

Seasonal Variation in Milk Composition

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Abstract

Most dairy producers and consultants in the United States are familiar with the consistent decline in milk yield and fat and protein tests during the summer. Oftentimes, these declines in production are ascribed as strictly a consequence of environmental factors, such as heat stress. While heat abatement strategies are incredibly important for maintaining health and productivity of dairy cows in the summer months, evidence suggests that summer declines in production may also be due to cows' inherent annual rhythms. It is important to consider these rhythms while setting goals and evaluating herd production. It is not entirely clear how to overcome these rhythms, but appropriately managing photoperiod is recommended.

Introduction

Rather than simply *responding* to an environmental stimulus, endogenous 'calendars' in the hypothalamus allow the animal to *anticipate* yearly environmental changes before they occur. This adaptation is especially useful for timing reproduction, allowing animals to synchronize parturition to the season with the greatest available resources for the offspring.

Annual rhythms are present in nearly all studied organisms as a mechanism to perceive and adapt to seasonal environmental changes.

For example, many mammals in northern climates hibernate over the winter. Bears, while not true hibernators, experience winter *torpor*, which is characterized by dramatically reduced body temperature and basal metabolic rate. Migrating birds undergo astonishing changes in metabolism prior to spring and fall migration, including initiation of nocturnal activity and accretion of body fat reserves. For example, rufous hummingbirds (*Selasphorus rufus*) gain 50 to 67% of their prior body weight (**BW**) in fat prior to migration (Carpenter et al., 1983). In production livestock, sheep display yearly rhythms of estrous behavior, leading to spring lambing (Malpaux et al., 1997). In most species that exhibit seasonality, annual changes in physiological persist even after animals are placed in constant day length (photoperiod).

Measuring Biological Rhythms

The analysis of biological rhythms, including annual rhythms, is typically performed using a technique called cosinor rhythmometry (Figure 1). This technique fits the linear form of a cosine function with a defined period length (for annual rhythms, this period is 12 months) to time course data. Comparing the fit of the linearized cosine function to a standard linear model is used to determine whether the response variable follows a biological rhythm. This approach is called a zero-amplitude test because it tests if the amplitude of a cosine function is statistically

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greater than zero, and therefore is rhythmic. Our lab has applied this approach using mixed models to separate random effects, such as animal, herd, or year, from the effects of the rhythm, and to compare rhythms between different treatment groups. In addition to determining rhythm fit, cosinor rhymometry is used to calculate the time that a rhythm peaks, termed acrophase, and the difference from peak to mean of the rhythm, called the amplitude. The acrophase of an annual rhythm indicates the time of the year when response is greatest, and differences in acrophase between groups indicate that they are entrained to a different cycle. The amplitude is a measure of the robustness of a rhythm, and twice the amplitude (double amplitude) is a measure of the difference between maximal and minimal response.

Annual Rhythms in the Dairy Cow

Yearly patterns of milk production have been recognized for over 40 years (Wood, 1970). Producers are familiar with summer declines in milk production, and recovery during the winter. When examining average monthly bulk tank records from U.S. Federal Milk Marketing Orders, the presence of an annual rhythm is apparent. Fat and protein concentration from the years 2000 to 2017 display repeating 12-month cycles that are remarkably consistent between years (Figure 2). These yearly patterns fit a robust cosine function, suggesting that they represent a biological rhythm ($P < 0.001$; Salfer et al., 2016). The rhythms of fat concentration peak between December 29 and January 18 in all regions except for Florida, which peaks on December 4. Protein concentration is even more consistent among regions, with a maximum between December 27 and January 6. The variation in milk fat concentration due to the annual rhythm is between 0.15% and 0.30%, depending on the region. Notably, annual fat concentration rhythms of regions in the southern

U.S., mainly Florida and Arizona-Las Vegas, seemed to have lower amplitude rhythms than further north regions, which may be related to their lower latitude and smaller change in photoperiod across the year. The amplitudes of milk protein concentration were more consistent among regions, with peak to trough difference being 0.16% to 0.20% (2x the amplitude).

