

**EFFECTS OF SEASON ON CIRCADIAN RHYTHMS OF BEHAVIOR AND
PRODUCTION, AND EFFECTS OF AUTOMATED FEEDING ROBOTS ON
RATION CONSISTENCY, COW BEHAVIOR, AND PERFORMANCE.**

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ABSTRACT

Dairy cows consistently exhibit patterns of feeding and milk synthesis. These patterns can be entrained by environmental cues and management practices. The study of behavior of cows is an essential practice to enhance cow well-being and milk production. Two studies were conducted to better understand the relationship between cow behavioral patterns, physiology, and performance. First, we investigated the effect of season on daily rhythms of body temperature, milk production and milk components. In this experiment, daily rhythms were observed in fall, summer, and spring but winter did not fit a rhythm ($P < 0.05$). Season also tended to affect the AM to PM ratio of milk yield ($P = 0.09$). However, season did not affect the AM to PM ratio of fat and protein concentration in milk. The effect of season on daily rhythms of temperature is significant while season's effect on daily rhythm of milk yield is minimal. Secondly, we examined the effect of using automated feeding robots on cow performance (milk production and milk components), cow behavior and total mixed ration consistency at the feed bunk. The use of feeding systems did not affect milk production and milk components except for lactose ($P = 0.02$). However, the use of automated feeding robot reduced variability in feed ration at the feed bunk. Ration consistency is an important factor in maintaining proper rumen health. Further research on the effects of automatic feeding systems on rumen health, ration consistency and dairy performance is recommended.

Keywords: Daily rhythms, Automated feeding systems, ration consistency

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1. LITERATURE REVIEW

AUTOMATED FEEDING AND MILKING SYSTEMS

Introduction

According to the Food and Agriculture Organization (FAO Milk and Dairy Products in Human Nutrition, 2013), the dairy industry has contributed to the growth of rural parts of North America, Europe, and the Oceania regions, while in other parts of the world, dairy production has been a key feature in national development programs. Dairy products are pivotal for the growing need for income and a source of macro and micro-nutrients as human population continues to grow globally. Subsistence dairy farmers are therefore transitioning to commercial farming, which requires advancement in production techniques (FAO Milk and Dairy Products in Human Nutrition, 2013).

Within the United States, dairy industry has experienced considerable change during the past century. These changes include improvements in genetics, nutrition and feeding strategies (Shook, 2006), housing designs (Hoglund and Albright, 1970), as well as increases in average herd size, and exports to international markets. Ultimately, these changes have led to increased efficiency and milk production (Capper et al., 2009; USDA NASS, 2021). However, several ongoing global and local issues create additional need for technological advancement. These issues include the population growth, climate change, labor security, increased focus on animal welfare, farm safety and security, and land availability (FAO Livestock long shadow, 2006; Croney and Anthony, 2011). Cropland acreage, for example, has also declined by approximately 10% due to urban sprawl (von Keyserlingk et al., 2013). Migration to cities and population growth are accompanied by

changes in food and nutritional preferences due to lifestyle changes. The disconnect between urban residents and dairy production means that these consumers must depend on the available information channels to make nutritional decisions (FAO Milk and Dairy Products in Human Nutrition, 2013). Consumer concerns and criticism about the welfare of dairy animals in intensive dairy production systems have arisen especially among the urban population, a situation that has led to the invention of competing non-dairy milk alternatives (Alonso et al., 2020; Schiano et al., 2022).

Large amounts of fossil fuels are consumed by farm machinery, which greatly contribute to greenhouse gas emissions. Carbon dioxide (CO₂) and methane (CH₄) are the two most important greenhouse gases in the livestock industry released either through direct respiratory emissions or through routine on-farm practices - especially the use of fossil fuels in transportation, fertilizers and feed production and heating (FAO Livestock long shadow, 2006). These issues have necessitated continuous research and innovation into precision dairy management technologies (von Keyserlingk et al., 2013). The use of precision dairy technology will help farmers cut the use of fossil fuels and nitrous fertilizers by helping to optimize milk productivity with reduced number of cows, or with high productivity from little farm input. Precision farming encompasses the use of modern information and communication technology (ICT) and other real-time dairy management monitors (Berckmans, 2015; Henschion et al., 2022). The coordination of information technology, data management and mechanization has been employed to optimize dairy production performance (Eastwood et al., 2012). Technology is currently used to monitor factors such as rumination time, rumen temperature, estrus detection, standing and lying time, milking time, and feeding and milking among other parameters (Borchers and

Bewley, 2015). Recently, automated feeding and milking systems have been adopted in dairy farm operations in the United States.

Automated feeding systems (AFS) and automated milking systems (AMS) provide flexibility in the feeding and milking times of cows, since the conventional systems of feeding and milking follow rigid daily set schedules (Wagner-Storch and Palmer, 2003). Cows naturally follow daily patterns of feeding and milking; therefore, the timing of feeding facilitates entrainment of these patterns (Asher and Schibler, 2011). The flexibility that comes with AMS and AFS may alter the conventional feeding-milking patterns primarily determined by the set schedules, therefore enabling the cows to freely establish their behavioral patterns. Previous research has shown that cows respond to the mode of feed delivery, and that fresh feed delivery stimulates cows to visit the feed-bunk, indicating that management patterns may greatly influence cow behavior (Phillips and Rind, 2001). Automated feeding systems collect and relay data, perform technical functions such as loading, mixing, pushing feed as well as supplying cows with fresh feed throughout the day and night, minimizing on-farm labor demands (Rodenburg, 2017). Surveys have shown that the primary reason why farmers choose to install automated systems is to have more flexibility in operating schedules by reducing labor requirements (Hogeveen et al., 2004; Belle et al., 2012). A survey conducted by Borchers and Bewley (2015) reported that choice of automated feeding system is based primarily on cost-benefit factors, although factors such as farmer preferences also greatly influence the decision towards automation.

Automated Feeding Systems

Historical Technological Developments of Dairy Cattle Feeding Systems

Naturally cows, like other herbivores, consume native vegetation. However, through domestication, the methods of feed access by cows have evolved tremendously (Vermeulen, 2015). The domestication process led to human-cow co-dependent relationship whereby cows provide draft power and/or animal products such as hide, meat and milk, and humans provided feed, water, and safety from predators (Hodgson, 1979). In the agrarian and industrial revolution periods, the demand for food due to increasing human population necessitated the improvement of dairy management systems to produce sufficient dairy products to feed the growing population (Capper et al., 2009). Growth in herd sizes, increased production per cow, and the shift from subsistence farming to commercial dairy farming required more efficient cattle handling systems (Hodgson, 1979). This led to the development of feed supplements for dairy cows as well as the mechanization of dairy farms (Ma et al., 2012; Medeiros et al., 2022).

In the United States, growing herd sizes and improved understanding of nutritional requirements led to the development of total mixed rations (TMRs), which are complete rations containing a blend of forages and concentrates that are formulated to provide all the required nutrients by cows. The use of TMRs for lactating cows dates to the 1950s. Prior to their use, cows were typically fed forages that were top dressed or supplemented with feed concentrates (Bickert and Light, 1982; Schingoethe, 2017). The development of TMRs significantly changed feed management systems by increasing the ability to control particle size distribution and moisture content. It also increased the need for feed mixer-wagons to optimize TMR mixing. The first feed-mixer wagons were developed in the late

1960s. TMR feeding became the conventional way of feed management in the United States and many other countries (Schingoethe, 2017). As of 2014, 90% of large dairy (500 or more cows) farms feed TMRs (USDA, 2016). Today, most TMRs are delivered to cows using driven or pulled mixer-feeder wagons that facilitate dumping feeds along the cow feeding fence (Buckmaster et al., 2014). Total mixed rations must ideally be homogenous to ensure that nutrients are dispersed equally within a ration and are accessible to all cows along the feeding fence. Additionally, their proper particle distribution enables the cow to receive all the feed ingredients in a single mouthful of feed since cows can sort long particles out of feed (Leonardi and Armentano, 2003; Feng et al., 2013), which may lead prevent consumption of nutrients required for maximum health and productivity (Miller-Cushon and DeVries, 2017). Total may also allow farmers to incorporate unpalatable but nutritious ingredients (Coppock, 1977).

In other circumstances, partial mixed rations (PMRs) may be fed instead of TMRs. A PMR contains the majority of the diet fed within a complete mix, but an additional animal-specific proportion of concentrate is provided separately, that is designed to fit the performance potential of the high producing cows (Winnicki et al., 2010). Partial mixed rations are commonly used in dairy farming systems that have adopted the automated milking system (AMS) with free flow parlor visits, since the AMS have pellet dispensers that motivate milking visits (Bach and Cabrera, 2017; Menajovsky et al., 2018).

To obtain homogeneity, rations are formulated using batch mixers which are designed to effectively mix the forages, concentrates, mineral salts, byproducts, and vitamins for dairy cows (Buckmaster, 2009). The development of feed batch mixers (or feed mixer wagons) improved efficiencies and uniformity in feed mixing and delivery. In

a study comparing different mixing feeding wagons, the standard deviations (SD) for mixer feeder wagons using horizontal augers were shown to be 33.7% while mixers with vertical augers 61.3%, suggesting that use of mechanized systems were more efficient feeding options (Vegricht et al., 2007). Improved feeding equipment also enabled capturing of feeding data as farm operations were growing, and ways to manage large herds were needed (Jordan, 2001).

The three main basic designs of feed mixers available in the market include auger mixers, reel mixers and tumble/drum mixers (Turner, 1990; Kammel, 1998). Auger mixer wagons have two or more vertical or horizontal augers that counter-rotate to optimize mixing of feed ingredients. Reel mixers have augers with knife edges that cut long forages, and reels that lift and tumble the feed to provide a mixing action. Tumble mixers also provide lift and tumble action around the circumference of the drum as the spiral action moves the feed towards one direction for remixing or exiting into delivery equipment. In the event of large herds, farmers require larger mixer equipment or more mixing units.

