Circadian Patterns of Feed Intake and Milk Composition Variability

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Summary

The dairy cow has a well-recognized natural daily pattern of feed intake and milk synthesis, but regulation of these circadian rhythms has not been well described in the literature or well considered in current dairy management. Even when a total mixed ration **(TMR)** is fed, ruminal fermentation varies due to circadian variation in feed consumption, resulting in more fermentable substrate in the rumen during the high intake period of the day. We propose that "circadian feeding and management strategies" can improve milk yield and efficiency by stabilizing rumen fermentation and temporally matching nutrient absorption and mammary requirements, thereby altering the interactions between central and peripheral circadian clocks. Although we are early in our research efforts, we have demonstrated that there is a daily pattern to milk fat and protein synthesis that is dependent on the timing of nutrient intake.

Introduction

Circadian rhythms are changes that occur over the day and repeat every day. It is easy to appreciate the importance of these rhythms when you think of your own activity over the day and how you feel when your schedule is disrupted. We don't often think about it, but it is easy to observe changes in cow behavior over the day simply by counting the number of cows eating, chewing their cud, or lying down at different times of the day. Regulation of these daily rhythms has a rich history that has been advanced greatly by recent discoveries of the molecular components of the circadian system at the cellular level. Although a timekeeping mechanism in the brain has been known for many years, recent discoveries have clearly described circadian time-keeping mechanisms in peripheral tissues that are responsive to environmental factors, such as timing of food availability. Interestingly, the timing of food intake can alter the synchronization between the central master timekeeper and peripheral clocks, and desynchronization increases the development of numerous disorders, including obesity, insulin resistance, and metabolic diseases (Takahashi et al., 2008). Some management strategies on dairy farms may desynchronize the central and mammary circadian timekeepers and/or desynchronize nutrient absorption and mammary synthesis of milk, resulting in reduced milk yield and efficiency. Recent evidence supports expression of circadian regulators in human and rat mammary tissue; however, the circadian pattern of milk synthesis and circadian regulation in the mammary gland of the cow has not been specifically investigated. Ruminants consume numerous meals over the course of the day, and the rumen creates a more constant rate of absorption of nutrients than non-ruminants, although a significant circadian rhythm of absorption is still observed, especially in dairy cows fed highly fermentable diets. Therefore, the timing of lighting, feeding, and milking times are expected to change the daily rhythms of the rumen and mammary gland and are important

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considerations in dairy management that interact to determine the rate of milk synthesis (Figure 1).

Biological Clocks

Endogenous circadian clocks are found in most tissues of virtually all organisms. Clocks enable each organism to synchronize their behaviors and physiological processes with changes in the external environment. Clocks also permit the coordination of internal activities in one organ of an organism with complementary processes that occur in a different organ, all within the same animal. In mammals, the dominant circadian pacemaker is located in the Suprachiasmatic Nucleus **(SCN)** of the hypothalamus, which organizes the temporal activity of peripheral clocks located throughout the different organs of the body by regulating a series of neural and hormonal signals (See review Dibner et al., 2010).

Many physiological variables in the cow follow a circadian pattern. Giannetto and Piccione (2009) reported 12 out of 25 physiological variables examined, including locomotor activity, respiratory rate, and body temperature, followed a circadian rhythm in the cow. Additionally, plasma glucose, non-esterified fatty acids **(NEFA)**, urea, total cholesterol, total lipids, and insulin also demonstrated significant circadian rhythms in the cow (Lefcourt et al., 1999; Giannetto and Picciano, 2009).

Mammalian Circadian System

The SCN in the brain serves as the master pacemaker in mammalian brains. However, recent evidence overwhelmingly has identified endogenous biological clocks in peripheral tissues, including liver, mammary, and adipose tissue (See reviews Dibner et al., 2010; Doherty and Kay, 2010; Asher and Schibler, 2011). Peripheral rhythms are normally synchronized by the SCN through its rhythmic regulation of neural or hormonal signals, and these include the regulation of prolactin, glucocorticoids, melatonin, insulin-like growth factor **(IGF-1)**, thyroid hormones, and growth hormone, thereby promoting rhythmic changes in physiological function of peripheral tissues.

