production and minimize disease in cows.

Almost all physiological and behavioral functions of animals are rhythmic, including hormone secretion patterns, sleep–wake cycles, metabolism, and core body temperature. These circadian rhythms (i.e., 24-h cycles of biochemical, physiological, or behavioral processes) evolved as a common strategy among animals to coordinate internal systems and synchronize these systems to the environment (Casey and Plaut, 2012; Plaut and Casey, 2012). In mammals, circadian clocks are regulated hierarchically, with the master circadian clock located centrally in the suprachiasmatic nuclei of the hypothalamus. In addition, peripheral clocks are distributed in every organ. Metabolic function and circadian clocks are tightly connected and reciprocally regulated, such that clocks drive metabolic processes and various metabolic parameters affect clocks. Dairy cattle display circadian rhythms of eating behavior; plasma concentrations of metabolic hormones, including insulin, somatotropin, cortisol, melatonin, and triiodothyronine (Dahl et al., 2012); as well as metabolites, such as plasma glucose, nonesterified fatty acids (**NEFA**), BHB, and urea nitrogen (Bitman et al., 1990; Lefcourt et al., 1999). Photoperiod and feeding behavior affect circadian rhythms of metabolism in cattle (Ogino et al., 2014), with light–dark information being the most important environmental cue for entraining rhythms established by the master clock in the suprachiasmatic nuclei. Moreover, photoperiod length affects ruminant metabolism and milk production. In particular, exposure to a long-day photoperiod during an established lactation increases milk yield in dairy cattle without initially altering feed intake (Peters et al., 1978), whereas exposure to a short-day photoperiod during the dry period enhances subsequent lactation performance (Dahl, 2008).

Human and rodent studies support a mechanistic link between circadian disruption and the development of metabolic diseases (Pillar and Shehadeh, 2008; Gangwisch, 2009; Möller-Levet et al., 2013). Of particular interest is that metabolism is altered and lactation performance is impaired in mice with mutations in the *CLOCK* gene, one of the genes that make up the core molecular clocks that generate circadian rhythms of gene expression (Dolatschad et al., 2006; Hoshino et al.,

2006; Casey et al., 2016). Here our aim was to determine whether the circadian timing system plays a role in maintaining homeostasis and regulating the onset of lactation in transition dairy cows. A common approach to understanding the role of a certain factor in a biological system is to disrupt the factor and determine the consequence to the animal. Environmental cues that function to set the time of circadian clocks are also capable of disrupting clocks when applied at unusual and inappropriate times. Under natural conditions, circadian rhythms are synchronized to the regular 24-h light–dark cycles that are generated by the rotation of Earth. Alterations of natural light–dark cycle timing can disrupt circadian clocks (Papagiannakopoulos et al., 2016; Skinner et al., 2019). In this study, a chronic jet-lag model was used to disrupt circadian clocks. In particular, to effectively knock out the function of the circadian timing system, the light–dark cycle to which cows were exposed was shifted by 6 h every 3 d. Previous studies in dairy cows showed that disrupting circadian clocks by exposure to chronic light–dark phase shifts during an established lactation decreased milk production and increased MUN (Casey et al., 2014). We hypothesized that disruption of the circadian timing system in dairy cows during the nonlactating dry period would decrease their ability to maintain homeostasis during the transition from pregnancy to lactation and cause metabolic disturbances, characterized by increased NEFA and BHB, and be related to decreased milk production. Our study objectives were to determine the effect of exposure to chronic shifts of the light–dark cycle during the last 5 wk of the prepartum nonlactating dry period on (1) circadian rhythms of body temperature and plasma melatonin and behavior (feed intake and activity), (2) blood concentrations of glucose, NEFA, BHB, and insulin, and (3) milk yield and milk composition.

MATERIALS AND METHODS

Animal Management, Housing, and Experimental Conditions

The animal study protocol (protocol no. 1701001523) was reviewed and approved by the Purdue University Institutional Animal Care and Use Committee before beginning the experiments. The study was performed at the Dairy Unit of the Purdue Animal Sciences Research and Education Center in West Lafayette, Indiana. Thirty-two multiparous Holstein cows were matched by parity [2.68 ± 1.19 for control (**CTR**) and 2.87 ± 1.01 for phase shifted (**PS**); mean $\pm$ SD], previous lactation milk yield ($12,578.5 \pm 1,685$ kg for CTR and $12,864 \pm 1,802$ kg for PS for 305-d milk), and previous metabolic

Figure 1. Schematic representation of the study design. Thirty-two multiparous Holstein cows were dried off 60 d before expected calving (BEC). On d 35 BEC, animals were moved to the experimental barn and housed in individual tiestalls, and photophase treatments were applied (dashed portion of the arrow) until 3 d BEC. Control cows were exposed to 16 h of light and 8 h of dark, with the lights turned on at 0430 h and off at 2030 h. Phase-shifted cows were exposed to 16 h of light and 8 h of dark, but the light phase was shifted forward 6 h every 3 d. From 3 d BEC to 3 d postparturition (PP), all animals in both treatments were housed in the maternity barn (boxstalls) and exposed to control lighting (16 h of light and 8 h of dark; white portion of the arrow). On d 3 PP, all cows from both treatments were moved back to individual tiestalls, where they were kept until 15 d PP and exposed to control lighting. Then, animals were moved to a freestall barn for the remainder of lactation. Feed intake and resting activity were individually measured from 35 d BEC to 15 DIM. Milk yield was recorded up to 60 DIM. Starting on d 23 and 9 BEC and d 5 PP, blood samples were collected every 4 h during a total of 48 h. Temperature was recorded intravaginally over 48 h on d 23 and 9 BEC.

and mammary-related diseases (ketosis, mastitis, hypocalcemia, metritis, retained placenta, and displaced abomasum) and were assigned to 1 of 2 treatments: CTR ($n = 16$) or PS ($n = 16$). Cows were dried off 60 d before expected calving (BEC).