Features of feed mixer wagons vary depending upon brand, which may be designed to incorporate different forms, shapes, and sizes of feed ingredients to obtain a desired feed mix. Other factors that may determine the type or design of feed mixers include power requirements of the unit, cost of unit or parts of it, maintenance, and repair services as well as after sale services offered by distributors. Portable feed mixers are more flexible and best used with flat silos whereas stationery mixers are better with vertical silos and require a conveyor equipment (Buckmaster, 2009).

The advancement of feeding systems came hand-in-hand with the changes in the barn designs and designs of the milking area (Bickert and Light, 1982). Dairy barn design

evolved to enable feeding of large herds. Stanchion barns, tie-stall barns, and free-stall barns are largely utilized in conventional farms. These designs have a feeding alley adjacent to the stall to facilitate housing of large herds and feeding of complete rations along a feeding fence (Schingoethe, 2017). Farms that utilize pasture frequently provide concentrates at the milking parlor and farms. Similarly, farms that utilize automated milking systems (AMS) provide concentrates to individual cows at the milking parlor to motivate cows to visit the milking system (Bach, 2014).

Modern Application of Automated Feeding Systems

Surveys show that 51% of American dairy farmers rely on immigrant farm workers, while approximately 40% of U.S. farm workers are non-U.S. citizens (Adcock et al., 2015). Immigrant workers cannot always be depended upon due to the rising political and social issues regarding race and unregistered workers among other issues. Agriculture is a major factor in the American economy and the inconsistencies associated with relying on immigrant workforce pose major labor availability problems. In fact, future dairy labor projections predict labor shortages and farms will be at risk of shutting down (Susanto et al., 2010). The use of new technology on dairy farms is expected to offset these labor predicaments and result in positive effects on farm growth, labor efficiency, and animal productivity (Gargiulo et al., 2018). Furthermore, animal welfare has become a top concern in the dairy industry as consumers and critics push for more sustainable, animal friendly, healthy, and environmentally friendly systems of dairy production (Alonso et al., 2020). Future production prospects is also another major concern for most dairy producers since

most farmers want to stay in business even as technology continues to advance. For this reason, automated feeding systems (AFS) have been developed.

Automatic feeding systems have been introduced to deliver fresh feed to the feed bunk frequently each day with minimal human labor (**Figure 1.1**). This contrasts with conventional feeding, where feed is manually delivered to cows typically 1 to 2 times per day (Mäntysaari et al., 2006; Mattachini et al., 2019). Automated feeding systems perform technical tasks and concurrently measure and record feeding data with increased precision compared to conventional methods of feeding. Automated feeding systems reduce labor demands by mixing and delivering TMRs to the cows. Additionally, frequent movement of the AFS around the barn may stimulate cow activity, increasing the number of visits to the feeding fence or to automatic milking systems (AMS) (Bisaglia et al., 2012). Feed delivery is known to stimulate cows visiting the feed bunk (King et al., 2016), and the availability of fresh feed at the feed bunk reduces the sorting, an effect that may be beneficial to rumen health (DeVries et al., 2005). Farms that have both the AFS and AMS give cows flexibility to feed and milk at any time of the day (Mattachini et al., 2019).

Automated feeding systems (AFS) were first commercially introduced in 2004 in the Netherlands (Hollander et al., 2005; Bisaglia et al., 2012). They can be either railed-guided or self-propelled feed mixers with programmable feed delivery systems. Initially, AFS mostly included rail-guided mixer wagons but wheel mounted wagons also were developed (Barmore, 2002). The self-propelled mixers have introduced feed delivery to dairy cows every day with minimized human involvement in the mixing or delivery process (Bisaglia et al., 2012). The use of AFS are widely incorporated into European dairy farming

(Bisaglia et al., 2012) and according to farmers and distributors, the AFS is increasingly being adopted by American dairy farmers.

The automated feeding systems for complete rations are available in different designs with varying operating principles from manufacturing companies mainly in North America, Europe, and Japan. They can be programmed to mix, move, and deliver feed at the feeding fence for cows' access, independent of human presence contrary to conventional feeding systems (Belle et al., 2012; Prakash et al., 2015). The primary operating principles for automated concentrate feeders that existed in the 1990s paved way for the development of the AFS (Kazumoto, 1999). In 2009, it was reported that about 300 to 400 farms in Northern Europe, Canada and Japan had adopted AFS (Nydegger and Grothmann, 2009).

Bisaglia et al. (2010) classified pre-industrial prototypes and commercially available AFS based on manufacturer, operating principles, the feeding method, and country of origin (**Table 1.1**). We developed an updated list of AFS systems using a systematic web search and through personal communication with professionals and researchers in the field (**Table 1.2**). The operating principles used by these systems include stationery systems, mobile systems, and self-propelled mixers. Stationery systems mix TMR and rely on conveyors to deliver feed to cattle and fit well where space was limited while mobile systems have temporary storage wagons which are either filled with feed overhead automatically, or by an operator. Mobile systems consist of feed mixing systems powered by line or batteries, as well as feed unloading devices. The self-propelled feeder mixers models consist of wagons fitted with mixing devices, navigation systems and engine, enabling them to be self-propelled as it delivers feed. The average working time of

AFS is estimated to be 50.6 manpower minutes per (MPmin/day) for 60 animals whereas for 120 animals it was 65.2 MPmin/day. One AFS is estimated to deliver feed between 1 time to 15 times in a day (Bisaglia et al., 2010, 2012; Prakash et al., 2015). Commercially available feeding robots use a fully computerized automated system designed to perform their mixing and feeding. The operator loads the “feed kitchen”, a temporary storage area, with different ingredients separately, then an automatic feeding crane mounted with a grabber, will grab feed on demand and load onto an automatic feed mixer wagon. The wagon sits on three wheels, is mounted with mixing augers that continuously blend the feed ingredients loaded until when the blended ration is delivered to the cows (AMS Galaxy USA; Lely Maassluis Netherlands). The robot has a rechargeable battery which facilitate power for mobility along the feeding fence. It is also mounted with ultrasonic sensors that define a route for the robot to follow whereas infra-red sensors detect spaces with low amounts of feed along the feeding fence that need to be refilled. The computerized system records and provides information of all the feeding activities it undertakes on the farm, therefore enabling the farmer to adjust robot functions accordingly.

Adjustments are performed to improve precision and efficiency in functioning and sufficient feed supply to all cows. The operator can set the time intervals for the robot to push the feed and scan the feeding fence to find spots that need additional fresh feed (AMS Galaxy USA; Lely Maassluis Netherlands). This constant rotation throughout the day ensures constant presence of fresh feed for the cows (Karn et al., 2019). Because of the novelty of self-propelled feeding robots within the dairy industry, there is currently a shortage of research looking at the effects of implementation of AFS on dairy farms.

Manufacturers frequently claim an increase in milk production, and milk components due to the increased feeding frequency. However, these results have not been validated.

Automated Milking Systems

Historical Technological Development of Milking Systems

Technologies used for harvesting milk from dairy cows have evolved over time, from the 1800's when cows were milked by hand until today whereby automated milking technology is used. The first known hand-held milking pump was invented by Lee O. Colvin of New York in 1860 (Colvin, 1868). In 1879, Anna C. Baldwin of New Jersey patented the first milking machine, the "Hygienic Glove-Milker", which used elastic rubber teat-cups connected to a bucket and tubes that connected to a suction-pump (Baldwin, 1879). This machine was upgraded into the "Thistle vacuum milker" in 1895 by adding a pulsator to replace the painful constant milking with an intermittent flow (Dick, 1896). The device became a huge success but struggled with sanitation issues. In the 1950s, pipeline milkers were developed for stanchion barns and the milking parlors. Many farms adopted the system despite it lacking the ability to weigh and sample each cow's milk (Senger, 1957). Over time, improvements in milk flow rates, efficiency, cow comfort and safety, and sanitation were made (Blake and McDaniel, 1978). In the 1970s, development towards a fully automated milking system (AMS) was taking shape, pushed mainly by labor limitations in handling large herds of dairy cattle. (Blake and McDaniel, 1978; Spencer, 1989).

In perspective, the conventional milking area comprises of centers that support the process leading to milking and post milking practices (Reinemann, 2021). There is a holding area for cows as they go to milk, the milking parlor, the milk room, a utility room,

and a supplies area. Conventional parlors are available in various designs. In herringbone or fishbone parlors, cows stand in an elevated area on two opposite sides facing away from the middle area where operators work at a 45° angle. Parallel parlors operate similarly to herringbone parlors, except that cows stand side-by-side at right angles to the employees. Rotary or carousel parlors are circular rotating platforms onto which cows walk to be milked. There are several other parlors designs utilized in the industry. These conventional parlors are highly labor dependent, often making up to half of the labor requirements of a dairy farm (Reinemann, 2021). Therefore, AMS were developed to improve functions efficiency. They also provided the additional benefit of maximizing cow comfort by removing holding areas, which represent stressful areas of the milking system, and they eliminate the need to push up all groups of cows to the milking parlor which may interfere with cows feeding and resting patterns (Hermans et al., 2003; Halachmi, 2004).