Recent evidence demonstrates that the time of day in which food is available also can entrain circadian rhythms in a variety of animals without affecting activity in the SCN. When the SCN is removed, mammals will typically exhibit more robust entrainment to feeding cycles, and the core molecular components of the intracellular circadian clock have been shown to serve as biological "sensors" of metabolic status (Rutter et al., 2002). Entrainment of peripheral tissue by feeding is key to the effect of "night shift work" in mice and humans where eating outside the natural pattern desynchronizes central and peripheral clocks and increased the incidence of obesity and metabolic disorders (See review by Bass and Takahashi, 2010).

Evidence of circadian regulation of milk synthesis in the dairy cow

Dairy farmers commonly recognize that morning and evening milking differ in milk yield and composition. Gilbert et al. (1972) reported 1.4 lb 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. Milk yield at individual milkings was extensively modeled with the development of AM/PM DHIA sampling methods in the 1960's where only one milking per month is sampled (Everett and Wadell, 1970a). The differences between morning and evening milkings were found to be dependent on the milking interval and cow days in milk with up to a 3.77 lb more milk at the morning milking in early lactation Holstein cows (Everett and Wadell, 1970b). More recently, Quist et al. (2008) conducted a large 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 repeated daily pattern over the 5 days sampled in herds that milked 2 and 3 x/day. Surprisingly, milk yield was highest and milk fat lowest in the AM milking of herds milked 2 x/day, but milk yield and milk fat concentration was lowest at the AM milking and highest at the night milking of herds milked 3 x/day. The difference in these rhythms may be due to differences in the length of time represented by each milking interval. However, their data demonstrated a rhythm of milk and milk fat and a possible effect of milking times. We have recently observed milk yield and composition at each milking while milking every 6 h and feeding cows 1 x/day at 0800 h or in 4 equal feedings every 6 h (0600, 1200, 1800, and 2400 h). There was an effect of time of day on milk and milk fat yield and milk fat and protein concentrations in cows milked every 6 h (Figure 2). This higher resolution experiment further demonstrates the circadian pattern of milk synthesis in high producing dairy cows (Mean milk yield = 105 lb/day). In addition, we have observed an effect of feeding pattern on milk synthesis that is discussed below.

Photoperiod is the length of the light phase of each day and has a well-characterized effect on milk synthesis (Reviewed by Dahl et al., 2000; Dahl and Petitclerc, 2003). Because the effects of photoperiod are normally dependent upon biological clocks, these data strongly support a role of the circadian system in milk synthesis. Milk yield is on average 5.5 lb/day higher with long-days (16 h light), although the mechanism responsible for this effect is not known (Dahl et al., 2000). The milk yield response to long photoperiod is lost when animals are placed under constant light, a condition that is known to abolish circadian rhythmicity (Dahl et al., 2000). Plasma IGF-1 is increased, but growth hormone **(GH)** does not change under long-photoperiods, implicating a change in the GH-IGF1 axis under these conditions (Dahl et al., 2000). Liver circadian regulators have been shown to be responsive to photoperiod in sheep (Andersson et al., 2005), and changes in photoperiod may explain changes in hepatic IGF-1 synthesis during long days. Lastly, short day photoperiods during the dry period increases milk yield in the subsequent lactation, providing further evidence of an impact of circadian rhythm on mammary epithelial cells (Dahl, 2008).

Most dairy farmers and nutritionists recognize a seasonal change in milk fat that is commonly attributed to changes in forage sources, weather, or herd days in milk. A very repeatable seasonal pattern is observed in milk fat and protein concentrations as seen in the monthly average milk fat percentages for the Mid-East Milk Order over the past 10 years (Figure 3). Milk fat and protein concentrations peak around December and January and reach a nadir around July and August, and the annual range for milk fat is approximately 0.25 percentage units. This highly repeatable pattern appears to be independent of year-to-year differences in forage quality and weather. A similar pattern is observed for other milk marketing orders in different regions of the US that experience more heat stress (e.g. Southwest). Dahl et al. (2000) summarized the production response to photoperiod and reported that 2 out of 9 studies decreased milk fat and 3 out of 9 studies increased milk protein. Seasonal variation in milk components may not be explained simply by long and short days, but changes in day length are one of the most repeatable changes that occurs through the year and requires further investigation. This seasonal variation should be incorporated into the expected milk fat concentration when setting production goals and troubleshooting milk fat production.