At 35 d BEC, cows were weighed and BCS were evaluated based on a 1-to-5 scale (Ferguson et al., 1994; Roche et al., 2009). Mean BW of the CTR cows (755 ± 18.66 kg) was not different from that of PS cows (757 ± 18.62 kg). The BCS of CTR cows (3.53 ± 0.06) was also not different from that of PS cows (3.42 ± 0.06). Following weighing on d 35 BEC, animals were moved to the experimental barn. Cows were housed in individual tiestalls and exposed to photophase treatments (either CTR or PS) in the experimental barn until 3 d BEC (Figure 1). For photophase treatments, CTR cows were exposed to 16 h of light and 8 h of dark, with lights turned on at 0430 h and off at 2030 h. The PS cows were exposed to 16 h of light and 8 h of dark, but the light phase was shifted forward 6 h every 3 d. In the experimental barn, the treatments were separated by a light-proof partition created by double draping fire-retardant tarps from floor to ceiling (Tarps Plus, Abadak Inc., Georgetown, TX). Natural light was eliminated from the barn by covering the windows with light-proof tarp and caulking around the doors. Used for the experiment were bright white light-emitting diode lights (Smart Electrician 5000 lm 46×6 LED Tread Plate Shop Light, Menards Inc., Eau Claire, WI), positioned in the experimental barn to provide 150 lx at the animals' eye level. Each cow in the PS treatment

was exposed to at least 10 light shifts during the treatment period (35 to 3 d BEC). The temperature in both treatment areas of the experimental barn was the same and varied no more than 5°C (10 – 15°C) from the start of the experiment (January 25, 2018) to the end (May 28, 2018) of the study. During the prepartum treatment period, animals were released from the tiestalls twice daily at 0430 and 1530 h into a gated holding pen outside the milking parlor for 30 min of exercise.

At approximately 3 d BEC, the photophase treatments of PS cows were stopped, and all animals were moved to the maternity barn. Cows were exposed to control lighting in the maternity barn and housed in boxstalls for calving until 3 d after parturition. Cows calved 2 ± 3.7 d (mean $\pm$ SD) before the expected calving date. Cows that showed signs of parturition before d 3 BEC (11 of the 32 cows) were moved to the maternity barn at the first sign of calving. At 3 DIM, all cows from both treatments were moved back to individual tiestalls until 15 DIM and exposed to control lighting. After 15 DIM, animals were moved to a freestall barn for the remainder of lactation.

Animals were fed individually for ad libitum intake once daily at 1600 h in the preparturition period and at 1100 h during the postparturition period. Feed was mixed in bulk, and individual daily feed intake was determined from 35 d BEC to 15 DIM by measuring feed offered and refused from the previous day. Cows were fed 110% of the calculated intake of the previous day. From 35 d BEC to parturition, animals were offered a prefresh ration (57.0% DM, 33.2% NDF, 15.8% CP,

Table 1. Diet ingredients and nutrient composition of prefresh and lactating diets

Item	Prefresh	Lactating
Ingredient ¹ (% of DM)		
Corn silage	32.7	24.6
Alfalfa silage	5.4	19.1
Rye hay	16.2	7.8
Ground corn	14.4	4.8
High-moisture shell corn		17.4
Soybean meal	6.4	5.5
Soyplus	3.6	4.8
Whole fuzzy cottonseed		6.0
Cottonseed hulls	6.6	
Distillers grains with solubles	0.7	
LysAAMet	0.6	0.8
QLF Blend 63-38	2.3	3.7
Calcium carbonate	1.2	1.1
Palmit 80		1.3
Biochlor	7.0	
Vitamin and mineral mix	2.9 ²	3.1 ³
Nutrient composition (% of DM)		
DM	57.0	49.9
NDF	33.2	28.6
CP	15.8	15.6
NFC	39.6	42.2
Fatty acids	3.9	5.4

¹Soyplus, Landus Cooperative, Ames, IA; LysAAMet, Perdue Agribusiness, Salisbury, MD; QLF Blend 63-38, Quality Liquid Feeds Inc., Dodgeville, MI; Palmit 80, Global Agri-trade Corporation, Rancho Dominguez, CA; Biochlor, Arm and Hammer, Ewing Township, NJ.

²Prefresh vitamin and mineral mix contained 28.55% MegalacR (Church and Dwight Co., Princeton, NJ), 13.25% Diamond V XP (Diamond V, Cedar Rapids, IA), 13.96% magnesium oxide, 9.11% monocalcium phosphate, 8.54% magnesium sulfate, 7.32% salt, 6.68% vitamin E 20,000 IU, 6.07% calcium sulfate, 5.94% mineral premix, 0.43% Rumensin 90 g/lb (40.8 g/kg; Elanco Animal Health, Greenfield, IN), and 0.15% vitamin A 30,000 IU.

³Lactating vitamin and mineral mix contained 27.07% sodium bicarbonate, 13.60% salt, 10.89% monocalcium phosphate, 10.72% DCAD Plus (Arm and Hammer), 6.68% Omnigen AF (Phibro Animal Health Corporation, Teaneck, NJ), 6.40% Diamond V XP (Diamond V), 6.59% magnesium oxide, 5.99% calcium sulfate, 5.90% fat yellow grease, 3.38% mineral premix, 1.80% ground corn, 0.78% vitamin E 20,000 IU, and 0.21% Rumensin 90 g/lb (40.8 g/kg; Elanco Animal Health).

39.6% NFC, and 3.9% fatty acids), and after parturition animals were offered a lactation ration (49.9% DM, 28.6% NDF, 15.6% CP, 42.2% NFC, and 5.4% fatty acids; Table 1).

Body Temperature and Resting Behavior Recordings

Calibrated temperature loggers (iButton DS1921H-F5#, iButtonLink Technology, Baulkham Hills, Australia) were used to measure body temperature (Burfeind et al., 2011, 2014). Each iButton was attached to a blank controlled internal drug release device (Eazi-Breed CIDR Cattle Insert, Zoetis Inc., Parsippany-Troy Hills, NJ). On d 23 and 9 BEC (average of 21 and 7 d before calving $\pm$ 3.7 d), starting at 0430 h, iButton controlled internal drug release devices were inserted

into the vagina for 48 h and set to record temperature every 30 min (Burdick et al., 2012; Johnson and Shade, 2017).