Automated milking systems (AMS)

The advancement in technology has brought about a ‘smart’ way of handling cattle to improve farm management (Henchion et al., 2022). Equipment such as electronic cow identification systems, herd management software, mastitis detection equipment among other technologies have been incorporated in milking systems to minimize labor requirements as the average herd size in farms are increasing, causing labor demands and management pressure to increase (Gargiulo et al., 2018). Particularly, automated milking systems have been developed to milking strategies on these large farms. Unlike conventional milking systems, automated systems have no fixed schedules but continuously work around the clock each day to milk, allowing room for optimal milking

intervals (Jensen et al., 2018). Constant improvements on automated facility designs is key to enable better management practices and to optimize profitability (Salfer et al., 2017).

Commercial AMS was first introduced in 1992 in the Netherlands. In 2009, about 8000 farms worldwide were using automatic milking systems (de Koning, 2010; Hogeveen and Steeneveld, 2013). Reports in 2015 showed that the number of farms using AMS had grown greatly to 25000 dairy farms (Barkema et al., 2015). European farmers who planned to adopt AMS farming technologies anticipated economic gains and flexibility in labor and reduced need for hired labor. They made adoption decisions based also on other key factors such as capital, industry trends and outlook, and compatibility with available farm resources and regulations (Dijkhuizen et al., 1997). Recent studies in Wisconsin and Minnesota have indicated that the main reasons for AMS adoption were flexibility in schedules which helped to improve lifestyle, labor efficiency and ability to grow without additional labor, and their ability to replace hired labor (Salfer et al., 2017).

Automated milking systems not only harvest milk but also collect data on factors such as milk production and milk components, somatic cell count (SCC), time of milking and milking intervals as well as concentrates offered per cow (Bach, 2014). Experiments conducted by (Svennersten-Sjaunja and Pettersson, 2008) showed that AMS reduced labor by about 18% and increased milk yield by 2-8%. The ability to milk more frequently in AMS has been shown to increase milk production (De Marchi et al., 2017) and to increase free fatty acids in milk associated with shorter milking intervals (Klei et al., 1997). Varying reports have been given on SCC with regards to milking frequency in AMS, including no effect on SCC (Mollenhorst et al., 2011) and increased SCC (Rasmussen et al., 2002).

The design and utilization of AMS goes hand-in-hand with barn designs to accommodate the flow of cows in and out of the milking equipment. Cows are allowed to voluntarily walk into the milking equipment, while additional concentrates are provided at the milking point to motivate cows to visit the AMS (Bach and Cabrera, 2017). The individual robotic device replaces the human operator, and includes automated teat detection and cleaning devices, automated teat cups attachment and milking devices, sensors, computerized control systems and software (de Koning, 2010). Every AMS is estimated to milk about 61 to 75 cows at a milking efficiency ranging between 1.47 to 1.67 kg/min (Aerts et al., 2022).

In AMS utilizing farms, feed management is crucial because it affects visiting patterns of cows to the milking robot. PMR is provided at the feed bank while additional concentrates are given at the AMS to motivate cows to visit as well as to maintain high milk flow per hour in the AMS (Penner et al., 2017). This is based on the findings showing that cows motivation to eat is higher compared to their motivation to milk at the AMS (Prescott et al., 1998), and that more concentrates at the AMS increase visits as well as milk yield. This however may not always be the case in a guided flow or forced traffic system where the amount of concentrate does not necessarily determine the voluntary visits (Salfer and Endres, 2016).

Feeding delivery strategies and cow performance, ration consistency and rumen health, cow behavior and daily rhythms

Cow performance

Dairy cow performance is measured in terms of milk quantity and milk quality. Several factors may determine optimal cow performance, and these include genetics, nutrition factors, cow comfort and health, stage of lactation, environment, herd management among other factors (Dobson et al., 2007). Today, majority of dairy cows in the US are kept in a large herds of over 1000 cows, grouped in dairy barns and rely on farm operators to provide feed (Coppock, 1977; Macdonald et al., 2020). Optimal nutrient availability to dairy cows is essential for maximum cow performance, and feeding strategies continue to be investigated as farming systems change. Automated feeding systems have been developed to replace human operators in feed mixing and delivery to the cows. Understanding variation in production between farms with and without AFS is an essential first step to understanding the value of AFS to dairy producers.

In a study on farms with both AMS and AFS, Belle et al. (2012) reported minimal effects of AFS on visits to the AMS, but reported no data related to milk production or efficiency. AFS feed more frequent times each day than conventional feeding, which may allow better access to fresh feed throughout the day (Mattachini et al., 2019). In experiments with increased frequency of feeding of both forages and concentrates to dairy cows Kaufmann (1976) observed an increase in total feed intake, milk yield and milk fat concentration. A review of 15 published reports on cattle growth and efficiency in food utilization found that increased feeding frequency (FF) significantly increased the average daily gain (growth rate) by about 16% and feed conversion rate by about 19%, (Gibson,

1981). Another review of 35 experiments from 23 research papers found that feeding 4 or more times a day increased food intake, increased average milk production by 2.7% and increased average milk fat by 7.3% (Gibson, 1984). In more recent studies higher FF saw increased feeding time and more visits to the feeding fence (DeVries et al., 2005), while comparing 1x, 2x, and 3x feeding, it was determined 3x feeding led to the highest DMI (Hart et al., 2014). More feeding time and more DMI intake may be associated with increased milk yield (Johnston and DeVries, 2018). Other reports show that higher FF increase milk fat concentration (Rottman et al., 2014). AFS reducing human-cow interactions thus maximize cow comfort, which is associated with increased concentrations of milk components.

Contradictory findings on FF have also been published in literature. For instance, feeding 2x, 4x and 22x showed no significant effect of FF on total feed intake, milk yield or fat concentration (Gill and Castle, 1983). Additionally, feeding 1x, 2x, 4x, and 8x did not indicate significant effects of FF of TMR on the average daily DM intake, milk yield, fat, and protein percent (Nocek and Braund, 1985). The ration formulations in these early studies were notably varied, where some looked at increased feeding frequencies of one supplement or a combination of a few or all feed ingredients such as concentrates, pellets, grains, and forages, whereas more recent feeding strategies mainly involve use of TMRs and PMRs. Understanding feeding frequency is important since multiple anecdotal and scientific hypothesis expect AFS to feed more times in a day than the conventional feeding.

Ration consistency and rumen health

Automated feeding systems were designed to mix and deliver TMRs. Rather than feeding high energy feeds and concentrates separately, TMR consistently supplies all essential nutrients to dairy cows and helps to optimize rumen functions (Coppock et al., 1981). Feeding strategies may also influence rumen health. A study on increased frequency of TMR feeding from 1 time a day to 5 times showed that higher feeding frequency tended to increase energy and protein conversion. Furthermore, frequent feeding reduces rumen ammonia fluctuation essential for microbial growth in the rumen, and water-soluble carbohydrate which is a source of energy (Mäntysaari et al., 2006). Increased feed delivery by AFS may lead to more consistent rations, more consistent feed intake, and therefore more stable rumen pH across the day. However, currently, there is limited research showing whether AFS affect ration consistency or rumen health.

Feed ration inconsistency may alter eating behavior and therefore impact ruminal fermentation patterns causing an increase in synthesis of trans 10, cis-12 CLA. Trans 10, cis-12 CLA limits de novo fatty acid synthesis and may result in milk fat depression. Maintaining optimal rumen fermentation enables a balanced rumen pH, optimizes microbial protein synthesis and increases milk synthesis (Bernard and Tao, 2019). Rumen pH is a major determinant for rumen health because essential microbial growth is best at optimum pH. Subacute ruminal acidosis occurs when ruminal pH levels remain below 5.6 for over 3 h each day. Rumen buffering and conditioning helps to prevent acidosis (Humer et al., 2018). The stabilization of rumen pH leads to less opportunity for the synthesis of trans 10, cis-12 CLA fatty acid isomers by rumen microbes, which lead to increased milk fat synthesis in the mammary gland (Bernard and Tao, 2019). Putting these rumen health

factors into consideration, researchers are beginning to question whether there are any differences in ration and nutrient consistencies at the feed bunk across the day between farms using AFS and CFS. In CFS, feed sorting may take place until the next delivery of fresh feed. Cows prefer to eat the concentrates in a TMR first over the forages (Hosseinkhani et al., 2008), while high intake of fermentable carbohydrates and less intake of effective fiber reduces rumen pH (Miller-Cushon and DeVries, 2017b). As with AFS it is still uncertain whether frequent feed supply to the cows encourages more sorting although we know that frequent feeding stimulates eating (Crossley et al., 2018).

Cow behavior

Feeding strategies may determine the extent of the sorting behavior, standing and lying activity and diurnal feeding and milking patterns. The delivery of fresh feed stimulates cow activity and feeding and affects the standing and lying patterns of dairy cows. Activity monitors are used to measure the standing and lying activities of the cows. When fresh feed is delivered around the milking time, cows stand for a longer period, while cows fed 6 h after milking have increased feeding time with no change in average lying time (DeVries and Von Keyserlingk, 2005). Particle distribution, dry matter content, amount of forage in the feed delivered to cows greatly affects feed sorting. The feed sorting behavior of cows has extensively been determined by researchers to reduce the nutritive value of the ration. TMR with proper particle distribution helps to minimize sorting by availing all ingredients in every feed bite by cows. However, there are many causes for feed sorting by cows besides feeding strategies (Miller-Cushon and DeVries, 2017). This includes the time of feeding at the feed bunk, availability of palatable concentrates at the

AMS and other herd management practices (Prescott et al., 1998). Observing behavior between cows fed using CFS and cows fed using AFS may help in determining differences in cow behavior.