The presence of yearly production rhythms was confirmed using 10 years of DHIA data from individual herds in Minnesota, Pennsylvania, Texas, and Florida (Salfer et al., 2017). Similar to the U.S. milk markets, milk fat and protein concentrations peak around January 1 and reach a nadir on July 1 in Minnesota, Pennsylvania, and Texas. Florida, on the other hand, had the greatest fat concentration in November and greatest protein concentration in October. These data provide further evidence to suggest that annual rhythms of production vary by geographical location. States in the northern U.S. have markedly greater amplitude rhythms of fat and protein concentration. For example, in Pennsylvania and Minnesota, the difference between peak and trough for fat concentration was 0.32% and 0.28%, respectively, while Texas was 0.16% and Florida's was 0.08%. To put this in perspective, a farm in Pennsylvania with a 3.6% butterfat test in July, should expect their fat test to increase to 3.92% in January merely due to the animal's annual rhythm. A farm in Florida with the same fat test in July, however, should only expect their January fat percent to equal 3.68%. In agreement with milk market data, DHIA herd-level data shows that the amount of oscillation in the annual protein concentration rhythm is generally less variable among regions of the U.S. (peak to trough 0.16 to 0.18% in Minnesota, Pennsylvania and Texas), but was distinctly lower in Florida (0.06%).

While fat and protein concentration both peak near the first of the year, the annual

rhythm of milk yield peaks between late March and early April, right around the vernal equinox (Salfer et al., 2017). Fat and protein yields peak between late February and early March. Contrary to the rhythms of fat and protein concentration, amplitudes of annual milk yield rhythms are greater in the southern U.S. compared to the north. Cosinor rhythmometry revealed that the peak to trough in Pennsylvania and Minnesota was 2.5 and 2.2 kg, respectively, while that of Texas and Florida were 6.3 and 7.4 kg, respectively. Fat and protein yields also oscillated more in the southern U.S. than the northern U.S. The mechanism causing the amplitudes of milk, fat, and protein yields to be greater in southern climates is unclear. Data from DHIA also revealed slight differences in annual production rhythms between breeds. The rhythm of milk peaks on April 1 in Holstein, while it peaks on May 11 for Jersey. While it is difficult to discern if this effect is actually due to genetic differences between breeds or simply an artifact of the analysis, it should be considered by producers.

Data from 11 individual herds in Pennsylvania has also been examined to determine which cow-level effects influence annual rhythms of milk production (Salfer et al., 2016). The diacylglycerol o-acyltransferase 1 (**DGAT1**) gene, responsible for 40% of the genetic variation in fat percentage, does not influence annual rhythms of fat concentration or fat yield (Winter et al., 2002). Similarly, rhythms of milk, fat, and protein yields, and fat and protein concentrations are not affected by parity. Data from these 11 herds also confirmed that fat and protein concentrations peak in late December and early January, milk yield peaks in late March and early April, and fat and protein yields peak in late February and early March. While rhythms of fat and protein concentrations were incredibly consistent among herds, 2 herds had very low amplitude rhythms of milk, fat, and

protein yields. There were not, however, any detectable differences among these herds and the rests, so the reason for the low-amplitude rhythms is unclear.

Naturally, environmental temperature is often blamed for causing the seasonal changes in milk production. While it is certainly a factor, our results suggest that an annual rhythm exists independent of temperature (Salfer et al., 2017). We compared the fit of a model containing daily maximum temperature within each state, to a model containing the linearized cosine function. Results suggest that the cosine function fits the data dramatically better than temperature, implying that the effect is not simply a function of temperature (P value of F -test <0.0001). Furthermore, a decline in fat and protein concentrations is observed *below* the fitted cosine function in July and August, especially in Pennsylvania and Minnesota (Figure 3). This phenomenon appears to suggest that heat stress is an additive effect, separate from the annual rhythm that causes additional production declines in the summer. A final piece of evidence to support the suggestion that the annual rhythm is independent of temperature is that milk yield reaches a minimum in late September, instead of during the middle of the summer when temperatures are lowest.

Additional support suggesting that dairy cows are affected by annual rhythms is provided by yearly patterns of circulating metabolites. Piccione et al. (2012) determined that plasma concentrations of bilirubin, creatinine, triglycerides, and β -hydroxybutyrate (**BHBA**) fit 12-month rhythms. They observed that BHBA peaks on April 1, total bilirubin peaks on July 14, creatinine peaks on June 12, and triglycerides peak June 16. The circulating concentrations of prolactin vary by season in cattle, with drastically greater concentrations in summer (46 ng/mL) than winter (7 ng/mL) (Petitclerc et al.,

1983). These effects persisted even after animals were blinded or pinealectomized, suggesting that the effect is endogenous. Furthermore, the season of the year affects the diurnal rhythm of body temperature in cows. Body temperature has greater daily fluctuations in the summer compared to the winter (Kendall and Webster, 2009). While cattle are not generally considered to be seasonal breeders, there are modest effects of season and photoperiod length on reproduction in cows (Hansen, 1985). While the common assumption is that domestication has removed the evolutionary drive for annual rhythms in cattle, there is ample evidence to suggest that they are still influenced by seasonal physiology.