Following twice-daily exercise, cows were herded through the milking parlor at 0500 h (primarily dark-phase recording for control) and 1600 h (primarily light-phase recording for control) to collect the resting data from individual pedometers (AfiAct II Leg Tag, Afimilk USA Inc., Fitchburg, WI). Pedometers were attached when cows were enrolled into the study at 35 d BEC and were used to record resting bouts (number of bouts), rest duration (average minutes of resting per bout), and total resting time (minutes) up to 15 DIM. To calculate the total daily rest duration, dark- and light-phase recordings were averaged. Daily data for total resting time and resting bouts were calculated as the summation of dark- and light-phase recordings.

Blood Sampling and Analysis of Glucose, BHB, NEFA, Insulin, and Melatonin

Blood samples were collected from the coccygeal vein of each cow between 0430 and 0530 h on d 35, 28, and 21 BEC, then every 2 d until 7 d BEC, and then daily until 15 d postparturition (PP). These samples were used to measure circulating glucose, BHB, NEFA, and insulin concentrations. To measure morning versus afternoon differences in blood NEFA levels during the first 9 DIM, plasma was also collected at 1700 h (p.m. sample). A random subset of cows (CTR, $n = 6$; PS, $n = 6$) were sampled for temporal analysis of circadian rhythms of NEFA and melatonin. Blood samples were taken from indwelling jugular catheters, inserted without local anesthesia, every 4 h over a 48-h period beginning at 0600 (1.5 h after the start of the light phase in the CTR treatment) at 23 and 9 d BEC and 5 d PP. All blood samples were transferred into EDTA tubes (Becton Dickinson, Rutherford, NJ) and centrifuged at 4°C for 15 min at $2,000 \times g$ immediately following collection. Plasma was transferred to microfuge tubes and stored at -20°C until analysis.

To measure changes of circulating glucose immediately following parturition, whole blood was sampled from a subset of cows in each treatment (CTR, $n = 6$; PS, $n = 6$; first animals added to the study in each treatment) every hour after parturition for 5 h. The blood samples were obtained from the coccygeal vein using a 1-mL syringe.

Whole-blood glucose and BHB concentrations were measured onsite using a Centrivet system (Acon Laboratories Inc., San Diego, CA) following the manufacturer's directions. Wako NEFA-HR(2) colorimetric assay [Fujifilm Wako Diagnostics USA, Mountain View, CA; intra-assay coefficient of variation (CV) =

3.65%; interassay CV = 5.47%] was used to measure NEFA concentration in plasma samples following the manufacturer's protocol. Plasma insulin was measured using a bovine insulin ELISA Kit (Alpco, Salem, NH; intra-assay CV = 4.07%; interassay CV = 4.12%) following the manufacturer's protocol. Plasma melatonin concentration was determined using liquid chromatography–tandem MS. Briefly, 500 μ L of plasma was extracted using acetonitrile (1:4 vol/vol), then vortexed and centrifuged at $3,220 \times g$ at room temperature for 10 min. Supernatant was collected, vacuum-dried, and stored at -80°C until analysis was performed. Chromatography was performed using an Intakt Intrada amino acid column (3 μm , 2×150 mm; (Chrom Tech Inc., Apple Valley, MN) as described and modified (Zhao et al., 2016). A standard curve was used to calculate melatonin concentrations ($R^2 = 0.99$).

Milk Yield and Milk Composition

Milk yield was recorded twice daily for the first 60 DIM at 0500 and 1600 h using Afimilk software (Afimilk USA Inc.). Milk records and samples were collected daily at the morning (0500 h) and afternoon (1600 h) milkings from 5 to 9 DIM and on d 15, 22, 30, and 60 and were sent to Dairy One (Ithaca, NY), and fat, protein, lactose, and MUN content and SCC were determined using infrared technology (MilkoScan 7RM, Foss, Hillerød, Denmark). Fat, protein, and lactose yields were calculated by multiplying the milk yield on that day by the respective percentage. Somatic cell counts were transformed into $\log_{10}$ scale. Daily milk yield was calculated as the summation between morning and afternoon records. Daily yield of the main milk components (fat, lactose, and protein) was calculated as the multiplication of the component concentration by the milk yield in each milking (a.m. and p.m.) and added by day. Then, the daily concentration of each component was calculated as the component daily yield divided by the yield of milk for the day.

Data and Statistical Analysis

Blood glucose, BHB, and NEFA concentration data were adjusted to the actual calving date and collapsed by week relative to parturition. Cosine fit analysis of 24-h rhythms of plasma NEFA and melatonin concentrations and core body temperature was performed with the cosinor package in R (RStudio 1.1.453, Boston, MA). Mesor, amplitude, and acrophase were outputs of the package algorithm and were calculated for each animal and within each 48-h period (at 23 and 9 d BEC for body temperature and at 23 and 9 d BEC and 5

d PP for NEFA and melatonin concentrations). These 3 parameters were used to calculate and visualize the fitted cosine curve for each animal based on the model

$$y_i = M + A \times \cos[2\pi(t_i - A\phi)/24],$$

where y_i is the value of the measured variable at time t_i ; M is the mean, or mesor, of y throughout a complete cycle; A is the amplitude of the cosine curve on either side of M (half the difference between the highest and the lowest calculated values); and $A\phi$ is the acrophase or phase angle of the maximum value at time t_i (time at which the highest value encountered in the cycle occurs). The coefficient of determination (R^2) and associated P -values were also calculated for each cow for each sampling period. Mesor, amplitude, and acrophase R^2 and P -values were used for statistical analysis. Thus, data presented in tables are means across treatments and not cosine fit of all samples. This approach captures the true variation across animals and the statistical significance of treatment and physiological state effect on fit of data to the 24-h cosine pattern. In contrast, using fitted data from each cow to calculate R^2 and P -value overestimates the goodness of fit of data to a 24-h rhythm (i.e., resulted in statically stronger difference). To calculate the area under the curve (AUC), the function area under the curve with spline interpolation in the package MESS (RStudio) was used.