Automated milking systems **(AMS)** provide an opportunity to observe a natural preference for milking time. Care is needed in interpretation of cow behavior in AMS because of the confounding factors of demand for the robot and the entrainment of behavior by lighting, feeding, and manager intervention. However, the frequency of cows entering the milking system appears to follow a circadian pattern. Wagner-Storch and Palmer (2003) reported 2% of cows in the holding area between 0000 and 0500 h compared to 8 to 12% of cows between 0800 and 1900 h. This preference for milking time may be due to a natural circadian synchronization of milking with physiology and other behaviors.

Evidence of circadian regulation in the mammary gland in other species

The circadian rhythm of milk synthesis has been investigated in other species. For example, a robust circadian rhythm was observed in milk fat, lactose, and protein concentrations in the donkey (Piccione et al., 2008). Milk fat had the largest amplitude with afternoon milk ~2.5% percentage units higher than morning milk. In addition, a daily pattern of milk fat concentration also was apparent in the horse (Matsui et al., 2003).

A circadian pattern of milk synthesis is also commonly recognized in breastfeeding women. The circadian pattern of milk fat is best characterized with the rhythm of milk fat concentration appearing to be dependent on diet composition and the timing of major meals (Prentice et al., 1981; Stafford et al., 1994). Interestingly, epidemiologists have also identified an association between nightshift work disruption of circadian rhythms and increased incidence of mammary cancers (Stevens, 2009).

The rat lactating mammary gland has a circadian rhythm of metabolism with a ~1 fold change in lactose synthesis and ~2 fold change in lipid synthesis occurring over the day (Carrick and Kuhn, 1978; Munday and Williamson, 1983). Casey et al. (2009) recently reported modified expression of a core circadian clock gene and 6 other clock related genes at one time point on day 1 of lactation compared to the last days of pregnancy in the rat. The authors also observed modification of core clock genes in liver and mammary tissues during the transition from pregnancy to lactation and suggest that the homeorhetic adaptions to lactation may be coordinated through molecular clocks in peripheral tissues. Although far from a complete characterization, the literature provides strong support for a functional role of the clock genes in mammary tissue.

Lastly, mammary gland function during lactation is regulated by multiple endocrine, autocrine, and paracrine factors, including GH, IGF-1, thyroid hormone, prolactin, and serotonin (Tucker, 2000). Interestingly, all of these factors are synthesized and secreted under the direct control of a circadian clock, resulting in variations in their concentration across the day.

Circadian pattern of intake and nutrient absorption

Discussion of the circadian rhythm of metabolism must be integrated with a discussion of the pattern of feed intake and nutrient absorption. Feeding behavior is centrally regulated through integration of many factors, including hunger, satiety, physiological state, environment, and endogenous circadian rhythms. Grazing cows have a well described "crepuscular" feeding pattern, with a large proportion of intake consumed at dawn and dusk (Reviewed by Albright, 1993). Feeding behavior of lactating dairy cows has more recently been studied using automated observation systems and found to follow a slightly modified crepuscular pattern (e.g. Dado and Allen, 1994; DeVries et al., 2003; Shabi et al., 2005). To provide insight into the circadian pattern of intake in high producing cows, we have plotted the rate of intake (% daily intake per h) by time after feeding of eight lactating cows fed a control ration [Figure 4; mean milk yield = 105 lb/day; (Harvatine and Allen, 2006)]. Fresh feed was offered once per day in the morning. Feed intake was observed with an automated feeding behavior system that monitored feedbunk weight every 5 seconds over 4 days (Dado and Allen, 1993). Over 16% of daily intake was consumed in the first 2 h after feeding, which is nearly twice steady state consumption. A second 6-hour period of increased feed intake was observed in the early evening and a third high intake period occurred in the morning. Others have recently reported a similar primary analysis of feed intake (e.g., DeVries et al., 2007; Hosseinkhani et al., 2008). These simple analyses clearly show different phases of feed intake over the day.