Potential Mechanisms of Seasonality

As discussed above, a primary role of annual rhythms is to coordinate reproduction with resource availability to maximize the likelihood of survival of the offspring. As an important component of reproduction, it is not implausible to expect that lactation is controlled through similar mechanisms. It stands to reason that producing more energy-dense milk with greater concentrations of fat and protein in the winter when energetic demands are greater would increase the likelihood of calf survival. In all mammalian species characterized, annual rhythms are controlled by a photoperiodic timer based on the duration of melatonin release (Lincoln and Hazlerigg, 2011). The synthesis of prolactin is also under the control of the photoperiod-based timing mechanism. Prolactin is released from the pituitary and is involved in feed intake and initiation of lactation in many mammalian species (Bauman and Bruce Currie, 1980; Lawrence et al., 2000).

Besides the melatonin-controlled day length timer, many species have evolved an endogenous timekeeping system that keeps

track of time in constant photoperiods. This system allow animals to anticipate seasonal changes and prepare for the upcoming climate. Furthermore, it allows migrating animals to continue to have a record of the time of year, even after moving to a geographical location with a different photoperiod (Lincoln and Hazlerigg, 2011). The combination of output from this endogenous timer and melatonin-based photoperiod signaling lead to the ultimate seasonal phenotype.

Effects of Photoperiod on Milk Production

Extensive research has examined the impact of altering photoperiod length on milk synthesis of the dairy cow. The first report of increased milk production after 16 h light: 8 h dark (16L:8D) photoperiod was made by H. Allen Tucker's lab at Michigan State (Peters et al., 1978). Since this initial discovery, several subsequent experiments have confirmed these findings (Stanisiewski et al., 1985; Dahl et al., 1997; Miller et al., 1999). Reksen et al. (1999) demonstrated that the effect occurs after implementation of any photoperiod greater than 12L: 12D; however, response is greatest at 16L: 8D.

The increase in milk production during long-day lighting has been associated with several hormonal changes that may be responsible for the observed effect. The duration of melatonin secretion is limited during artificial long-day lighting. Insulin like-growth factor-1 (**IGF-1**) is an effector molecule in the somatotropic axis. While the direct role of IGF-1 on milk synthesis is unclear, its concentration is increased after exogenous treatment with recombinant bovine somatotropin (Bauman and Vernon, 1993). Increased milk synthesis due to artificial 18L:6D photoperiod is associated with increased circulating IGF-1 concentrations (Dahl et al., 1997). Another hormone targeted

for its potential role in the lactational response to photoperiod is prolactin. Plasma prolactin concentrations increase during long days and decrease during short days (Tucker et al., 1984; Lacasse et al., 2014). Feeding melatonin to mimic the dark phase also decreases the concentrations of plasma prolactin (Buchanan et al., 1993). However, effects of prolactin on milk synthesis do not appear to be direct because no effects on milk production are observed after administration of exogenous prolactin (Plaut et al., 1987).

The results of photoperiod experiments and annual rhythms of production are seemingly paradoxical. While long-day lighting consistently increases milk synthesis, the cow's natural annual rhythm dictates that milk production increases when the duration of the light cycle is shorter than 12 hr. This anomaly is difficult to explain using current knowledge of annual rhythms in dairy cattle. One potential explanation is that long-day lighting may induce *photorefractoriness* to the annual rhythm of milk synthesis. Photorefractoriness is a phenomenon observed in other mammalian species, through which long-term exposure to a constant photoperiod leads to spontaneous reversion of a seasonal physiological response to the state expected in the opposite photoperiod (Lincoln et al., 2005). In other species, a fixed photoperiod must be applied for a long period of time (4 to 12 weeks) before switching of the physiological response occurs. In cows, the increase in milk yield after long days typically does not manifest until after 4 weeks of administration (Dahl et al., 2000). While this mechanism seems promising as a possible explanation for the observed effects of long-day lighting, it has not yet been studied in cows and further research must be done to test if it is related to the milk yield response.