We used PROC MIXED of SAS (version 9.4; SAS Institute Inc., Cary, NC) to compare mesor, amplitude, acrophase, R^2 , and P -value between treatments. Tukey-Kramer test was used to evaluate differences in cosine parameters between treatments. Values were considered significant at $P < 0.05$, and P -values > 0.05 but ≤ 0.1 were discussed as a trend.

Milk yield, milk composition, blood metabolites (glucose, ketones, and NEFA), feed intake, and resting behavior data were analyzed using PROC MIXED with repeated measure using AR(1) covariance structure in SAS. Rest-activity, blood components, and milk composition data were analyzed using the following model:

$$Y_{ijk} = \mu + T_i + D_j + JDate + (T_i \times D_j) + C(T)_{ik} + e_{ijk},$$

where Y_{ijk} is the dependent variable; μ is the overall mean; T_i is the fixed effect of treatment ($i = \text{CTR}$ or PS); D_j is the fixed effect of day of milk ($j = 1$ to 60); $JDate$ is the covariate of Julian date; $T_i \times D_j$ is the interaction between treatment and day of milk; $C(T)_{ik}$ is the random effect of cow nested within treatment; and e_{ijk} is the random error. Treatment effects

were declared significant at $P \leq 0.05$, and trends were declared at $P \leq 0.10$. All data were expressed as least squares means and standard errors of the mean.

RESULTS

Body Temperature

The CTR cows exhibited circadian rhythms of core body temperature at 3 and 1 wk BEC, with the acrophase peak occurring just after onset of the dark phase of the light–dark cycle during both sampling periods ($2238 \text{ h} \pm 57 \text{ min}$ and $2150 \text{ h} \pm 54 \text{ min}$, respectively). A minor peak in body temperature was also evident approximately 2.5 h after the start of a.m. and p.m. exercise (0700 and 1800 h, respectively) in both sampling periods. The R^2 of cosinor-fit analysis increased from 3 wk to 1 wk BEC in CTR cows (0.30 ± 0.06 at 23 d BEC and 0.50 ± 0.06 at 9 d BEC; $P < 0.05$). Mesor and amplitude of body temperature increased between 23 and 9 d BEC in CTR cows (Figure 2; Table 2). The PS animals exhibited a trend ($P = 0.1$) for circadian rhythmicity of core body temperature during both sampling periods. Similar to CTR cows, the mesor of core body temperature increased between 23 d BEC ($39.01 \pm 0.07^\circ\text{C}$) and 9 d BEC ($39.26 \pm 0.04^\circ\text{C}$) in PS cows. There was no treatment effect on body temperature mesor. However, unlike in CTR cows, amplitude of body temperature circadian rhythm did not increase in PS cows between 3 and 1 wk BEC. The time of temperature acrophase for PS cattle was similar between

23 and 9 d BEC ($0322 \text{ h} \pm 54 \text{ min}$ and $0422 \text{ h} \pm 49 \text{ min}$, respectively) and was different from that for CTR cows ($P < 0.01$). The AUC of core body temperature was not different between periods or treatments.

Melatonin

Temporal analysis of plasma melatonin concentration rhythms revealed highly similar patterns across the 3 sampling periods (23 and 9 d BEC and 5 d PP; Table 3; Figure 3) in CTR cows. Least squares means of R^2 and corresponding P -values across the 3 periods (0.73, 0.78, and 0.77 for 23 d BEC, 9 d BEC, and 5 d PP, respectively) showed a good fit (all $P < 0.05$) to a 24-h cosine curve. The time of plasma melatonin acrophase (peak) was approximately 3 h after the start of the dark phase in CTR cows. Plasma melatonin concentrations changed dynamically across the day in CTR cows, with low concentration during the light phase and greater concentration during the dark phase (Figure 3), and were reflected by high amplitude values.

Cosine fit analysis of plasma melatonin concentration found that PS cows fit 24-h rhythms only at 3 wk BEC (Figure 3; Table 3). The time of peak melatonin was not different between treatments, but the acrophase standard errors showed greater variation in PS cows. There was a trend for an overall effect of treatment on melatonin mesor (32.95 ± 0.35 and $41.27 \pm 2.72 \text{ pg/mL}$ for CTR and PS, respectively; $P = 0.08$) and AUC ($1,615.10 \pm 194.10$ and $1,986.81 \pm 352.27$ for CTR and PS, respectively; $P = 0.09$).

Figure 2. Body temperature in nonlactating dairy cows at d 23 and 9 before expected calving (BEC) under (A) the control treatment (exposed to 16 h of light and 8 h of dark) or (B) the phase-shifted treatment (exposed to 16 h of light and 8 h dark, with the photophase shifted 6 h every 3 d). Dashed and solid lines represent d 23 and 9 BEC fitted cosine curves, respectively. Filled symbols = d 23 BEC; empty = d 9 BEC; dots = control cows; squares = phase-shifted cows. Bars under graph A represent the light (white) and dark (black) phases. Dashed bars under graph B represent the shifted photoperiod for phase-shifted cows.

Table 2. Cosine-fit analysis of core body temperature (°C) at 23 and 9 d before expected calving (BEC) in control (CTR; n = 16) and phase-shifted (PS; n = 16) cows¹

Time point and treatment	Mesor (LSM ± SE)	Amplitude ² (LSM ± SE)	Acrophase ³	R ² ⁴	P-value ⁵	AUC ⁶ (LSM ± SE)
23 d BEC						
CTR	39.04 ± 0.043 ^a	0.15 ± 0.025 ^a	2238 ± 15 ^a	0.30 ± 0.063 ^a	0.01	1,889.6 ± 11.70
PS	39.01 ± 0.078 ^a	0.12 ± 0.023 ^a	0322 ± 67 ^b	0.26 ± 0.064 ^a	0.1	1,888.6 ± 11.00
9 d BEC						
CTR	39.19 ± 0.076 ^b	0.23 ± 0.027 ^b	2150 ± 23 ^a	0.50 ± 0.062 ^b	<0.01	1,896.2 ± 20.23
PS	39.26 ± 0.041 ^b	0.14 ± 0.025 ^a	0422 ± 75 ^b	0.27 ± 0.067 ^a	0.1	1,896.6 ± 18.46

^{a,b}Values within a column with different superscripts are different ($P < 0.05$).