Physiological significance of the circadian pattern of intake

The ruminant has a more consistent absorption of nutrients over the day because of the large amount of digesta stored in the rumen, the slow rate of ruminal digestion, and presumably constant ruminal digesta outflow. However, highly fermentable diets are commonly fed to dairy cows to maximize energy intake and ruminal microbial protein production. Highly fermentable feeds result in a rapid production of volatile fatty acids **(VFA)** after consumption that reduces ruminal pH when the rate of VFA production exceeds absorption (Allen, 1997). The resulting low ruminal pH depresses ruminal fiber digestibility and microbial growth and can cause milk fat depression, bloat, laminitis, displaced abomasums, and metabolic acidosis (Owens et al., 1998; Krause and Oetzel, 2006). Total mixed ration feeding was developed to provide a consistent *concentration* of fermentable substrate in each meal over the day and has become the standard practice for feeding dairy cattle, although TMR feeding was reported to be associated with an increased odds ratio of cow mortality (McConnel et al., 2008). The common perception is that the rumen is a steady-state fermenter, and the TMR is a complete substrate that is continuously added to maintain constant fermentation and maximal microbial growth (Coppock et al., 1981). However, differences in the rate of feed intake over the day results in a large difference in the *amount* of fermentable substrate entering the rumen.

To provide insight into the effect of feeding patterns on ruminal pH, we plotted the hourly mean ruminal pH of the 8 cows whose pattern of intake is shown in Figure 4. Ruminal pH was determined every 5 seconds over 4 days by an indwelling pH probe (Harvatine and Allen, 2006). The high rate of intake immediately after feeding resulted in a decrease in ruminal pH that reached a nadir approximately 10 h after initial feed offering, coinciding with the end of the 6 h period of high feed intake. Mean ruminal pH was decreased by over 0.35 units (below the expected threshold for inhibition of fiber digestion) and was not rescued until 15 hours after the feed offering. During the low intake period from 12 to 20 h after feed offering, mean ruminal pH progressively increased, presumably due to a lower rate of fermentable substrate entering the rumen and more time available for rumination. Ruminal pH subsequently decreased again after the feeding period associated with the morning milking. Recently, others have provided similar preliminary analysis of hourly means of ruminal pH (e.g. Devries et al., 2007; Hosseinkhani et al., 2008). The daily pattern of rumen pH demonstrates the impact of the circadian pattern of intake on the rumen environment and gives an indication of the circadian dynamics of VFA production.

Nutritionists balance the composition of the diet, but the biologically relevant point is the composition of ruminal digesta because it is the substrate available to rumen microbes. The effect of the pattern of feed intake on ruminal digesta weight and composition can be seen in ruminal contents 1.5 h before feeding compared to 4 h after feeding (Harvatine and Allen, Michigan STate University, Unpublished). Consistent with the increased rate of intake, ruminal digesta weight increased 24% after feeding. More importantly, ruminal starch concentration was 87% higher after feeding and the concentration of indigestible NDF and lignin decreased approximately 14% after feeding. This clearly demonstrated a large difference in the fermentability of the substrate available to ruminal microbes during the high intake period of the day. We are not aware of a characterization of the circadian pattern of the flow rate or composition of duodenal flow, but we expect significant differences based on the changes observed in the amount and composition of ruminal digesta.