Implications for Producers

While a greater understanding of the mechanisms responsible for the annual rhythm of milk production must still be developed, there are practical considerations dairy producers can make now to better manage for seasonal changes in production. An acceptance of the annual rhythm as a biological phenomenon that cannot be altered by nutrition or management can help producers adjust expectations across the year. As discussed above, the annual rhythm of milk production is responsible for a large amount of the variation in milk production and components. Using parameters derived from the linearized cosine function, we have calculated variables to adjust monthly milk, fat and protein yields and fat and protein concentrations based on their annual rhythms (Table 1). These adjustment factors can be added to monthly production to remove the effect of the annual rhythm and standardize production across the year.

Conclusions

Seasonal rhythms are controlled by timekeeping systems within an animal and allow adaptations before environmental weather and feed availability changes occur. The cow is not as seasonal as small ruminants that will only breed at certain times of the year, but a modest seasonal rhythm in milk and milk component yields is observed. The amplitude of the rhythm appears to be decreased in more southern regions of the US. Predicting the seasonal rhythm will allow a more precise evaluation of herd production. Long photoperiods are a well demonstrated method to increase milk and milk component yields and may actually work to reverse some of the negative effects of lengthening days, but more research is needed to clearly understand this phenomenon.

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Table 1. Values to normalize milk, fat, and protein yields, and fat and protein concentrations to account for the annual rhythm of these variables. Monthly production should be added to the appropriate value in the table.

	Milk lb	Fat, %	Protein, %	Fat, lb	Protein, lb
Jan	0.0	-0.13	-0.10	-0.09	-0.04
Feb	-1.5	-0.12	-0.08	-0.14	-0.08
Mar	-2.6	-0.07	-0.04	-0.15	-0.09
Apr	-3.1	0.00	0.00	-0.13	-0.08
May	-2.7	0.08	0.04	-0.07	-0.05
Jun	-1.7	0.14	0.06	0.00	0.00
Jul	-0.2	0.17	0.07	0.07	0.05
Aug	1.3	0.15	0.05	0.12	0.08
Sep	2.4	0.11	0.01	0.13	0.09
Oct	2.8	0.03	-0.03	0.11	0.08
Nov	2.5	-0.04	-0.07	0.05	0.05
Dec	1.5	-0.10	-0.10	-0.02	0.00