The daily behavioral patterns of dairy cows may be affected by management factors such as the time of feeding and milking, frequency of feeding, pushing up cows to milk among other handling practices. Naturally, grazing cattle display diurnal feeding and milking patterns similar to grouped housing cattle (Sheahan et al., 2013). Precision technology has made it possible to accurately monitor these patterns over time and to characterize alongside their causes. Traditionally, operators would observe and identify animal behavior (Dutta et al., 2015). Researchers have found that dairy cows follow particular daily patterns of feed intake and milk synthesis. The timing of feeding helps to entrain these patterns of feed intake and milk synthesis in dairy cows (Rottman et al., 2014). The number of times feed is delivered to the cows in conventional feeding ranges between 1 to 2 times a day while AFS has a higher FF. The high feeding frequency by AFS may stimulate cow activity since feed delivery to cows stimulates feeding (Phillips and Rind, 2001). Additionally, a more evenly distributed pattern is obtained with increased feeding frequency (DeVries et al., 2005). Research in diurnal patterns of dairy cows have indicated that providing roughages 2 times a day was characterized by peak visits at feed bunks immediately after feed delivery (DeVries et al., 2003). Other practices such as cleaning the AMS and fetching cows have also been associated with affecting visiting patterns to the AMS (Wagner-Storch and Palmer, 2003). The entrainment of daily and seasonal body rhythms of dairy cow's body temperature and milk production by feeding have not been sufficiently explored.

Daily rhythms of dairy cows

Daily rhythms are behavioral and physiological patterns observed by living organisms in a 24-hr cycle in response to light-dark changes (Connor and Gracey, 2011). Circadian rhythms are specific types of daily rhythms that are endogenous to the animal and persist in the presence of constant environmental conditions. The term “circadian” was adapted from Latin; *circa*, meaning ‘about’; and *diēm*, meaning ‘day’, by Franz Halberg in 1959 at the University of Minnesota (Hallberg, 1959). Living organisms have natural timing devices called circadian clocks that develop anticipatory cues in response to daily changes in these environmental factors. The process of forming these anticipatory physiological and behavioral cues to daily patterns is referred to as entrainment (Triqueneaux et al., 2004). Entrainment can be performed by temporal changes such as light-dark cycles or feeding-fasting cycles.

Circadian clocks are hierarchical in nature, with the central biological clock, also known as the “master clock” being in the in the Suprachiasmatic Nucleus (SCN) of the hypothalamus, whereas peripheral clocks are found in the body tissues (Brown, 1976). Endogenous mammalian biological rhythms are orchestrated by the master clock, which perceives time, and predicts optimal environmental factors essential for survival, predation, hibernation, feeding as well as resting (Pickel and Sung, 2020). The master clock consists of two clusters of nerve cells on each side of the brain which fire in self-induced rhythmic patterns involving both activation and repressions of the genes that code for the clock proteins. These transcriptional-translational feedback loops play a significant role in adapting to and maintaining rhythmicity (Koike et al).

There are also peripheral circadian clocks throughout body cells and tissues that drive various system in organs such as the liver and in cardiovascular systems, among others (Mohawk et al., 2012).In dairy cows, orchestration of various metabolic and behavioral function by biological rhythms have been observed. Studies have shown that several physiological variables follow daily rhythms, for example, locomotor activity has been shown to peak at mid light phase, while body temperature follows a rhythm when feeding is restricted to either night or day in dairy cows (Giannetto and Piccione, 2009; Salfer and Harvatine, 2020).

Figures

Figure 1.1 A Lely Vector automated feeding robot delivering TMR and pushing feed closer to the cows along the feeding fence (photo credit: K. Kamau).



Figure 1.2 Daily rhythms of physiological variables within a 24-hr period. (Adapted from Salfer et al. 2021).

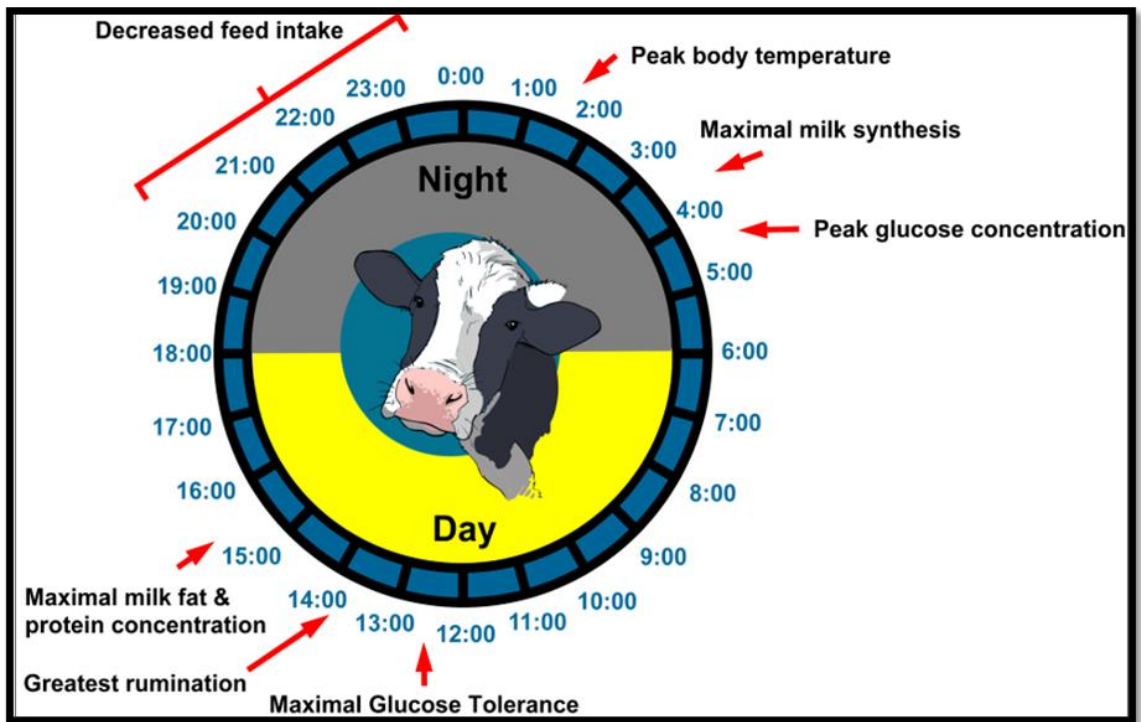
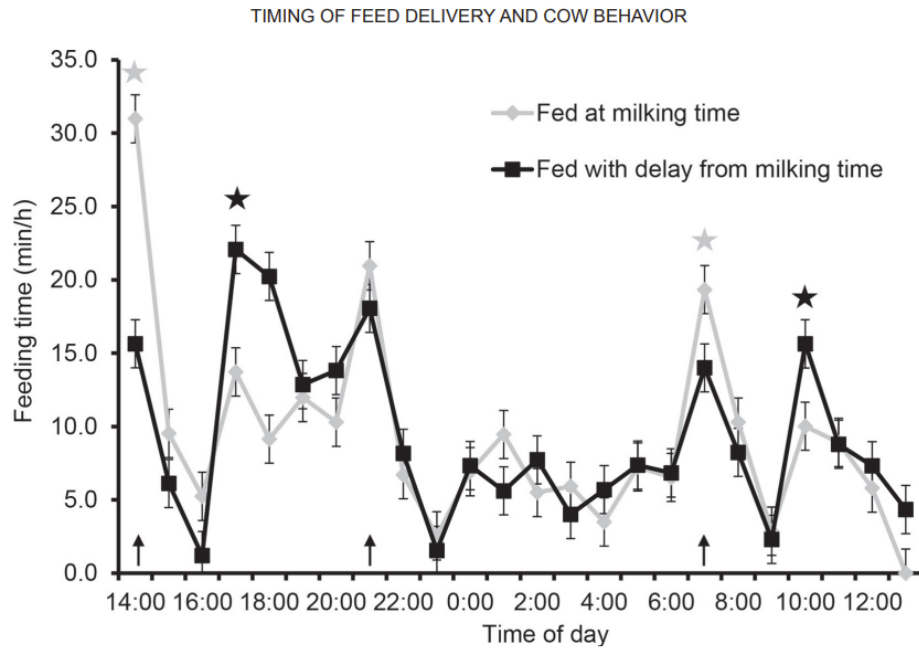


Figure 1.3. Time of feeding affects the daily behavioral patterns of cows (Adapted from King et al., 2016).



Tables

Table 1.1. Commercially available and prototype automatic TMR mixers and feeder devices Adapted from Bisaglia et al. (2010).

Make	Model(s)	Feeding method	Country
Agro Contact	SM 2000, MS 3000	Group	Canada
Agro X	One2Feed	Group	Denmark
Airabo	R.T.M.i	Group	Canada
Cormall	Multimix MTX: (Conveyor and Multi-feeder)	Group	Denmark
De Laval	Optimat	Group	Sweden
Hetwin	Futterungsroboter	Group	Austria
Lely	Atlantis	Individual	Netherland
Mullerp	Mix feeder		Denmark
Pellon	(Mixing device, Conveyor, Filling device, Feeder Robot)	Group	Finland
Rioh	Sputnic	Group	Denmark
Rovibec	Dec DP, Dec SR	Group	Canada
Schauer	Transfeed Dec	Group	Austria
Schuitemaker	Innovado	Group	Netherland
Trioliet	Triomatic	Group	Netherland
ValMetal	D.A.F.	Group	Canada
Wasserbauer	MixMeister	Group	Austria

Table 1.2 Models of Automated TMR feeding robots in 2022, sources from individual company websites

Company	Item/Model	Functions	Mode of operation/ Power system	Information Source/ Website
AMS Galaxy, USA	Aranom Mix	Weighing, Cutting, Mixing, Dosing, and Feed Pushing	Battery Powered Self-propelled	https://amsgalaxy.com/ robotic-cow-feeding/
	Aranom Cut & Mix	Weighing, Mixing, Feeding and Feed Pushing	Battery Powered Self-propelled	
Lely	Lely Vector	Feed mixing, Feed delivery, and pushing feed	Battery powered Self-propelled	https://www.lely.com/u s/
Schauer Perfect Farming Systems	Feedo Feed Belt	Feeding	Radio remote controlled	https://en.schauer- agrotronic.com/schauer
	Transfeed Dec Feeding Robot	Mixing and feeding	Rail-guided	
	Transfeed Rover Feeding Robot	Mixing, cutting, feeding	Battery Powered Self-propelled	