Modification of the circadian pattern of feed intake

The dairy cow appears to prefer a crepuscular pattern of feed intake, but milking times are also commonly at dawn and dusk, making a conclusion of the stimulus difficult. Many factors impact feeding behavior of dairy cow, including barn and feedbunk design, social interaction, stress, illness, etc. (Reviewed by von Keyserlingk and Weary, 2010). The dairy cow may have a preferred feeding rhythm, but she must also consume a large amount of feed to meet energy demands and adapt to feeding and milking times selected by farm management. The effect of feeding time on milk yield and intake has been investigated in a limited number of experiments. Piccione et al. (2007) demonstrated that the timing of feed availability entrained the rhythm of urea synthesis in restricted fed cows. Nikkhah et al. (2008) investigated the effect of feeding 1 x/day at 0900 or 2100 h and the interaction with diet fermentablity and cow parity and found that cows fed at 2100 h consumed 37% of the daily intake in the first 3 hours after feeding compared to 27% by cows fed at 0900 h. Cows fed at 2100 h also had lower plasma glucose after feeding. There was no effect of feeding time on milk and milk protein yields, but feeding at 2100 h increased milk fat yield and percentage by 16 and 22%, respectively. Ominski et al. (2002) observed a small increase in feed intake from 2 to 12 h after feeding 1 x/day at 2030 h compare to 0830 h; however, cows fed at 2030 h had decreased milk fat concentration. Although modest changes in intake relative to feeding time are observed, feeding pattern appears to rapidly entrain to feeding time.

Pushing feed closer to cows and offering fresh feed multiple times a day have been proposed as mechanisms to stimulate a more even rate of feed intake. Using presence at the feedbunk as a measure of feeding behavior, DeVries et al. (2003) observed that offering fresh feed and returning from the milking parlor resulted in a large increase in feeding, but feed push-ups did not increase the number of cows at the feed bunk. Multiple research groups have investigated the effect of increasing feeding frequency using diverse methodology (Summarized by DeVries et al., 2005a). In general, increasing feeding frequency up to 5 times per day changes the feeding pattern by creating more spikes in feeding behavior around the time of each feeding (Nocek and Braund, 1985; DeVries et al., 2005b; Mantysaari et al., 2006). Offering fresh feed more than 4 times per day increased feed efficiency (Mantysaari et al., 2006; Nocek and Braund, 1985) and reduced variation in ruminal pH (French and Kennelly, 1990; Shabi et al., 2005). However, Mantysaari et al. (2006) reported increased restlessness and decreased lying time and Phillips and Rind (2001) reported decreased time spent ruminating and disruption of the circadian lying pattern. Thus, feeding at very high frequencies ($\geq$ 4x/day) is not realistic for on-farm application and disruption of normal behavior makes the approach much less desirable.

Effect of the circadian pattern of nutrient absorption on milk synthesis

Theoretically, feed efficiency is maximized when the animal's nutrient requirements are exactly met by the diet, and nutritionists traditionally use a stoichiometric approach to match *daily* nutrient requirements with predicted nutrient absorption. A day is a meaningful unit, but the rate of milk synthesis varies over the day and is dependent on minute-to-minute availability of substrate. If the rhythm of milk synthesis is not synchronized with the rhythm of nutrient absorption, milk synthesis is limited during the period of nutrient deficit and excess nutrients are partitioned to non-productive functions during period of nutrient excess (Illustrated in Figure 5).

To investigate the effect of the circadian pattern of intake on milk synthesis, we recently conducted an experiment testing the effect of feeding the same TMR 1 x/day at 0800 h or feeding ad libitum in equal meals every 6 h. The circadian rhythm of milk synthesis was observed by milking cows every 6 h for the last 7 days of each experimental period. As previously discussed, we observed a circadian rhythm of milk, milk fat, and milk protein synthesis. Feeding equal meals every 6 h decreased the amplitude the circadian rhythm for milk fat percent by ~50% and increased daily milk fat yield by 8.3% ($P < 0.001$). Increasing the frequency of offering feed from 2 to 4 or 6 x/day was intensively investigated in the 1980's and normally resulted in little effect on milk yield, but more commonly, increased milk fat concentration occurred, especially when diets induced moderate or severe milk fat depression (Reviewed by Gibson, 1984; Sutton, 1989). Milk fat synthesis is decreased by the production of bioactive *trans* fatty acids formed as intermediates of ruminal biohydrogenation of polyunsaturated fatty acids under conditions of unstable ruminal fermentation (See review Harvatine et al., 2009). Increasing feeding frequency is expected to stabilize ruminal fermentation and decrease production of bioactive fatty acids. Loor et al. (2004) reported a circadian pattern of ruminal trans fatty acids isomers in rumen fluid of cows fed high oil diets, and thus, the circadian rhythm of milk fat synthesis may be due to daily dynamics of trans fatty acid absorption. Additionally, milk fatty acid profile will allow determination of the rhythm of de novo fatty acid synthesis and preformed fatty acid incorporation into milk fat.