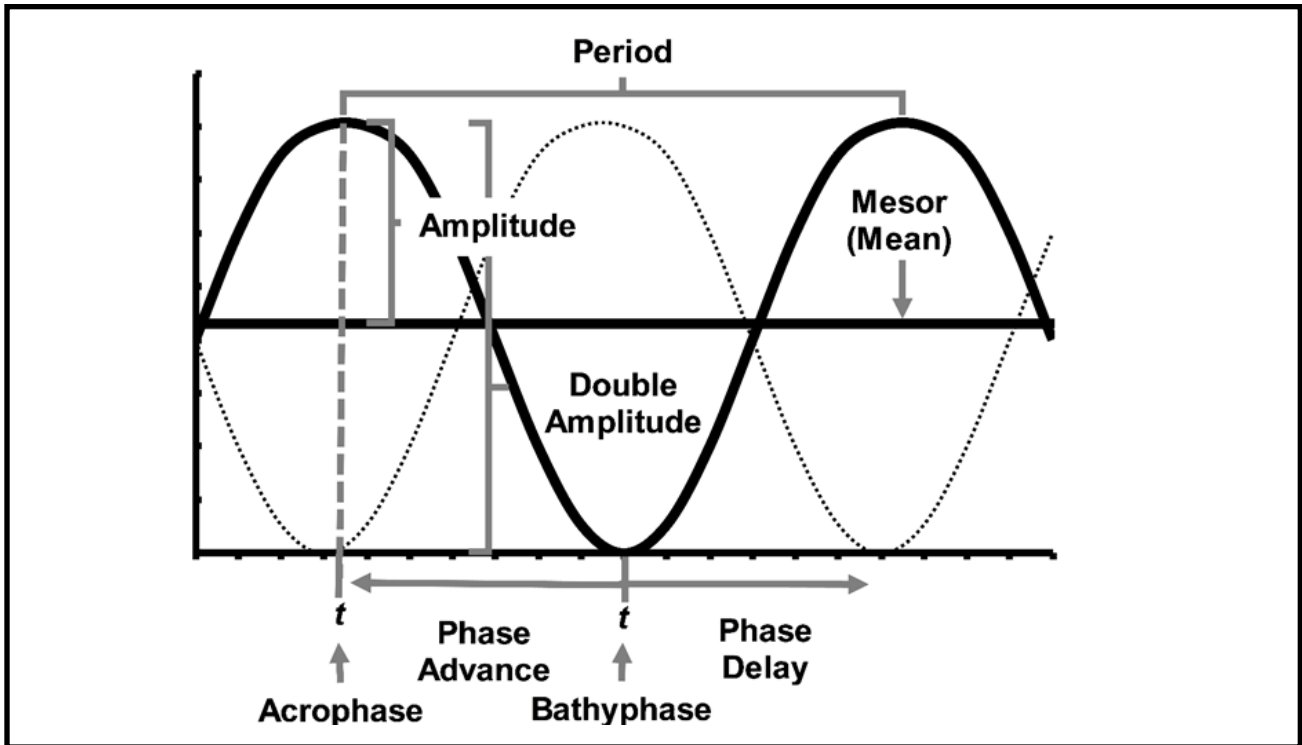


Figure 1. Parameters relevant to characterization of biological rhythms. Period refers to the length of time to complete one cycle of the rhythm. Amplitude is the difference between peak and mean. Double amplitude is the difference between peak and trough. Acrophase is the time at peak. Bathyphase is the time at trough. Phase advance refers to shifting the rhythm curve so that the acrophase occurs earlier than it was previously. Phase delay refers to shifting the rhythm curve so that acrophase occurs later than it was previously.

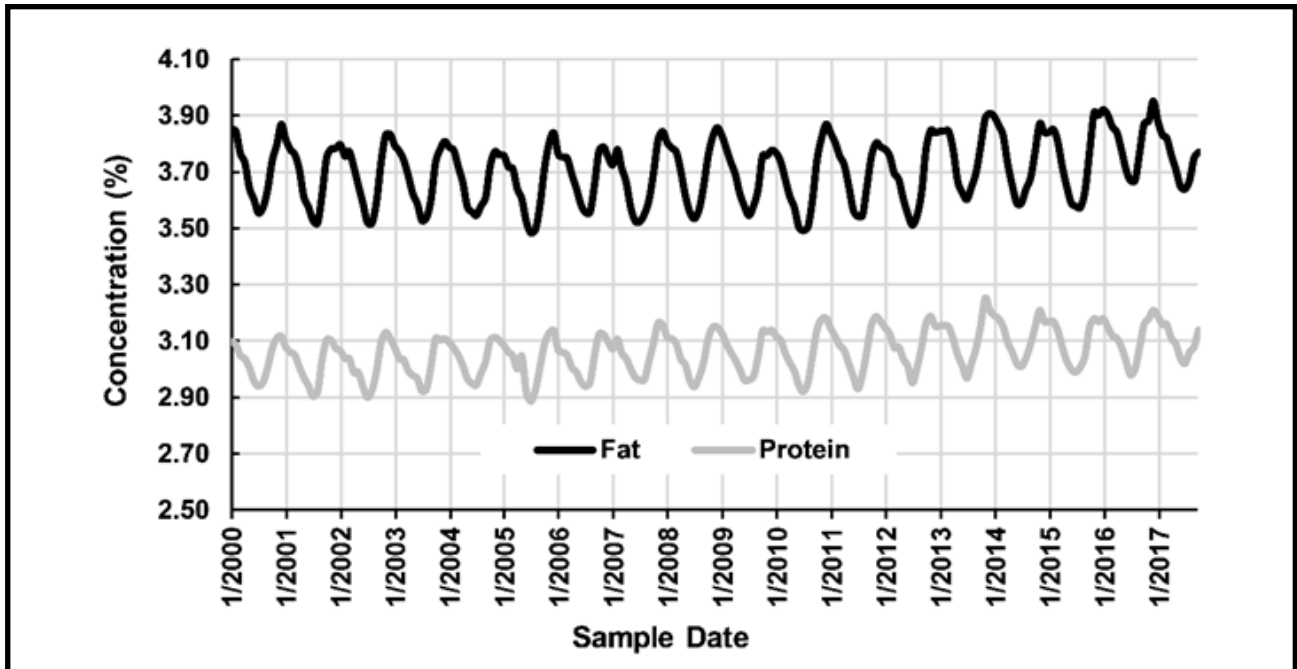


Figure 2. Yearly patterns of milk fat and protein concentration in the Mideast Milk Market. Rhythms are very repeatable between years.