Trioliet Feeding Technology	Triomatic WB battery-powered robot	Feed mixing, feed delivery and feed pushing	Battery Powered Self propelled	https://www.trioliet.us/products/automatic-feeding-systems
	Triomatic WP Wheel Driven Feeding Robot	Mixing, feeding and feed pushing	Uses Overhead Power rail	
	Triomatic HP Suspended Feeding Robot	Feed mixing, cutting, feed delivery and feed pushing		
DeLaval	DeLaval Optimat™ system	Feed mixing and feed distribution	Rail suspended distribution wagons (DeLaval FS1600 or DeLaval RA135)	https://www.delaval.com/en-gb/discover-our-farm-solutions/feeding/delaval-optimat/
	DeLaval Optimat™ master	Cutting, mixing and feed distribution	Rail-suspended wagon	
Wasser Bauer	Shuttle Eco	Weighing, mixing and feed delivery	Battery Powered Self Propelled	https://wasserbauer.at/en/products/shuttle-eco
Rovibec Agrisolutions	Rover Feeding Robot	Loading, mixing and distributing feed	Rail-guided	https://rovibecagrisolutions.com/en/produit/feeding_robot_autonomus_rover
Cormall	Multimix MTX: (Conveyor and Multi-feeder)	Mixing, feed delivery and feed pushing	Uses Fuel Self-Propelled	https://www.cormall.dk/produkt/feed-robot-multifeeder/
Agro Contact	SMD5100	Mixing and feed delivery and pushing feed	Hybrid Power (Gasoline and Battery)	https://www.agrocontact.net/produit/smd5100/

2. THE IMPACT OF SEASON ON THE DAILY RHYTHMS OF BODY TEMPERATURE, MILK YIELD, MILK COMPONENTS

Abstract

Suggestions have been made that daily rhythms of temperature and behavior in dairy cows differ among seasons. The photoperiod and temperature changes impact different aspects of daily dairy cow patterns. This experiment therefore intended to investigate the effects of season on daily rhythms of body temperature and milk synthesis in dairy cows. The hypothesis of the study was that the daily rhythm of body temperature and the morning to evening ratio milk of yield would differ by season. We expected that the highest amplitude of body temperature rhythm would occur in summer and that the lowest amplitude would be in the winter season. We measured body temperature, standing and lying behavior, and milk production of cows during each of the 4 seasons. We also hypothesized that cows would spend less lying time during the summer than in the winter. In this experiment, 147 mid-lactation Holstein cows were housed in a tie stall barn and were grouped into a randomized block design for a period of 1 year covering all the four seasons (n = 30 to 44 cows per season). Body temperature data and activity were collected. Milk yield was collected at 0500 AM and 1700 PM, and the ratio of AM to PM milk yield and milk components was used as a proxy for the daily rhythm of milk synthesis. Daily rhythms of body temperature and daily rhythms of cow activity were determined by fitting data to the linear form of cosine functions with a 24 h period in R version 4.0.2. A zero-amplitude test was used to determine the fit of daily rhythms. We observed that body temperature fit a rhythm in the fall, spring, and summer but not in the winter, with spring

having a lower amplitude rhythm than fall and summer ($P < 0.05$). The peak of the daily rhythm in summer (0025) occurred 2 and 4 hours later than spring and fall, respectively ($P < 0.05$). Season tended to affect the AM:PM ratio of milk yield ($P = 0.09$). Season did not affect the AM:PM ratio of fat or protein yield or concentrations ($P > 0.10$). These results suggest that while season impacts daily rhythms of body temperature, it has minimal effects on daily rhythms of milk yield.

Introduction

The biological rhythms of various physiological and behavioral factors that occur in most living things are entrained by environmental events such as light-dark cycles, photoperiods in the various seasons, oxygen levels, temperature among other factors (Zheng et al., 2021). These rhythms occur so that living things can naturally anticipate changes in their environment in order to adapt to their surroundings (Duffy and Wright, 2005; Panda, 2016). Natural expression of daily rhythms and seasonal rhythms occur when light-dark cycles, feeding cues and temperature cycles interact with cell-autonomous biological time clocks in the body. The major role of these clocks is the orchestration of metabolic processes in the body (Dibner and Schibler, 2015). Management factors like feeding schedules also entrain peripheral rhythms of reproduction, metabolic functions and predator-prey interactions (Damiola et al., 2000; Dominoni et al., 2016). Dairy cows naturally demonstrate consistent patterns of daily feeding and milk synthesis, and changes in metabolic signals may affect these patterns.

Chronobiological research has demonstrated several factors that determine cow performance exhibit daily rhythms. For example, rectal temperature has a robust daily rhythm that peaks at 2037 h (dark phase), the locomotor activity peaks in the mid-light phase of the day while blood metabolites urea peaks early light-phase (Giannetto and Piccione, 2009). The daily rhythms of body temperature observed in domesticated dairy cows differ between different seasons of the year, and is consistent with findings on human rectal temperature (Bitman et al., 1984; Honma et al., 1992; Giannetto and Piccione, 2009). Furthermore, milk yield, and milk fat, lactose, and protein concentration all have been shown to fit daily rhythms (Salfer and Harvatine, 2020).

Entrainment of peripheral rhythms may occur as a consequence of management factors on dairy farms. For instance, cattle disturbances caused by push-up to milk or during sorting and moving of cows, and the standing-lying behavior of cows impact milk productivity by interrupting their daily rhythms (Ramón-Moragues et al., 2021). The effects of time of feeding, feed intake and time of milking on rhythms in cows have been substantially characterized. Cows fed total mixed rations have exhibited diurnal patterns of feed intake (DeVries et al., 2005). Milk yield peaks in the morning and milk components peak in the evening when feeding cows is restricted to daytime, whereas, milk peaks in the evening and components in the morning when feeding cows is restricted to night time (Salfer and Harvatine, 2020).

Timing of feeding therefore affects time of peak milk synthesis, a key determinant of milk production, and also modifies the peak in feeding behavior, plasma metabolite and temperature (Stafford et al., 1994; Rottman et al., 2014). Daily peak feed intake in cows occurs twice, at dawn and at dusk (Albright, 1993). Time of food intake can also alter coordination between the master time clock in the brain and the body peripheral clocks resulting in metabolic disorders like insulin resistance and obesity (Honma et al., 1983; Takahashi S. Joseph et al., 2008). Short time frequent milking at intervals of 4hrs for 2 days exhibit distinct patterns of milk synthesis, a factor may be useful for robotic milking systems where cow milking is voluntary (Van Der Lest and Hillerton, 1989).

Seasonal photoperiodic and temperatures changes impact social behavior, nutrition, reproduction, and productivity of cows. Patterns of milk production in 4 regions of the United States indicate that peak milk yield occurs in spring while peak fat and protein occur in winter (Salfer et al., 2020). Cows' feed intake decreases in the summer while cows spend

more time lying down in fall and winter compared to summer (Cerné et al., 2016; Rejeb et al., 2016). Season therefore influences the daily behavior and physiology of dairy cows (Provolo and Riva, 2008).

The mechanisms underlying daily rhythms in cows have been studied for years, but there is increasing interest to determine ways to incorporate rhythms in day-to-day cow management in order to increase farm efficiency. Reports by producers and consultants suggest that daily rhythms of cow body temperature and cow activity differ between seasons of the year. In this experiment therefore we intended to investigate whether daily rhythm of body temperature, milk yield and milk components differ with seasons.

Materials and Methods

Experimental facility and animals

The experiment was conducted over 1 year beginning in Spring 2017 through Winter of 2018, at The Pennsylvania State University (University Park, PA). All procedures using animals were approved by The Pennsylvania State University Institutional Animal Care and Use Committee. The cows were housed in a free stall barn with natural ventilation all year round. One hundred forty-seven mid-lactation Holstein cows were utilized in this experiment randomized block design, with 30 to 44 cows used per season. Within each season, cows were assigned to one of three blocks of time, with each block lasting one-week, beginning at approximately the start of each season. Ambient temperature, humidity, and light intensity of the barn were monitored during each data collection period using remote data loggers (HOBO Pendant Temperature/Light 64K Data Logger; Onset Technologies; Bourne, MA). Natural light was utilized during the day, while

controlled lights were turned on approximately at 1700 h and turned off at approximately 2400 h. Cows were fed a TMR 1x/d at 0800 h and orts were removed each day. All the cows were milked twice each day at 0500 h and 1700 h using conventional milking systems.

Data Collection

Vaginal temperature was continuously monitored using temperature probe loggers (iBCod; Alpha Mach, Inc.; Sainte-Julie, QC, CAN) mounted on indwelling progesterone-free controlled internal drug release devices (CIDR; Zoetis Inc.; Parsippany-Troy Hills, NJ). Temperature was observed at 10-minute intervals for 3 weeks beginning on the first day of the experiment. The vaginal temperature probe was used to eliminate possible bias from interaction with humans, and to reduce labor.

Total standing time, lying time, and lying bouts was collected using 3-dimensional accelerometers called Hobo Pendant G data loggers (Onset Computer Corp., Bourne, MA) at 60 seconds recording intervals. The HOBO data logger device was attached to the lower part of the right hind leg of each cow using an adhesive medical bandage wrap and was set to record the x-axis, y-axis, and z-axis tilts for the entire experimental period. Data were downloaded using Onset HOBOWare Software and exported to Microsoft Excel®.