Conclusions

Most physiological processes exhibit circadian rhythms that are driven by central and peripheral timekeepers in order to improve fitness by synchronizing physiological processes so that they occur at the optimum time of day. Disruption of central and peripheral rhythms has significant implications for metabolism and health in rodents and humans. It is reasonable to expect that circadian rhythms in the cow are entrained by cues such as feeding, milking, and nutrient absorption. The impact of this should be considered in dairy management and further experiments will be required to specifically define the role of each factor on the dairy cow.

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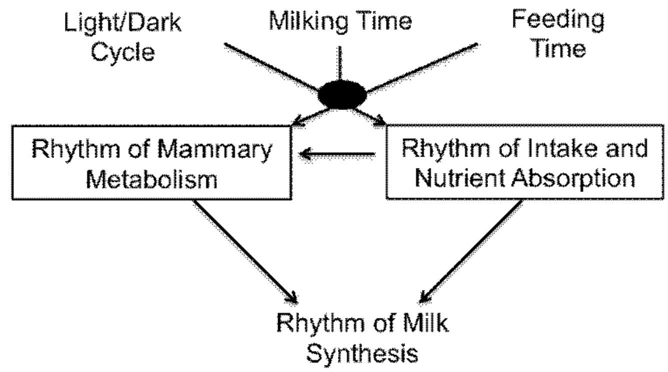

Figure 1. Integrated illustration of environmental cues and interaction of the circadian rhythm of intake, mammary metabolism, and milk synthesis.

