Milk components, nutrition, and circadian rhythms of cows

Isaac J. Salfer, Ph.D.

Assistant Professor of Dairy Nutritional Physiology
University of Minnesota

Take-Home Message

- Dairy cows have daily rhythms of feed intake and milk synthesis. Maximal feed intake occurs in the morning and afternoon, maximal milk yield occurs in the morning, and maximal milk components occurs in the evening
- Matching nutrient intake with circadian rhythms of the mammary gland and other metabolic tissues may help improve feed efficiency in dairy cows
- 'Chronotherapeutic' strategies to treating cows at optimal times based on their circadian rhythm may improve recovery from metabolic or infectious disease
- Automated feeding and milking systems create greater opportunities to implement time-based feeding strategies

Introduction

Throughout nature, biological rhythms allow organisms to coordinate their physiology and behavior with daily or yearly changes in their environment. Occurring at time scales ranging from days (circadian rhythms) to years (circannual rhythms), they are an integral component of an animal's physiology, and are driven by 'molecular clocks" within every cell of the body. While daily changes in dairy cow production and behavior have long been appreciated by dairy farmers, we are only beginning to understand the impact that circadian rhythms have in regulating dairy cow metabolism. Recent work has characterized biological rhythms of feed intake, milk production, and systemic metabolism in cattle. The daily pattern of feed intake results in differences in absorbed nutrients across the day. Furthermore, the mammary gland has a daily pattern that may or may not be synchronized with the daily pattern of intake. Improving the synchrony between absorbed nutrients and mammary gland rhythms provides opportunities to improve the efficiency of milk production.

Circadian Rhythms

The primary role of circadian rhythms is to maximize survival of an organism and their offspring by allowing them to predict and prepare for regularly-occurring environmental changes such as food availability/quality, risk of predators, and opportunities for sexual reproduction. Circadian rhythms are generated within individual cells of the body through a contingent of clock proteins that cycle over 24 h and act as the 'gears' of the clock (Ko and Takahashi, 2006). The timing of the expression of these clock proteins can be 'set' by light and nutrient availability, in a process called *entrainment*. Furthermore, the clock proteins regulate the expression of a host of *clock-controlled genes*, generating rhythms of physiological and behavioral outputs. The primary site of entrainment by the light-dark cycle occurs in the hypothalamus, which then communicates the time of day to other tissues through neurotransmitters and hormones such as melatonin and prolactin (Honma, 2018). However, rhythms in peripheral tissues – particularly metabolically important tissues like the liver, adipose tissue and pancreas - can be entrained by food intake independently of the light dark cycle (Mistlberger, 2020). Desynchronization of light and food intake has been implicated as a cause of several metabolic disorders in humans including obesity, high blood pressure and heart disease (Golombek et al., 2013). This desynchronization is one of the driving causes of disease states associated with chronic jet lag and shift work disorder. Recently, ‘intermittent fasting’ has become a popular dietary trend in humans, where all food is consumed during an 8 to 12 h window within the day. This dietary regimen has been associated with positive metabolic outcomes, with the principle being that it improves coordination of circadian rhythms of food intake and the light-dark cycle.

It is important to note that a *true* circadian rhythm must persist in constant environmental conditions (i.e., in the absence of changes in the light-dark cycle, feed availability, or any other cue that can indicate time of day). These constant conditions are impossible to achieve outside of very controlled laboratory settings. When a rhythm exists in the *presence* of changes in the light-dark cycle or feed availability, the appropriate term is *daily* or *diurnal* rhythm. These terms are used to distinguish between rhythms that are known to be endogenous in the animal (circadian), versus those that may be endogenous, may be a reaction to external cycles, or likely are a combination of both (daily/diurnal). Often, the terms are used interchangeably, but the difference is important for understanding animal physiology, and for knowing how management can influence rhythmic behavior.