¹Control cows were exposed to 16 h of light and 8 h of dark. Phase-shifted cows were exposed to 16 h of light and 8 h of dark, and the photophase was shifted 6 h every 3 d.

²Difference between mesor and peak, calculated as half the difference between peak and trough.

³Time of the peak, expressed as time of the day (h) ± SE in minutes.

⁴Variability explained by the following model: temperature = mesor + amplitude × cos [2π × (hour – acrophase)/24].

⁵Calculated between the observed data and the fitted curve data.

⁶Area under the curve.

Feed Intake and Rest–Activity

Treatment did not affect DMI (16.52 ± 0.331 and 16.24 ± 0.339 kg/d for CTR and PS, respectively; $P = 0.56$; Supplemental Figure S1, <https://doi.org/10.3168/jds.2019-16980>). Feed intake was greater in the postpartum period than in the prepartum period in both treatments ($P < 0.01$; 15.70 ± 0.422 and 15.48 ± 0.423 kg/d for CTR and PS, respectively, in the prepartum period; 18.67 ± 0.558 and 18.321 ± 0.615 kg/d for CTR and PS, respectively, in the postpartum period).

Total resting time was greater during the prepartum period than during the postpartum period (Supplemental Figure S2, <https://doi.org/10.3168/jds.2019-16980>). The PS cows rested more each day than the

CTR cows (649.95 ± 11.87 and 700.14 ± 12.33 min for CTR and PS, respectively; $P < 0.01$; Table 4). Treatment by day analysis showed that the main differences in rest–activity were in the preparturition period (Supplemental Figure S2). Diurnal differences in total rest time were evident for both treatments, with dark phase (1600–0500 h) resting time being greater ($P < 0.01$) than light phase (0500–1600 h) resting time. Number of rest bouts was greater ($P < 0.01$) in the dark-phase recording than in the light-phase recording for both treatments. Number of rest bouts across a day was greater in PS cow (10.21 ± 0.38 and 11.76 ± 0.39 for CTR and PS, respectively; $P < 0.01$), with multiple treatment by day differences evident in the preparturition period (Supplemental Figure S3, <https://doi.org/10.3168/jds.2019-16980>).

Table 3. Cosine-fit analysis of melatonin (pg/mL) at 23 and 9 d before expected calving (BEC) and 5 d postparturition in control (CTR; n = 6) and phase-shifted (PS; n = 6) animals¹

Time point and treatment	Mesor (LSM ± SE)	Amplitude ² (LSM ± SE)	Acrophase ³	R ² ⁴	P-value ⁵	AUC ⁶ (LSM ± SE)
23 d BEC						
CTR	31.42 ± 3.053	32.66 ± 4.573	0010 ± 17	0.73 ± 0.025 ^a	<0.01	1,522.25 ± 174.687
PS	42.01 ± 8.568	35.10 ± 8.659	0112 ± 159	0.79 ± 0.031 ^a	<0.01	1,938.09 ± 322.403
9 d BEC						
CTR	31.71 ± 2.835	32.68 ± 5.768	0006 ± 25	0.78 ± 0.025 ^a	<0.01	1,511.34 ± 160.512
PS	42.47 ± 7.219	32.94 ± 6.546	0026 ± 170	0.55 ± 0.065 ^b	0.06	2,085.53 ± 384.394
5 d PP						
CTR	35.72 ± 4.572	41.18 ± 8.322	0000 ± 21	0.77 ± 0.018 ^a	<0.01	1,811.72 ± 247.085
PS	39.31 ± 7.845	34.64 ± 11.133	0241 ± 160	0.60 ± 0.112 ^b	0.08	1,936.81 ± 386.014

^{a,b}Values with different superscripts are different ($P < 0.05$) between treatments or time points.

¹Control cows were exposed to 16 h of light and 8 h of dark. Phase-shifted cows were exposed to 16 h of light and 8 h of dark, and the photophase was shifted 6 h every 3 d.

²Difference between mesor and peak, calculated as half the difference between peak and trough.

³Time of the peak, expressed as time of the day (h) ± SE in minutes.

⁴Variability explained by the following model: temperature = mesor + amplitude × cos [2π × (hour – acrophase)/24].

⁵Calculated between the observed data and the fitted curve data.

⁶Area under the curve.

Table 4. Daily light period (L^1) and dark period (D^2) resting bouts (RB), resting duration (RD), and resting time (RT) in control (CTR) and phase-shifted (PS) dairy cows³

Item	CTR	PS	SEM ⁴	<i>P</i> -value	CTR				PS			
					L	D	SEM ⁴	<i>P</i> -value	L	D	SEM ⁴	<i>P</i> -value
RB (no. of bouts)	10.21	11.76	0.395	<0.01	4.85	5.67	0.102	<0.01	5.72	6.52	0.164	<0.01
RD (min/bout)	67.65	61.83	1.482	<0.01	70.37	65.80	1.346	0.02	62.01	61.90	1.575	0.96
RT (min)	649.95	700.14	12.333	<0.01	306.31	341.44	4.593	<0.01	321.02	359.84	5.919	<0.01

¹Light phase data were collected at 1600 h and represent data recorded from 0500 to 1600 h (the primary dark phase in CTR cows).

²Dark phase data were collected at 0500 h and represent resting data recorded from 1600 to 0500 h (the primary light phase in CTR cows).

³Control cows were exposed to 16 h of light and 8 h of dark. Phase-shifted cows were exposed to 16 h of light and 8 h of dark, and the photophase was shifted 6 h every 3 d.

⁴Largest SE among treatments.