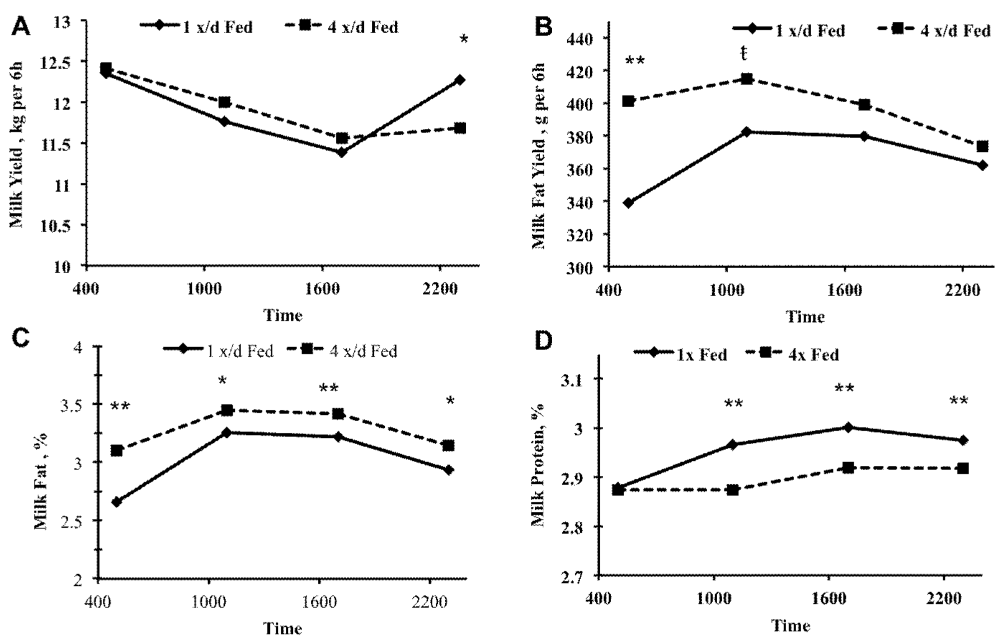

Figure 2. Milk production response by milking of cows fed 1 x/day at 0800 h or in 4 equal meals every 6 h (0600, 1200, 1800, and 2400 h). Model tested effect of feeding frequency (**F**), time (**T**), and their interaction (FxT). Panel A: Milk yield (F, $P = 0.84$; T, $P < 0.001$; and FxT, $P = 0.12$); Panel B: Milk fat yield (F, $P < 0.001$; T, $P = 0.02$; and FxT, $P = 0.18$); Panel C: Milk fat concentration (F, $P < 0.001$; T, $P < 0.001$; and FxT, $P = 0.12$), and Panel C: Milk protein concentration (F, $P < 0.001$; T, $P < 0.001$; and FxT, $P < 0.001$). Treatment differences at each time point are shown (g $P < 0.10$, * $P < 0.05$, and ** $P < 0.01$).

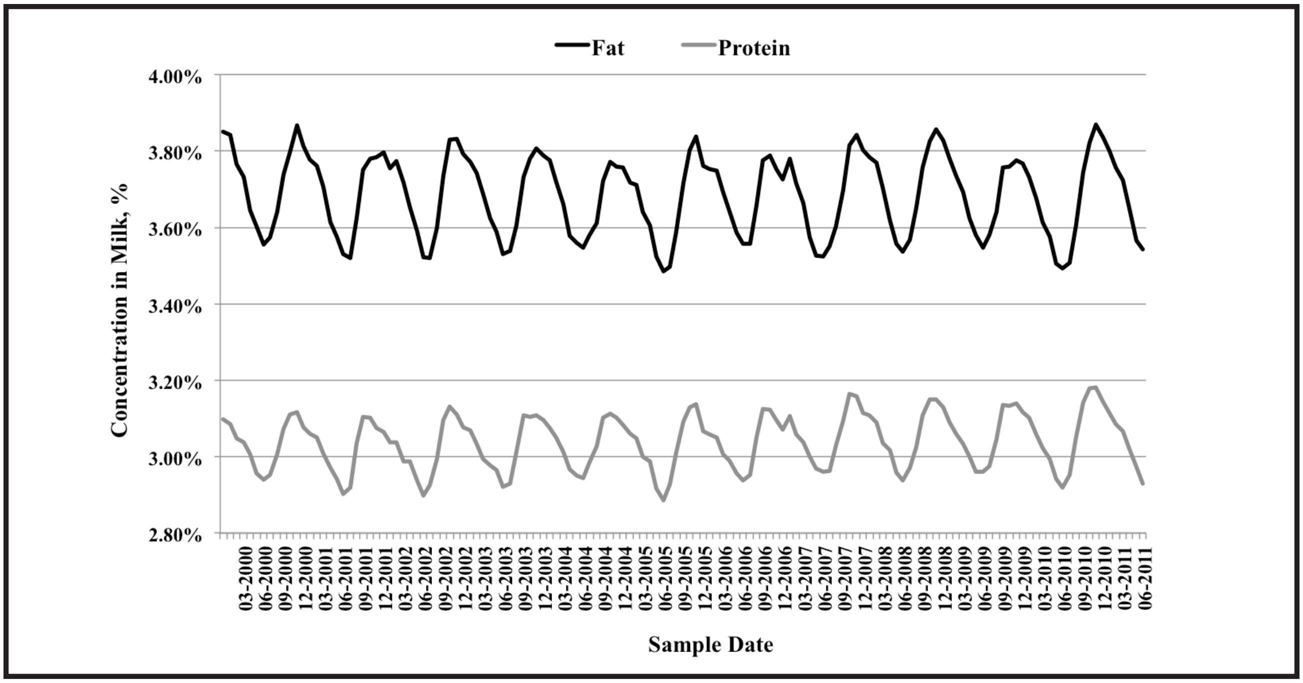

Figure 3. Seasonal pattern of milk fat and protein percentages in the Mid-East Milk Market Order over the past 10 years.