Daily Rhythms and Feeding

Dairy cows typically spend 3 to 5 hours per day eating, and consume majority of their feed in 8 to 14 meals across the day (Johnston and DeVries, 2018). Naturally, the feeding pattern of cows on pasture is nearly crepuscular, with the highest frequency and size of meals occurring in the early morning (~ 6 AM to 10 AM), and mid-afternoon (~3 PM to 5 PM) with minimal feed intake overnight (Albright, 1993). It is likely that this pattern of intake evolved to help protect wild cattle against nocturnal predators such as wolves and coyotes, and to potentially help them select for higher-energy forage. In confinement systems where cows are fed a TMR, the daily pattern of feed intake is modulated by management decisions like feeding and milking time, with the delivery of fresh feed and returning to the parlor after milking both stimulating feed intake (DeVries and von Keyserlingk, 2005).

Despite management influences, the underlying daily pattern of feed intake persists even in TMR-fed cattle. We have examined the effects of putting lactating cows under an 8 h fast either overnight, when intake is naturally low, or during the middle of the afternoon, when intake is generally high (Salfer and Harvatine, 2020). Even after adapting cows to this feeding/fasting schedule for 14 d, the cows that were fasted during the afternoon had a much stronger response to fasting, with a 3x greater increase in fat mobilization at the end of the fasting period than cows fasted at night. We also monitored the feeding behavior of these cows using an automated feed weight-recording system. While cows on both treatments at their biggest meals after feeding, the day-fasted cows ate a much higher percentage of their diet in the 2 h following the fasting time than the night-fasted cows, suggesting a greater hunger response at this time (Figure 1). The effect of night feeding on the rate of feed intake occurs even when cows are not fasted for any period during the day, albeit to a lesser extent (Niu et al., 2014).

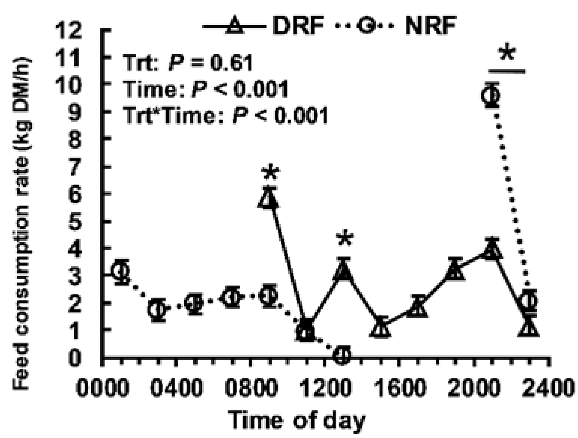

Figure 1. The impact of day-restricted feeding (DRF) versus night-restricted feeding on the daily pattern of feed intake. Cows on DRF were fasted for 8 h from 11 PM to 7 AM and cows on NRF were fasted for 8 h from 11 AM to 7 PM. Asterisk (*) denotes differences in rate of feed intake at each time point (Salfer and Harvatine, 2020).

While feeding a TMR is designed to ensure that cows have steady feed intake, the daily pattern of feed intake and feed sorting lead to variations in the amount of nutrients entering into the rumen across the day. This reality stands in contrast with many nutritional models which assume steady-state rumen conditions (Fox et al., 2004). We have observed daily variations in rumen pool sizes of dry matter, starch and NDF, with all reaching their lowest levels prior to feeding (Ying et al., 2015). Moreover, in the same experiment, authors observed clear daily patterns of ruminal ammonia, and VFA concentrations, with ammonia peaking in the morning (~7 AM) and VFA peaking in the evening (~7 PM). Rumen pH also follows a daily pattern, that typically peaks in immediately before feeding time and reaching a minimum 10 to 12 h later (Krause et al., 2002; Yang and Beauchemin, 2006). Methane production from the rumen follows a daily pattern as well, which closely resembles of pattern of VFA concentration (Brask et al., 2015).