Milk samples were collected at each milking, at 0500 h and 1700 h and stored at 4°C. Milk samples for analysis of fat and protein were collected on the last day of each block of each season. Fourier-transform Infrared spectroscopy was used to analyze fat, protein, lactose, and milk urea nitrogen concentrations (Dairy One DHIA, PA). Total daily milk yield for all the seasons was compiled for analysis.

Statistical Analysis

Data were analyzed using the linear mixed effects model *lme* package in R, version 4.0.2. Cosine rhythmometry was used to analyze the daily rhythms of body temperature. Time-course data were fit into the linear form of cosine functions with a 24 h period (Bourdon et al., 1995). Fixed effects of season and random effects of block nested in season, cow, and period were included in the models. The mean, amplitude and acrophase of rhythms of body temperature were determined via cosinor analysis and used to describe rhythms. The mean is the midline of the rhythm within each season, the while the amplitude is the difference between peak and mean of each daily rhythm, and the acrophases is the time at which the rhythm peaks as illustrated on **Figure 2.1**. A zero-amplitude test of fit of rhythm and rhythm robustness was conducted by comparing the full model with a reduced model using *F-test*. The ratio of AM to PM milk yield and milk components was used as a proxy for the daily rhythm of milk synthesis using the following equation:

$$\text{AM: PM ratio} = \frac{\text{Morning Yield or Components}}{\text{Evening yield or components}}$$

A *P*-value of < 0.05 was considered significant while 0.05 < *P* < 0.10 was a tendency for significance.

Results and Discussion

The daily behavior and patterns of dairy cows is affected by seasonal changes in photoperiods and temperature. Environmental temperature influences the physiological response of cows and is used to measure thermal stress. Higher external temperatures

increases cows' respiratory rate and rectal temperature, while the ability of cows to cool by dissipating heat reduces (Das et al., 2016; Leliveld et al., 2022). Excessive temperature-humidity index reduces dry matter intake (DMI) and milk yield. Seasonal variation in temperature also causes the variation of milk composition throughout the year, with lower fat and protein content observed in the summer than in winter (Finch, 1986; West, 2003). The effect of season on the daily variation of milk production, milk components and body temperature have not been well-documented, hence this study.

In the current experiment, body temperature fit a daily rhythm in fall ($P < 0.0001$), spring ($P < 0.0001$), and summer ($P < 0.0001$), but winter did not have a significant rhythm ($P < 0.12$; **Table 2.1**). The amplitude of winter and spring were less robust while the amplitude of fall and summer were more robust ($P < 0.0001$). The zero-amplitude test indicates that the seasonal body temperature changes for spring, summer and fall fit a daily rhythm while that of winter did not fit a rhythm ($P < 0.12$). The acrophase (time at peak) occurred at approximately the same time period in fall (2047 h), winter (1856 h) and spring (2104 h), while the peak of rhythm in Summer occurs slightly after midnight (0028 h ($P < 0.05$)). The illustration on Figure 2.1 shows the seasonal temperature means, time of peak and nadir occurrence and the amplitude robustness by season. There was a tendency of season to affect the daily morning to evening ration of milk yield ($P = 0.09$) Figure 2.3. The >1 (greater than 1) ratio indicates that changes in season seemingly causes a higher morning milk yield than evening milk yield, especially in the summer fall and winter, than spring. There was no indication of potential effects of season on the daily variation of milk components (fat, protein, lactose, and milk urea nitrogen (MUN) ($P < 0.05$)).

The lying and standing behavior of cows is often used as an indicator for health and well-being. The lying duration of cows is generally shorter in the day than night and in the summer because the summer heat stress causes cows to stand to increase surface area for cooling (Zähner et al., 2004; Leliveld et al., 2022). In this study, we found no effects of season on the standing-lying activity and lying bouts of cows. The average standing time (hours) and lying bouts in the summer were expectedly higher than in the fall, winter, and spring. Animals under heat stress spend less time resting, typically 9 to 12 hours per day (Tullo et al., 2019; Ramón-Moragues et al., 2021). Animal standing and lying behavior is often a good indicator of cow comfort (Overton et al., 2002).

Conclusions

Seasonal changes cause daily temperature variation that may affect the physiological responses by dairy cows. It was evident in this study that temperature changes by season affect the daily rhythms of body temperature in dairy cows. The largest temperature daily rhythm amplitude was observed in summer while winter did not fit a daily rhythm. We did not observe any effects of season on the daily rhythms of milk components, but season tended to affect daily rhythm of milk yield. The average daily standing-lying time and daily lying bouts were not significantly affected by season.

Figures

Figure 2.1. An illustration identifying rhythm parameters. The period indicates the time used to complete one cycle. The amplitude refers to the peak of the rhythm from the mean or (peak minus mean), while acrophase is the specific time at which the peak occurs.

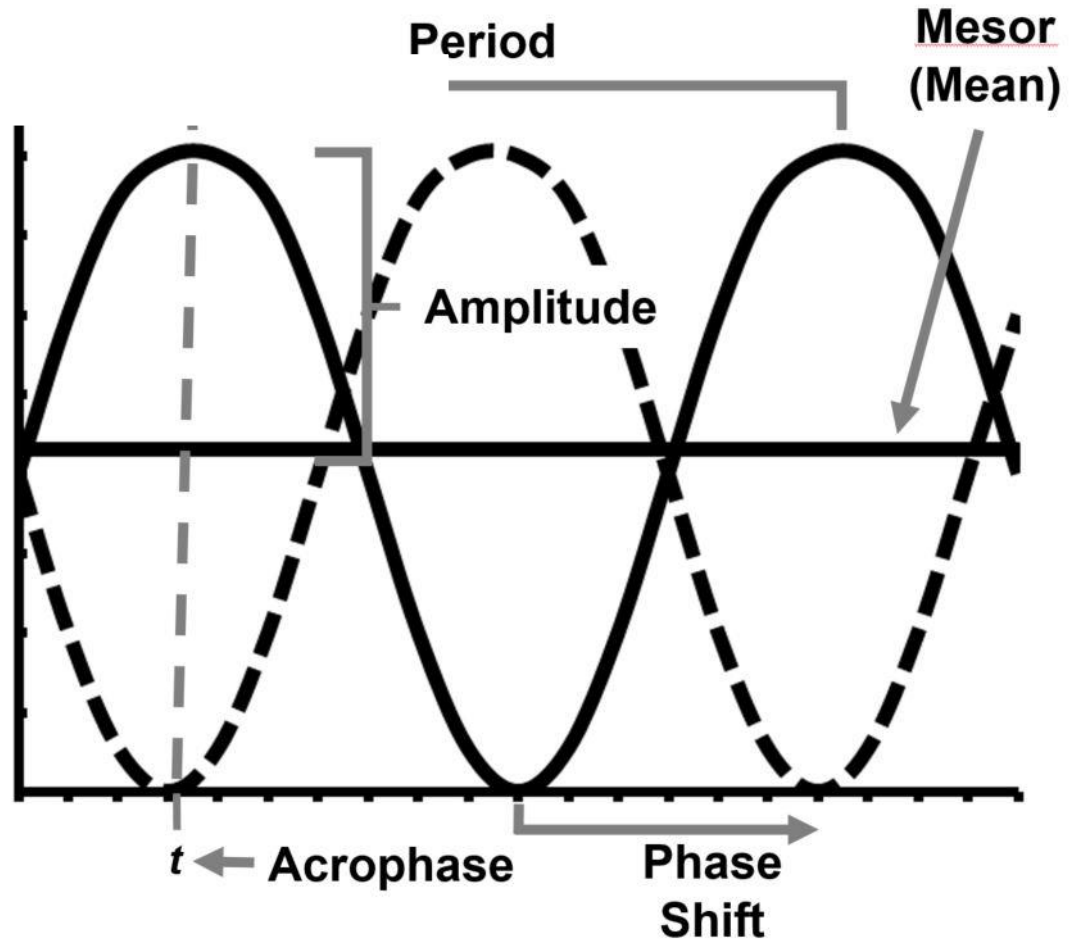


Figure 2.2 Daily body temperature rhythm characteristics with respect to season.