[//doi.org/10.3168/jds.2019-16980](https://doi.org/10.3168/jds.2019-16980)). The PS cows had fewer minutes of rest duration per bout than the CTR cows (67.65 ± 1.41 and 61.83 ± 1.48 min in CTR and PS, respectively; $P < 0.01$), and this effect was more pronounced in the preparturition treatment period (Supplemental Figure S4, <https://doi.org/10.3168/jds.2019-16980>).

Blood Glucose, Ketones, NEFA, and Insulin

Mean blood glucose concentrations were greater in the pre- versus postparturition sampling periods for

both CTR cows (78.52 ± 0.39 and 68.00 ± 0.75 mg/dL in pre- and postparturition, respectively) and PS cows (73.90 ± 0.51 and 62.81 ± 0.61 mg/dL in pre- and postparturition, respectively; Figure 4A). Treatment had an overall effect ($P < 0.05$) on blood glucose, with PS cows having lower glucose than CTR cows (73.06 ± 0.64 and 69.61 ± 0.61 mg/dL for CTR and PS, respectively). Treatment by week analysis revealed differences between treatments at 2 wk before and 2 wk after parturition. Analysis of blood glucose concentration in the hours immediately after parturition found lower concentrations in PS animals compared

Figure 3. Melatonin concentration (pg/mL) in periparturient dairy cows at d 23 before expected calving (BEC) and d 5 postparturition (PP). Graphs show melatonin concentration of plasma samples collected every 4 h from (top row) cows in the control treatment (exposed to 16 h of light and 8 h of dark; $n = 6$) at (A) d 23 BEC, (B) d 9 BEC, and (C) d 5 PP and (bottom row) cows in the phase-shifted treatment (exposed to 16 h of light and 8 h of dark, with the photophase shifted 6 h every 3 d; $n = 6$) at (D) d 23 BEC, (E) d 9 BEC, and (F) d 5 PP. Different shapes represent individual cows used in the study. Bars under graphs in panels A, B, and C represent the light (white) and dark (black) phases. Dashed bars under graphs in panels D, E, and F represent the light-dark phase shifts.

Figure 4. Blood glucose, BHB, and nonesterified fatty acid (NEFA) concentrations in the periparturient period in dairy cattle in the control treatment (exposed to 16 h of light and 8 h of dark; $n = 16$; black bars) or the phase-shifted treatment (exposed to 16 h of light and 8 h of dark, with the photophase shifted 6 h every 3 d; $n = 16$; dashed bars). (A) Weekly blood glucose concentration (mg/dL) in control and phase-shifted cows. (B) Blood glucose concentration (mg/dL) after parturition in a subset of control ($n = 6$) and phase-shifted ($n = 6$) cows. (C) Weekly blood BHB concentration (mmol/dL) in control and phase-shifted cows. (D) Weekly plasma NEFA concentration (mEq/L) in control and phase-shifted cows. Asterisks represent treatment by week or hour effect ($P < 0.05$). Data are presented as LSM $\pm$ SEM.

with CTR animals (Figure 4B). In both treatments, the peak of glucose concentration occurred between 1 and 2 h postparturition.

Physiological state had a significant effect on BHB and NEFA concentrations, with concentrations being greater in the postpartum period than in the prepartum period (Figure 4C and D, respectively). There was no effect of treatment on BHB or NEFA metabolites in blood at the 0600 h sampling. Cosinor analysis of plasma NEFA concentrations found no significant evidence for circadian rhythms at 23 d BEC, 9 d BEC, and 5 d PP in either treatment ($P > 0.05$; Figure 5). The NEFA concentration was greater in the morning than in the afternoon in both treatments ($P = 0.03$; 0.72 ± 0.09 and 0.48 ± 0.08 mEq/mL in CTR a.m. and p.m., respectively; 0.89 ± 0.08 and 0.69 ± 0.08

mEq/mL in PS a.m. and p.m., respectively; Figure 6). The PS cows tended ($P = 0.06$) to have greater NEFA concentrations during the first 9 d of lactation (0.59 ± 0.07 and 0.79 ± 0.06 for CTR and PS, respectively).

Treatment did not have an overall effect on insulin concentration, but on day of calving, CTR cows had greater insulin concentration than PS cows (Figure 7). Physiological state affected insulin concentration in both treatments ($P < 0.05$), with greater concentrations in the prepartum versus postpartum period.

Milk Yield and Composition

There was an overall treatment effect on milk yield to 60 DIM. The PS cows produced an average of 2.8 kg/d more milk than the CTR cows (Table 5; Figure

Figure 5. Nonesterified fatty acid (NEFA) plasma concentration (mEq/L) in periparturient dairy cows at d 23 and 9 before expected calving (BEC) and d 5 postparturition (PP). Graphs show NEFA concentration measured in plasma collected every 4 h across 48 h from (top row) cows in the control treatment (exposed to 16 h of light and 8 h of dark) at (A) d 23 BEC, (B) d 9 BEC, and (C) d 5 PP and (bottom row) cows in the phase-shifted treatment (exposed to 16 h of light and 8 h of dark, with the photophase shifted 6 h every 3 d) at (D) d 23 BEC, (E) d 9 BEC, and (F) d 5 PP. Bars under graphs in panels A, B, and C represent the light (white) and dark (black) phases. Dashed bars under graphs in panels D, E, and F represent the shifted photophase to which phase-shifted cows were exposed.

8). Although there were no differences in percent fat, protein, or lactose between treatments, daily yield of fat was 220 g/d greater in PS cows ($P = 0.02$). Milk urea nitrogen and SCC were not different between treatments.

DISCUSSION

Exposing dairy cows to chronic light–dark phase shifts beginning 5 wk BEC to parturition attenuated rhythms of 2 primary master clock outputs (body temperature and melatonin concentration). However, the experimental manipulation did not have detrimental effects on metabolism or milk production as hypothesized. In fact, the light–dark phase shift treatment increased milk yield up to 60 DIM, suggesting that a more constant daily milieu of hormones and metabolites may be conducive for lactogenesis.