The daily pattern of intake results in daily changes in the abundance of microbial species within the rumen. Total bacterial abundance increases after feeding as more substrate is available. We recently observed that total bacteria, total protozoa, total fungi, and several well-characterized microbial species follow distinct daily patterns of relative abundance in the rumen (Salfer et al., 2021). Using 16S amplicon sequencing, also determined that overall microbial diversity varied across the day, with greatest diversity occurring shortly after feeding (Gomez & Salfer, 2021). In this experiment, we also noticed an interaction of diet and time, with lower-fiber diets causing a smaller daily variation in diversity. Finally, a daily pattern of fecal nutrient composition has been reported in the literature. Maulfair et al. (2011) discovered variation in fecal particle size, NDF, iNDF and starch across the day. This result implies that there is a daily pattern of nutrient absorption across the day.

Daily Rhythms and Nutrient Metabolism

While circadian rhythms regulate feed intake to help maximize the quality of feed consumed while avoiding activity when predators are active, they also affect the timing of metabolic pathways within tissues. This allows the animal to ensure that energy and structural metabolites are available at the times when they are most needed. Typically, catabolism is synchronized to the active period of the day, so fuels can be burned to provide energy needed for movement. Alternatively, anabolism typically occurs overnight to store energy for later use (Green et al., 2008). This circadian coordination of metabolism prevents inefficiency related to futile cycling of nutrients, ensuring that storage molecules are not being built and degraded simultaneously.

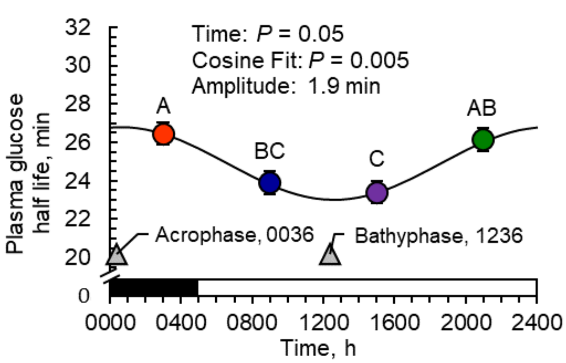

Figure 2. Comparison of glucose half-life at 4 time points across the day. Data is presented as mean glucose half-life at each time point with a fitted 24-h rhythm. Effect of time and cosine fit, time at peak (acrophase) and time at nadir (bathyphase) are denoted. Maximum insulin sensitivity occurs at 12:36 PM, the point where glucose half-life is lowest.

Several circulating metabolites follow daily rhythms in dairy cows. Giannetto and Piccione (2009) observed rhythms of glucose, hemoglobin, creatinine, urea nitrogen (PUN), cholesterol, phospholipids, non-esterified fatty acids (NEFA), phosphorus, and magnesium in Italian Brown Cattle. These observations agree with results from our lab in modern Holsteins which demonstrated rhythms of glucose, insulin, NEFA and PUN (Salfer and Harvatine, 2020). Feeding time influences these rhythms, with night-feeding shifting the peaks of glucose, insulin and PUN by approximately 12 h (Salfer and Harvatine, 2020). Furthermore, body temperature follows a consistent daily rhythm that peaks overnight (Bitman et al., 1984). We recently observed that the daily rhythm of body temperature is dependent on season, with the rhythm being much more robust in the summer (Kamau et al., *Unpublished*).

We looked at the impact of time of day on insulin-stimulated glucose uptake by conducting glucose-tolerance tests at 4 evenly-spaced intervals across the day. In this experiment, insulin sensitivity followed a very distinct daily rhythm, with maximal insulin sensitivity occurring at just after noon (Salfer et al., 2020). Notably, in this experiment, cows were not influenced by the daily pattern of feed intake, because they were feed for stable feed intake by delivering fresh feed every 2 h. These results indicate a potential benefit to time-based therapies to fresh cows. For example, providing oral propylene glycol or intravenous glucose may be more effective at relieving ketosis if administered around noon versus in the morning. We plan to continue work looking at 'chronotherapeutics' in dairy cows to determine if there are optimal times for certain therapies. Recently, compelling data suggests that immune function of dairy cows follows a daily rhythm, providing additional support to suggest that there may be beneficial effects of treating cows with consideration to their circadian rhythm (Alhussien and Dang, 2018).