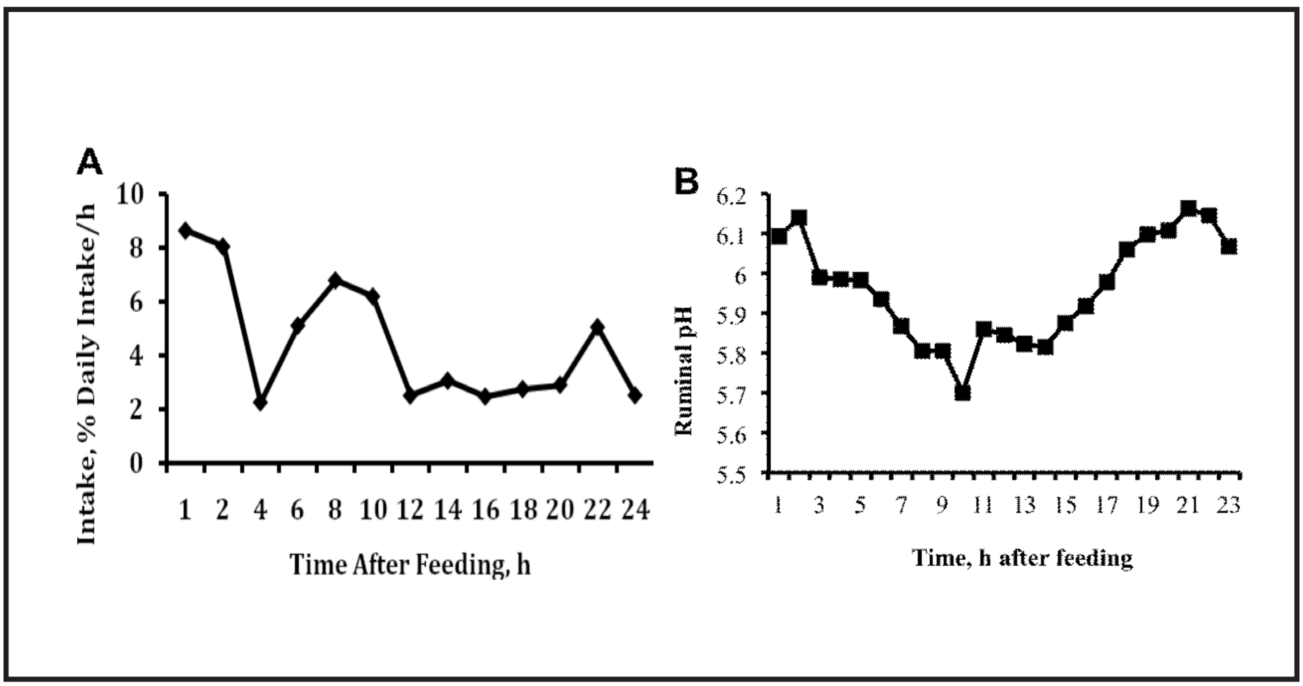

Figure 4. Circadian pattern of the rate of feed intake and ruminal pH of high producing dairy cows fed a control TMR once daily (n = 8).

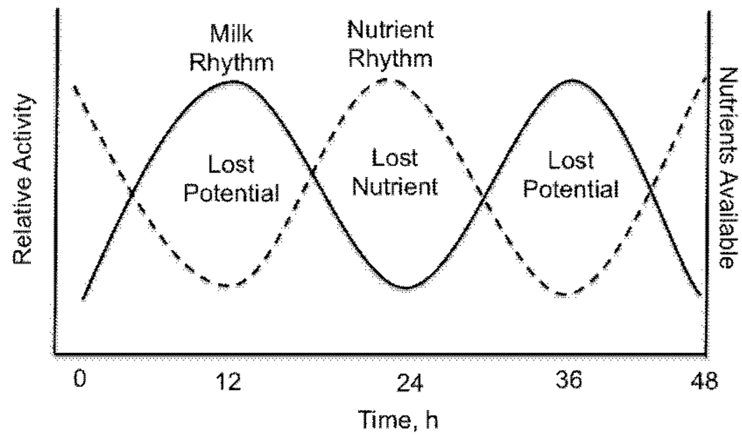

Figure 5. Illustration of the impact of unsynchronized rhythms of nutrient absorption and milk synthesis.