Circadian Disruption Changed Rhythms of Body Temperature, Melatonin, and Resting Behavior

Core body temperature and melatonin serve as indicators of suprachiasmatic nuclei function (Mohawk et al., 2012). Differences in the circadian rhythms of

body temperature and melatonin between treatments suggested that the continuous light–dark cycle phase shifting affected the master clock. The acrophase of PS cows was shifted 5 to 6 h relative to CTR cows. Peak of core body temperature (acrophase) was approximately 2 h after initiation of the dark phase in the CTR treatment, which was similar to findings of others (Burfeind et al., 2011, 2014), whereas the time of core body temperature peak (acrophase) in PS cattle was 0322 and 0422 h for 23 and 9 d BEC, respectively. The shift in timing of PS temperature rhythms likely reflected adjustment of the circadian timing system to other reliable, regularly occurring cues, which in the present study was time of first daily exercise at 0430 h.

In CTR cows, a significant increase in core body temperature amplitude and mesor occurred between 3 and 1 wk BEC. The increase in mesor was likely related to thermogenesis associated with the progression of pregnancy and fetal growth (Schrader et al., 2009; Gamo et al., 2013). Similar to CTR cows, mesor of core body temperature of PS cows increased from 23 to 9 d BEC. Increase in core body temperature between 23 and 9 d BEC was accompanied by an increase in circadian rhythm amplitude in CTR cows. However, no increase in body temperature rhythm amplitude occurred be-

Figure 6. Morning (solid lines) and afternoon (dashed lines) blood nonesterified fatty acid (NEFA) concentrations during the first 9 DIM in 32 dairy cows in the control treatment (exposed to 16 h of light and 8 h of dark; ●) or the phase-shifted treatment (exposed to 16 h of light and 8 h of dark, with the photophase shifted 6 h every 3 d; ■) during the preparturient period. Data are presented as LSM.

tween 9 and 23 d BEC in PS cows. The amplitude reflects the degree of fluctuation of the circadian rhythm and is the difference between mesor and peak. There was a 0.5°C difference in peak and trough of core body temperature in CTR cows, whereas the difference in peak and trough of core body temperature range was 0.3°C for PS cows. This finding supports a dampening or attenuation of rhythms in the PS treatment relative to the CTR treatment.

Melatonin synthesis is inhibited by light, and changes in circadian rhythms of plasma melatonin reflect this response in CTR cows, with levels being almost undetectable during the light phase. The increased mesor and greater AUC of melatonin in PS versus CTR cows supports that PS cows had greater daily exposure to melatonin. The increase in milk yield due to exposure to a short-day photoperiod during the nonlactating dry period is also associated with greater circulating melatonin levels (Velasco et al., 2008; Dahl et al., 2012), suggesting that there may be a relationship between melatonin concentrations and milk yield. However, feeding melatonin to increase plasma concentrations did not increase milk yield (Lacasse et al., 2014). Thus, greater melatonin in PS alone may not account for the increased milk yield.

Cattle in both treatments had greater total resting time and rest durations per bout in the prepartum versus postpartum periods. This is consistent with findings of others, who reported that the reduction in resting time in the postpartum versus prepartum period is due to increased time spent eating and in the milking parlor (Kaufman et al., 2016). The PS treatment affected behavior, with differences between treatments in total rest time, number of rest bouts, and duration of rest bouts in the preparturition period. The increase in number of bouts but decrease in resting bout duration suggests less consolidation of resting in a 24-h rest-activity cycle. There were no differences between treatments in rest variables in the postparturition period, and so changes in PS treatment behavior were likely a direct effect of light-dark phase shifts and thus consistent with disruption of the circadian timing system preparturition.

Circadian Disruption During Preparturition Decreased Blood Glucose Pre- and Postparturition

Blood glucose decreased from pregnancy to lactation in both treatments, and these changes are consistent with previous findings (Reverchon et al., 2014;

Table 5. Daily milk production (d 1–60) and milk composition (d 5–9, 15, 22, 30, and 60) of cows exposed to the control (CTR) and phase-shifted (PS) treatments during the nonlactating period

Item	Treatment ¹		SEM ²	P-value
	CTR	PS		
Milk yield ³ (kg/d)	40.30	43.10	0.995	0.05
Milk fat yield (kg/d)	1.67	1.89	0.061	0.02
Milk fat (%)	4.70	4.89	0.127	0.30
Milk protein yield (kg/d)	1.12	1.19	0.043	0.24
Milk protein (%)	3.10	3.07	0.037	0.55
Milk lactose yield (kg/d)	1.70	1.83	0.066	0.18
Milk lactose (%)	4.62	4.63	0.036	0.87
MUN (mg/dL)	8.37	8.29	0.239	0.86
SCC (log ₁₀)	4.79	4.70	0.049	0.21

¹Control cows were exposed to 16 h of light and 8 h of dark. Phase-shifted cows were exposed to 16 h of light and 8 h of dark, and the photophase was shifted 6 h every 3 d.

²Largest SE among treatments.

³Daily milk yield from d 1 to 60 postparturition.

Hernández-Castellano et al., 2017; Ruoff et al., 2017). The decrease in circulating glucose concentrations likely reflects the increased demand for glucose by the lactating mammary gland, which increases 2.5-fold over the gravid uterus in late pregnancy (Bell and Bauman, 1997).

Blood glucose was reduced in PS cows relative to CTR cows during the last 2 wk of pregnancy and the

first 2 wk after calving. Lower blood glucose concentrations were not accompanied by increased mobilization of fat reserves based on the lack of differences in NEFA and BHB concentration between CTR and PS cows. In dairy cows, increased glucose requirements during late pregnancy and lactation are met in part by decreased uptake of glucose by adipose tissue, skeletal muscle, and other tissues through a temporary increase of peripheral insulin resistance (Bell and Bauman, 1997; De Koster and Opsomer, 2013). Thus, PS treatment may have diminished changes that lead to peripheral insulin resistance and allowed greater uptake of glucose from circulating pools by peripheral tissues before parturition. During lactation, the lower glucose concentration in PS cows may be due to their higher milk production and thus a greater requirement for glucose. Previous studies in humans and rodents showed that the disruption of the circadian timing system induced hyperglycemia and insulin resistance (Froy, 2007; Shi et al., 2013; Qian and Scheer, 2016). However, circadian disruption of nonpregnant ewes using a rotating shift-work model that inverted feeding time with a 12-h shift in light–dark phase resulted in hypoglycemia with no effect on insulin sensitivity (Varcoe et al., 2014). However, when the same research group exposed pregnant ewes to the shift-work paradigm, hyperglycemia and insulin resistance ensued in a subset of animals (Gatford et al.,