Long-term circadian disruption through chronic shifts of the light-dark cycle can have major impacts on metabolism in dairy cows. McCabe et al. (2021) observed that shifting the light cycle by 6 h every 3 d in the prepartum period caused decreased insulin sensitivity during, which was accompanied with decreased mammary development and decreased milk production in the subsequent lactation. The same laboratory group also demonstrated that chronic phase shift decreased circadian rhythms of body temperature and melatonin (Suarez-Trujillo et al., 2020). Moreover, Wagner et al. (2021) discovered that changes in the circadian rhythm of body temperature can be an accurate indicator of negative health events in cows.

Daily Rhythms and Milk Composition

Milk production follows a daily rhythm with total milk volume typically peaking in the morning, with milk fat and protein percentage peaking in the evening (Harvatine, 2012). This adaptation likely developed in wild ruminants to provide nursing offspring with nutrient-dense milk at night when activity of the dam and the calf is reduced. Beyond traditional milk components, other molecules oscillate across the day within milk. For example, melatonin concentration of milk is greatest overnight (Asher and Sassone-Corsi, 2015). Data from other species suggests that this mechanism is designed to help synchronize the daily rhythms of the dam and offspring (Recio et al., 1997). Recently, Teng et al. (2021) used metabolomics and lipidomics to determine that 36 different metabolites, including immune markers (IL-6, IFN-γ, HSP70, HSP90), adrenaline, and various intermediates of the urea cycle and fatty acid fatty acid synthesis differ between day-milk and night milk.

Feeding time and feeding frequency can have major effects on the daily pattern of milk synthesis. Rottman et al. (2014) examined the effects of 4x/d feeding on the daily rhythm of milk yield, and found that the daily pattern of milk fat and protein concentrations were attenuated compared to 1x/d feeding. During our previously described day- versus night-fasting experiment, shifting the time of feed delivery to the evening inverted the rhythms of milk synthesis, causing a peak in milk volume in the evening and a peak of milk components in the morning (Salfer and Harvatine, 2020). In a separate experiment, we used mammary biopsies to compare daily patterns of the expression of 'clock' genes during day-feeding versus night-restricted feeding (Salfer and Harvatine, 2018). We observed that rhythms several key circadian transcription factors were shifted by feeding time, suggesting that changes in the rhythms of milk synthesis due to feeding may be at least partially modulated by changes in the cellular circadian clock of the mammary gland.

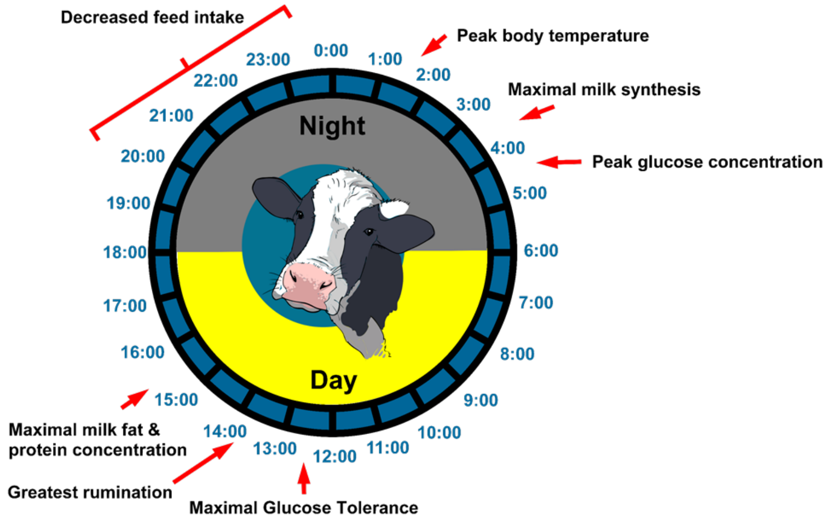

Figure 3. A clock illustrating a cow's circadian cycle of production and physiology across the day.