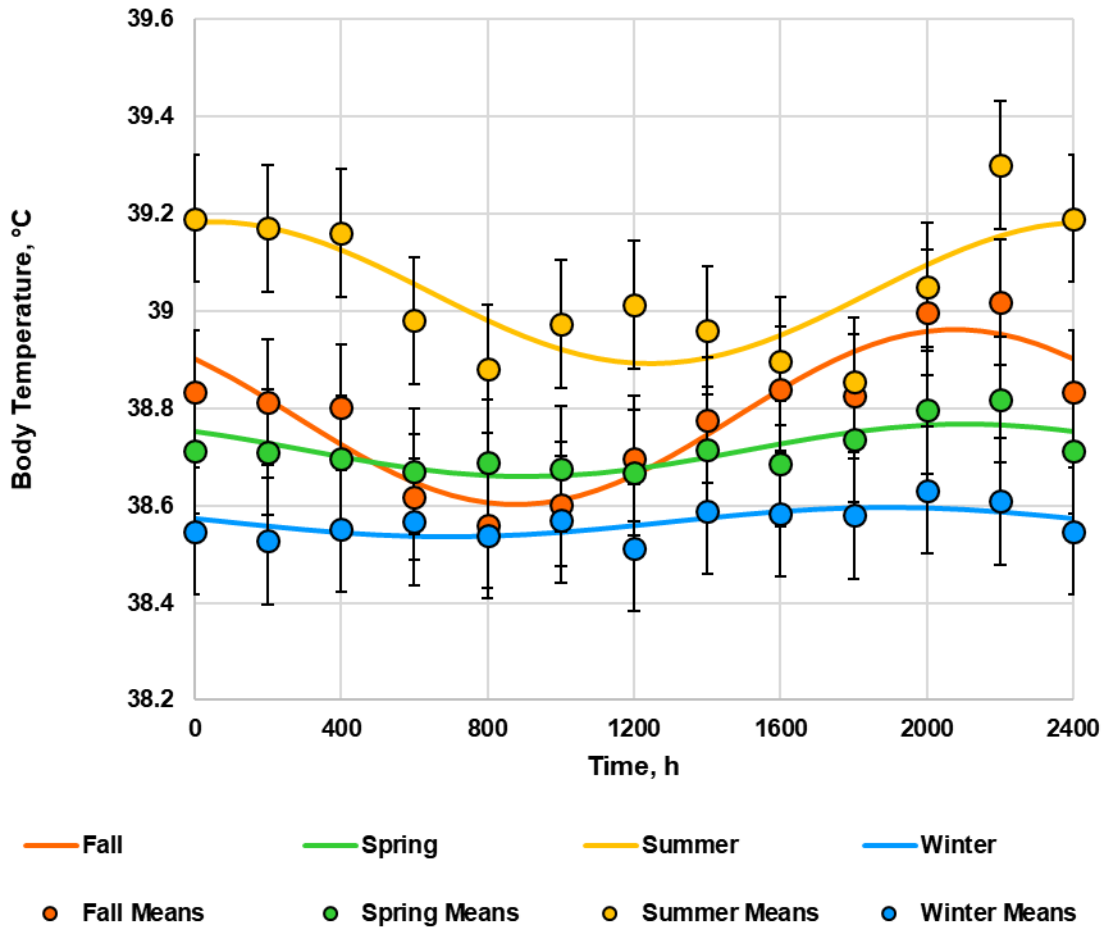


Figure 2.3 A graph comparing the daily ratio of morning to evening milk production between seasons.

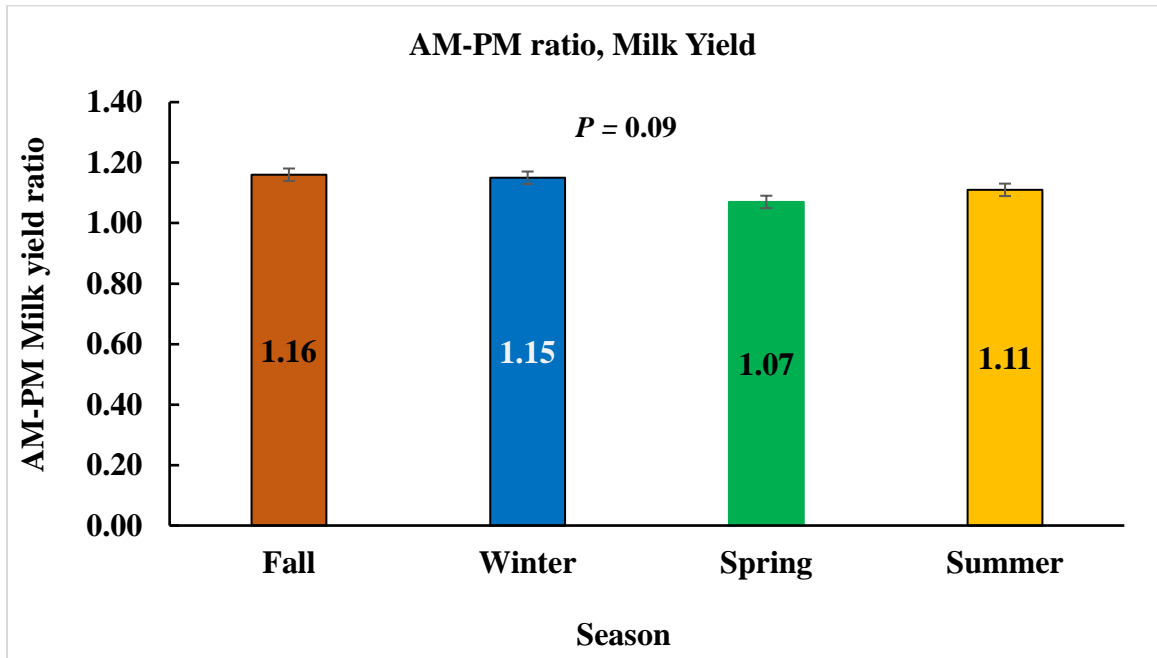


Figure 2.4 A graph comparing the morning to evening ration of daily milk fat percent between seasons.

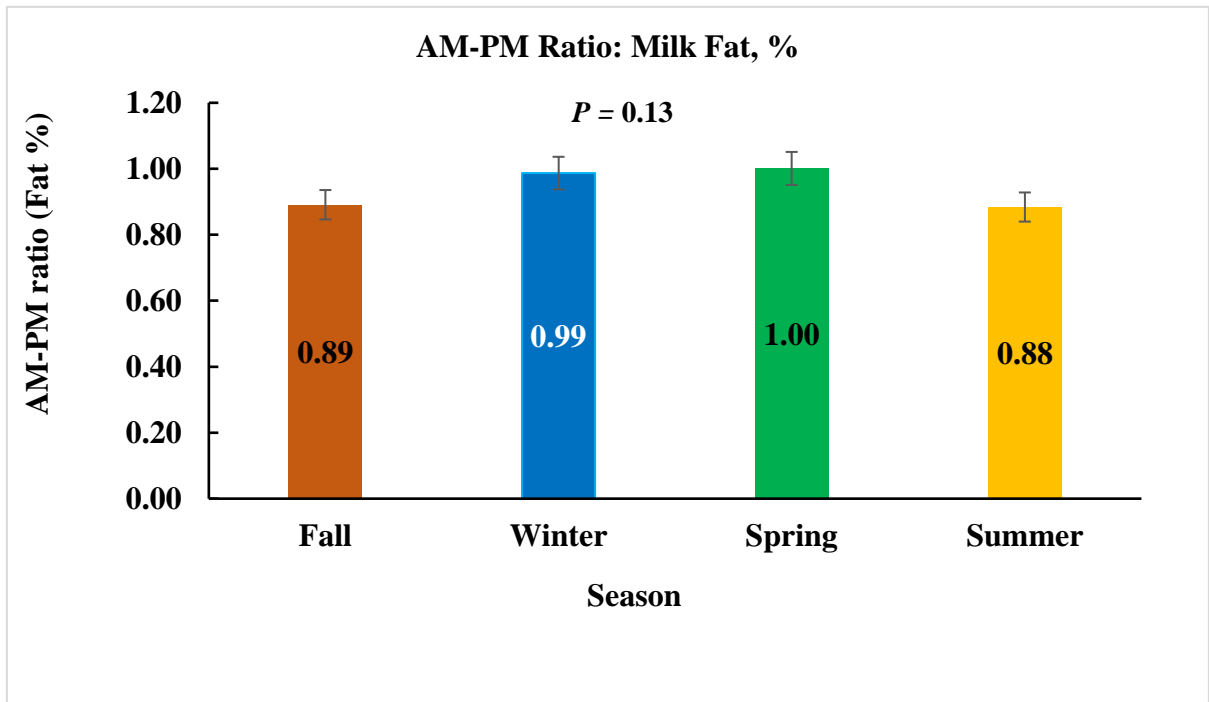


Figure 2.5 A graph comparing the morning to evening ration of daily milk protein percent between seasons.

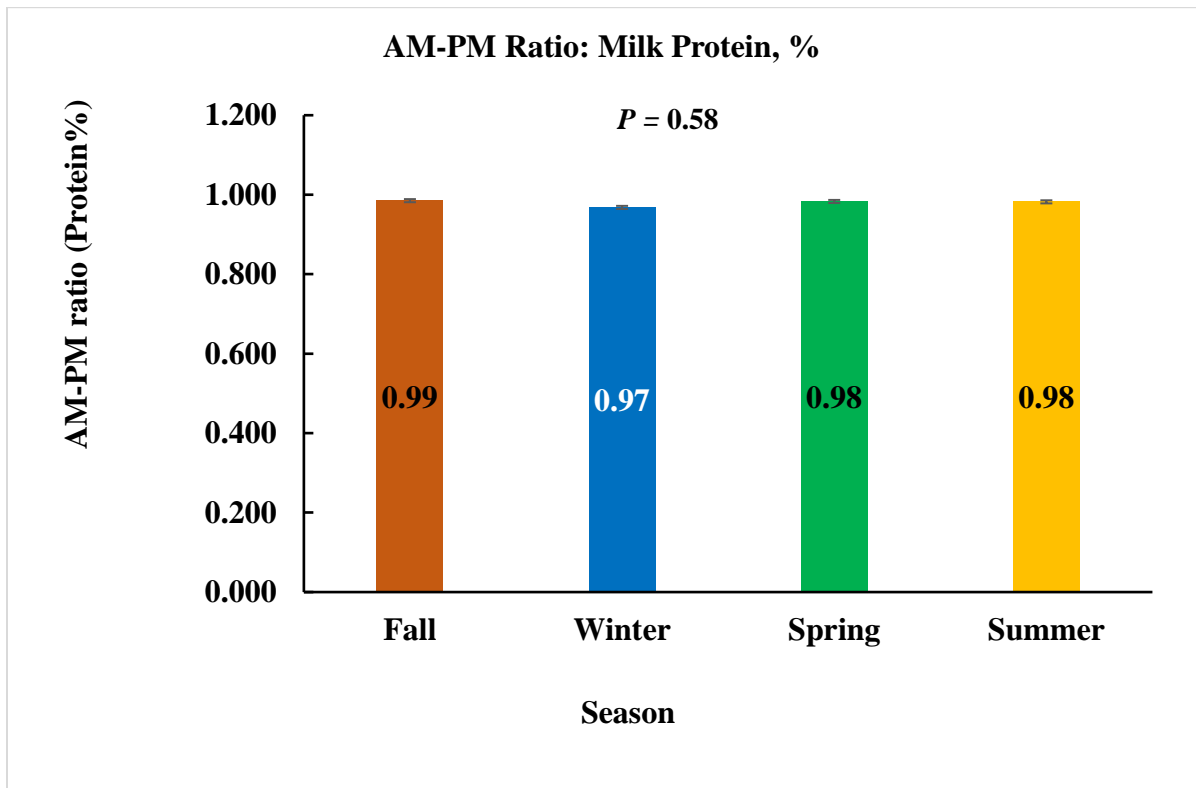


Figure 2.6 A graph showing the daily morning to evening ratio of milk lactose between seasons.