Figure 7. Plasma insulin in periparturient dairy cows. Multiparous dairy cows ($n = 32$) were assigned to either the control treatment (exposed to 16 h of light and 8 h of dark; $n = 16$; solid line) or the phase-shifted treatment (exposed to 16 h of light and 8 h of dark, with the photophase shifted 6 h every 3 d; $n = 16$; dashed line) and sampled on d 35, 21, and 2 before calving and d 0, 2, 5, 9, 15, and 21 postparturition. Data are presented as LSM $\pm$ SEM.

2019). In ruminants, the main source of glucose is from gluconeogenesis in the liver, whereas in monogastric animals circulating glucose is reflected in dietary intake. The contrasting findings in glycemic index suggest that monogastrics and ruminants respond to circadian system disruption differently. However, tissue-specific deletion of *BMAL1* core clock gene in the liver resulted in disturbances in glucose metabolism associated with hypoglycemia (Lamia et al., 2008), and so the resulting changes in glucose may depend on the model system used to determine the role of the circadian timing system in the regulation of physiology.

NEFA Did Not Show Circadian Rhythms in Blood but Showed Diurnal Variations in Both Treatments in Early Lactation

Temporal analysis of NEFA blood concentrations did not reveal circadian rhythms at any of the 3 sampling periods (23 d BEC, 9 d BEC, and 5 d PP). However, differences in NEFA concentrations were found in approximately 12-h intervals of a.m. and p.m. samples taken the first 9 DIM. Nutrition influences concentration of blood metabolites, with eating pattern (times and duration of intake, rumination, and fasting) affecting circulating nutrients. Lack of a circadian rhythm in NEFA has also been reported for mid-lactation cows despite evidence for rhythms in plasma glucose, BUN, and insulin (Niu et al., 2017; Niu and Harvatine, 2018).

However, when timing of feed dispersal was limited, circadian rhythms of NEFA were revealed in lactating dairy cows and were affected by time of day cows were fed (Niu et al., 2014). A study of mid-lactation dairy cows (Bitman et al., 1990) found spikes in circulating free fatty acids every 2 to 3 h, which was correlated with balancing between the entry and removal of lipids from the blood stream. Together, these findings suggest that circulating NEFA concentrations may primarily reflect demand and feeding behavior rather than be driven by circadian clocks. Further studies are needed to better understand blood NEFA regulation.

Circadian Disruption During Preparturition Increased Milk and Milk Fat Yields

Milk yield is determined by the number and metabolic activity of mammary epithelial cells. The mammary gland undergoes significant remodeling during the nonlactating dry period, including the replenishment of secretory epithelium (Sordillo and Nickerson, 1988; Capuco et al., 1997; De Vries et al., 2010). Varying exposures to differing management and environmental factors, such as photoperiod and heat stress, during the nonlactating dry period affect the number and metabolic activity of the secretory epithelium and thus the milk production capacity of the cow (Dahl, 2008; do Amaral et al., 2009; Mabjeesh et al., 2013). The magnitude of increase in milk yield in the PS

Figure 8. Milk yield of dairy cows exposed to the control treatment (exposed to 16 h of light and 8 h of dark; solid line) or the phase-shifted treatment (exposed to 16 h of light and 8 h of dark, with the photophase shifted 6 h every 3 d; dashed line) from 5 wk prepartum to 3 d before parturition. Data were recorded up to 60 DIM and are expressed as LSM $\pm$ SEM.

treatment versus the CTR treatment was similar to what is reported for cattle exposed to short-day versus long-day photoperiod during the nonlactating dry period (Dahl, 2008). A short-day photoperiod during the nonlactating dry period increased expression of genes that regulate proliferation and differentiation of cells in the mammary gland (Bentley et al., 2015); it also attenuated rhythms of expression of core clock genes in mammary tissue of goats (Casey et al., 2018). Our work in cells and rodents supports a role for the circadian clocks in regulation of mammary epithelial growth and differentiation (Casey et al., 2016). Thus, it is possible that attenuation of circadian rhythms in the PS treatment affected mammary-specific clocks in a manner that increased proliferation of the mammary tissue preparturition and is reflected as an increase in milk yield during lactation.

Feed intake was reduced around parturition and recovered to levels greater than the prepartum period over the first 2 wk after calving in both treatments. This pattern of feed intake was expected and consistent with previous studies of transition-period cows (Fairfield et al., 2007; Shi et al., 2019). Daily feed intake was not different between CTR and PS cows across the pre- and postpartum periods. However, due to constraints in animal housing management, individual feed intake measures were limited to 35 d BEC to 15 DIM, when animals were in tiestalls. Treatments that increase milk production find that milk yield increase often precedes changes in feed intake (Peters et al., 1978; Sechen et al., 1989; McDowell, 1991). However, in the longer term, treatment-induced higher milk production needs to be supported by greater feed intake of dairy cattle. Therefore, we expect that the greater milk production to 60 DIM of PS versus CTR cows was supported by greater feed intake when animals entered the freestall barn.

CONCLUSIONS

We hypothesized that disruption of the circadian timing system during the preparturition period would affect nutrient metabolism and reduce milk production. Findings confirmed that the chronic light-dark phase shift treatment affected the central clock outputs, resting behavior, glucose metabolism, and milk production; however, results were contrary to our hypothesis. Analysis of body temperature and melatonin revealed attenuation of these circadian rhythms in PS cows. Further studies need to be performed to elucidate the effect of chronic light-dark phase shifts on tissue-specific clocks and metabolism, although our findings suggest that attenuation of rhythms likely results in a more constant milieu of hormones and metabolites.

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