In addition to the timing of TMR delivery, the timing of absorption of individual nutrients can impact milk production. We conducted a series of infusion experiments to look at the timing of fatty acids, amino acids, and acetate on daily rhythms of milk production and circulating metabolite concentrations. In each experiment, these nutrients were infused for either continuously for 24 h/d, for 8 h during the day (9 AM to 5 PM), or for 8 h overnight (9 PM to 5 AM). Interestingly, the time of protein availability to the small intestine impacted overall milk fat yield, with day-infusion increasing milk yield compared to day-infusion and continuous infusion. This result implies that perhaps feeding a high protein diet in the morning and a low-protein diet in the evening may have beneficial effects on milk production (Salfer et al., 2019). This change in total daily milk fat yield, was accompanied by an increase in the robustness of the daily rhythm of milk fat yield. The time of abomasal infusions of fatty acids affected milk fat percentage, with night-infusion causing a 0.24 percentage point increase than continuous infusion (Salfer and Harvatine, 2019). However, this increase in milk fat percentage was offset by a decrease in total milk yield, resulting in no difference in milk fat yield. The timing of ruminal acetate infusion had minimal effects on daily milk production, but impacted the daily rhythms of milk yield and milk components (Matamoros et al., 2020). We plan to conduct future research with more applied approaches to examine the effects of feeding multiple diets differing in nutrient composition across the day.

Circadian Rhythms and Precision Feeding

The implementation of automated feeding and milking technologies provides additional opportunities to take advantage of 'circadian' feeding and management strategies. Automated milking systems (AMS) are becoming increasingly popular, especially in the upper Midwest, as labor challenges increase. The economic viability of AMS is maximized when greatest throughput through the robotic parlor is achieved (Salfer et al., 2017). Hogeveen et al. (2001) observed a decline in robot visits in the early morning, and another smaller decline in the early afternoon. In contrast, we examined the distribution of robot visits in herds using an automated milking system, and observed that the frequency of cows visiting the robot closely matched the daily pattern of feed intake, with cow visits peaking at around 6 AM and about 4 PM (Salfer et al. *Unpublished*). We are continuing to research factors that influence when cows prefer to be milked based on their circadian rhythms. Improving our understanding of the circadian rhythms of cow behavior may allow for interventions to maximize the flow of cows through the AMS.

In addition to AMS, there is some increased interest in implementation of automated feeding systems. These systems provide the opportunity to greatly increase feeding frequency, which can result in more stable rumen fermentation (DeVries et al., 2005). Furthermore, both automated milking and feeding systems also provide opportunities to more practically apply time-based feeding strategies. Increased utilization of these technologies provides exciting opportunities to feed multiple diets across the day to better match nutrient intake with the circadian rhythm of the mammary gland.

References

Albright, J.L. 1993. Feeding behavior of dairy cattle. J. Dairy Sci. 76:485–498. doi:10.3168/jds.S0022-0302(93)77369-5.

Alhussien, M.N., and A.K. Dang. 2018. Diurnal rhythm in the counts and types of milk somatic cells, neutrophil phagocytosis and plasma cortisol levels in Karan Fries cows during different seasons and parity. Biol. Rhythm Res. 49:187–199. doi:10.1080/09291016.2017.1350442.

Asher, G., and P. Sassone-Corsi. 2015. Time for food: The intimate interplay between nutrition, metabolism, and the circadian clock. Cell 161:84–92. doi:10.1016/j.cell.2015.03.015.

Bitman, J., a Lefcourt, D.L. Wood, and B. Stroud. 1984. Circadian and ultradian temperature rhythms of lactating dairy cows.. J. Dairy Sci. 67:1014–23. doi:10.3168/jds.S0022-0302(84)81400-9.

Brask, M., M.R. Weisbjerg, A.L.F. Hellwing, A. Bannink, and P. Lund. 2015. Methane production and diurnal variation measured in dairy cows and predicted from fermentation pattern and nutrient or carbon flow. Animal 9:1795–1806. doi:10.1017/S1751731115001184.

DeVries, T.J., and M.A.G. von Keyserlingk. 2005. Time of feed delivery affects the feeding and lying patterns of dairy cows. J. Dairy Sci. 88:625–631. doi:10.3168/jds.S0022-0302(05)72726-0.