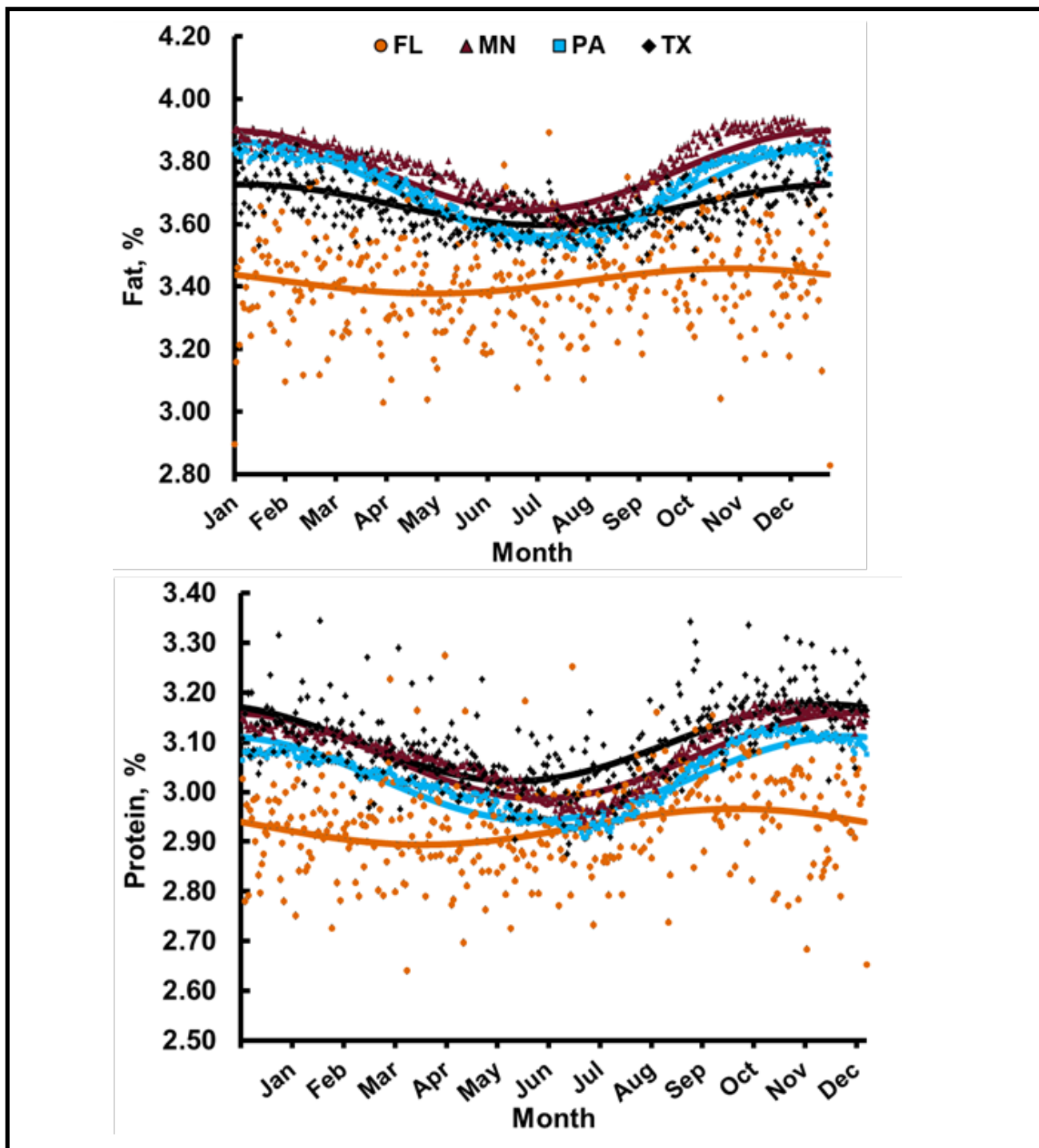


Figure 3. Yearly rhythms of fat and protein concentration from DHIA herd records in Minnesota, Pennsylvania, Florida and Texas from 2004 to 2016 (Salfer et al., 2017).