DeVries, T.J., M.A.G. von Keyserlingk, and K.A. Beauchemin. 2005. Frequency of feed delivery affects the behavior of lactating dairy cows. J. Dairy Sci. 88:3553–3562. doi:10.3168/jds.S0022-0302(05)73040-X.

Fox, D.G., L.O. Tedeschi, T.P. Tylutki, J.B. Russell, M.E. Van Amburgh, L.E. Chase, A.N. Pell, and T.R. Overton. 2004. The Cornell Net Carbohydrate and Protein System model for evaluating herd nutrition and nutrient excretion. Anim. Feed Sci. Technol. 112:29–78. doi:10.1016/j.anifeedsci.2003.10.006.

Giannetto, C., and G. Piccione. 2009. Daily rhythms of 25 physiological variables in Bos taurus maintained under natural conditions. J. Appl. Biomed. 7:55–61. doi:doi.org/10.32725/jab.2009.005.

Golombek, D.A., L.P. Casiraghi, P. V. Agostino, N. Paladino, J.M. Duhart, S.A. Plano, and J.J. Chiesa. 2013. The times they're a-changing: Effects of circadian desynchronization on physiology and disease. J. Physiol. Paris 107:310–322. doi:10.1016/j.jphysparis.2013.03.007.

Green, C.B., J.S. Takahashi, and J. Bass. 2008. The meter of metabolism. Cell 134:728–742. doi:10.1016/j.cell.2008.08.022.

Harvatine, K.J. 2012. Circadian patterns of feed intake and milk composition variability 43–55.

Hogeveen, H., W. Ouweltjes, C.J.A.. de Koning, and K. Stelwagen. 2001. Milking interval, milk production and milk flow-rate in an automatic milking system. Livest. Prod. Sci. 72:157–167. doi:10.1016/S0301-6226(01)00276-7.

Honma, S. 2018. The mammalian circadian system: a hierarchical multi-oscillator structure for generating circadian rhythm. J. Physiol. Sci. 2018 683 68:207–219. doi:10.1007/S12576-018-0597-5.

Johnston, C., and T.J. DeVries. 2018. Short communication: Associations of feeding behavior and milk production in dairy cows. J. Dairy Sci. 101:3367–3373. doi:10.3168/jds.2017-13743.

Ko, C.H., and J.S. Takahashi. 2006. Molecular components of the mammalian circadian clock.. Hum. Mol. Genet. 15 Spec No:R271-7. doi:10.1093/hmg/ddl207.

Krause, K.M., D.K. Combs, and K.A. Beauchemin. 2002. Effects of forage particle size and grain fermentability in midlactation cows. II. Ruminal pH and chewing activity. J. Dairy Sci. 85:1947–1957. doi:10.3168/jds.S0022-0302(02)74271-9.

Matamoros, C., I. Salfer, and K. Harvatine. 2020. Effect of acetate absorption on the daily rhythm of milk synthesis and plasma hormones and metabolites in dairy cows.

Maulfair, D.D., M. Fustini, and A.J. Heinrichs. 2011. Effect of varying total mixed ration particle size on rumen digesta and fecal particle size and digestibility in lactating dairy cows. J. Dairy Sci. 94:3527–3536. doi:10.3168/jds.2010-3718.

McCabe, C.J., A. Suarez-Trujillo, K.A. Teeple, T.M. Casey, and J.P. Boerman. 2021. Chronic prepartum light-dark phase shifts in cattle disrupt circadian clocks, decrease insulin sensitivity and mammary development, and are associated with lower milk yield through 60 days postpartum. J. Dairy Sci. 104:2422–2437. doi:10.3168/jds.2020-19250.

Mistlberger, R.E. 2020. Food as circadian time cue for appetitive behavior. F1000Research 9:61. doi:10.12688/f1000research.20829.1.

Niu, M., Y. Ying, P.A. Bartell, and K.J. Harvatine. 2014. 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. J. Dairy Sci. 97:7764–7776. doi:10.3168/jds.2014-8261.

Recio, J., J.M. Miguez, O.M. Buxton, and E. Challet. 1997. Synchronizing circadian rhythms in early infancy. Med. Hypotheses 49:229–234. doi:https://doi.org/10.1016/S0306-9877(97)90207-3.

Rottman, L.W., Y. Ying, K. Zhou, P.A. Bartell, and K.J. Harvatine. 2014. The daily rhythm of milk synthesis is dependent on the timing of feed intake in dairy cows. Physiol. Rep. 2:1–12. doi:10.14814/phy2.12049.

Salfer, I., and K. Harvatine. 2019. 313 Effect of fatty acid absorption on the daily rhythms of milk synthesis and plasma hormones and metabolites in dairy cows. J. Dairy Sci. 102:369.

Salfer, I., C. Matamoros, R. Bomberger, and K. Harvatine. 2019. 311 Effect of protein absorption on the daily rhythms of milk synthesis and plasma hormones and metabolites in dairy cows. J. Dairy Sci. 102:269.

Salfer, I., C. Matamoros, and K. Harvatine. 2020. Glucose tolerance appears to follow a daily rhythm in dairy cows. Page in American Dairy Science Association Annual Meeting.

Salfer, I.J., C.E. Crawford, L.W. Rottman, and K.J. Harvatine. 2021. The effects of feeding rations that differ in neutral detergent fiber and starch within a day on the daily pattern of key rumen microbial populations. JDS Commun.. doi:10.3168/jds.2021-0099.

Salfer, I.J., and K.J. Harvatine. 2018. 480 The effect of night restricted feeding on the molecular circadian clock of the mammary gland. J. Dairy Sci. 101:405.

Salfer, I.J., and K.J. Harvatine. 2020. Night-restricted feeding of dairy cows modifies daily rhythms of feed intake, milk synthesis and plasma metabolites compared with day-restricted feeding. Br. J. Nutr. 123:849–858. doi:10.1017/S0007114520000057.

Salfer, J.A., K. Minegishi, W. Lazarus, E. Berning, and M.I. Endres. 2017. Finances and returns for robotic dairies. J. Dairy Sci. 100:7739–7749. doi:10.3168/jds.2016-11976.

Suarez-Trujillo, A., G. Wernert, H. Sun, T.S. Steckler, K. Huff, S. Cummings, J. Franco, R.N. Klopp, J.R. Townsend, M. Grott, J.S. Johnson, K. Plaut, J.P. Boerman, and T.M. Casey. 2020. 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. J. Dairy Sci. 103:2784–2799. doi:10.3168/jds.2019-16980.

Teng, Z.W., G.Q. Yang, L.F. Wang, T. Fu, H.X. Lian, Y. Sun, L.Q. Han, L.Y. Zhang, and T.Y. Gao. 2021. Effects of the circadian rhythm on milk composition in dairy cows: Does day milk differ from night milk?. J. Dairy Sci. 104:8301–8313. doi:10.3168/jds.2020-19679.

Wagner, N., M.M. Mialon, K.H. Sloth, R. Lardy, D. Ledoux, M. Silberberg, A. de Boyer des Roches, and I. Veissier. 2021. Detection of changes in the circadian rhythm of cattle in relation to disease, stress, and reproductive events. Methods 186:14–21. doi:10.1016/j.ymeth.2020.09.003.

Yang, W.Z., and K.A. Beauchemin. 2006. Effects of physically effective fiber on chewing activity and ruminal pH of dairy cows fed diets based on barley silage. J. Dairy Sci. 89:217–228. doi:10.3168/jds.S0022-0302(06)72086-0.

Ying, Y., L.W. Rottman, C. Crawford, P.A. Bartell, and K.J. Harvatine. 2015. The effects of feeding rations that differ in neutral detergent fiber and starch concentration within a day on rumen digesta nutrient concentration, pH, and fermentation products in dairy cows. J. Dairy Sci. 98:4673–4684. doi:10.3168/jds.2014-8873.