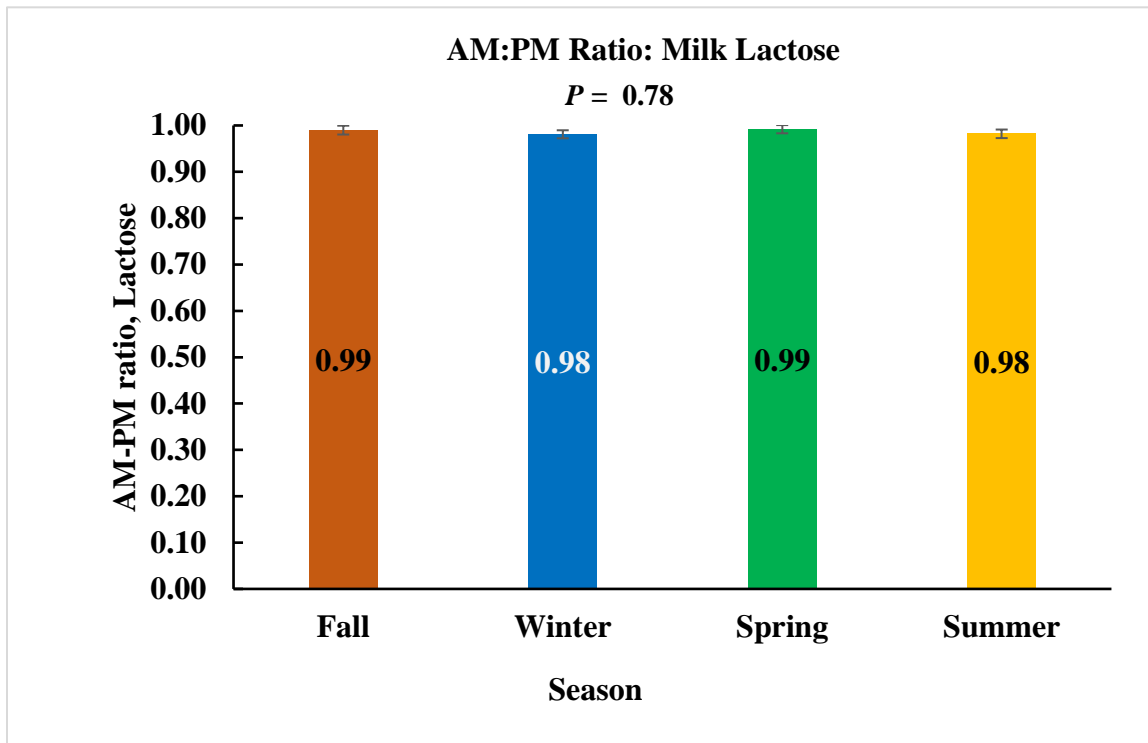


Figure 2.7. A graph showing the effects of season on lying bouts (number of times a cow lies down in a day).

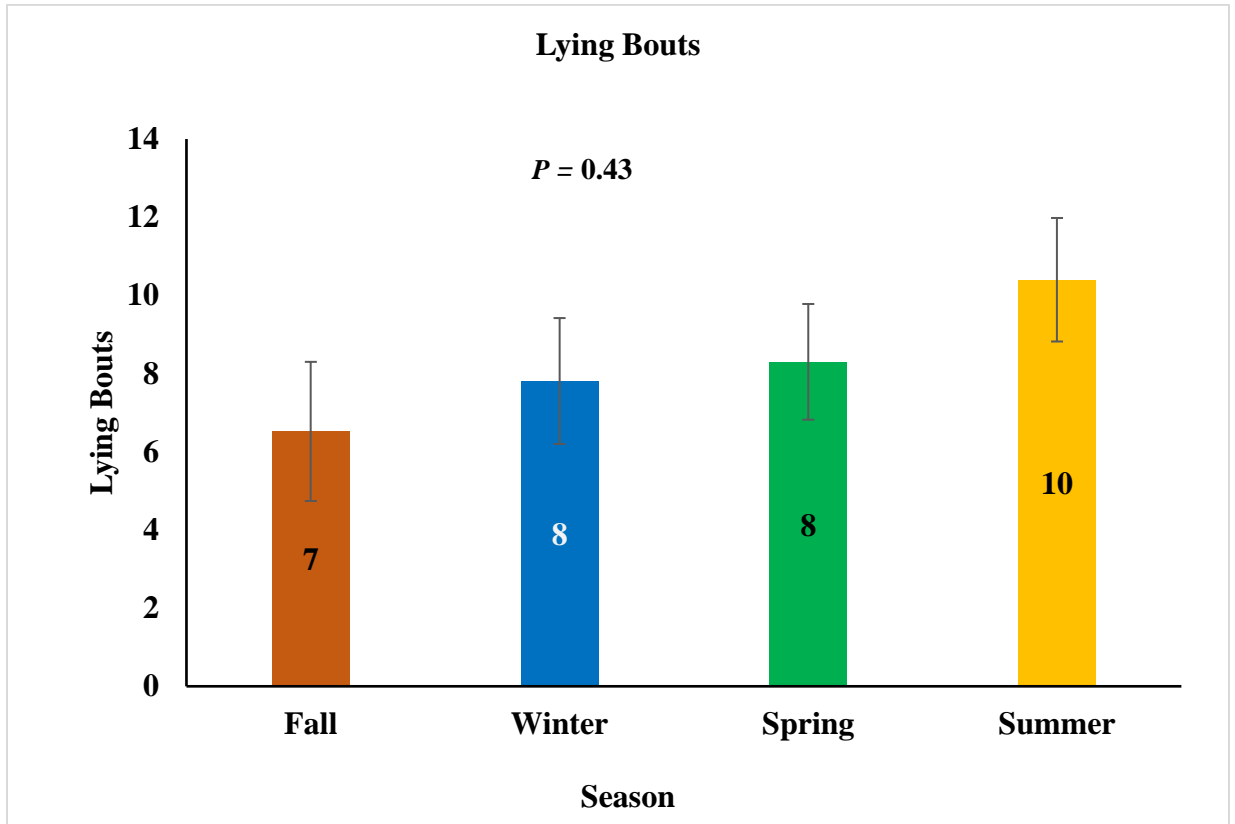
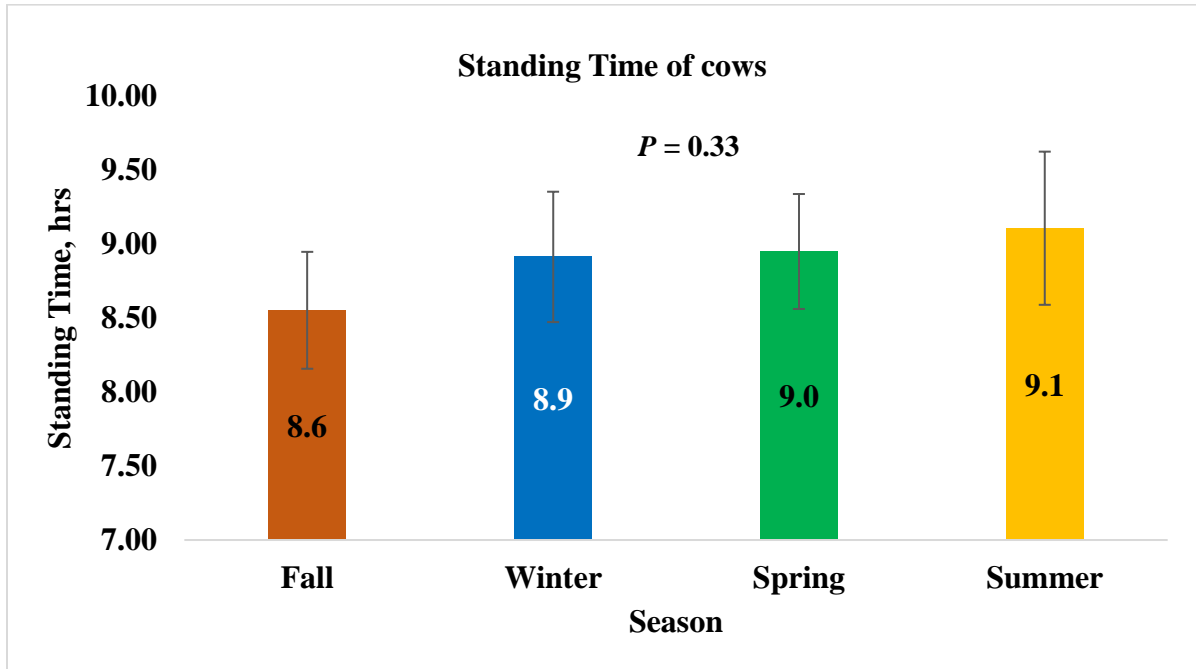


Figure 2.8. A graph showing the effects of season on the daily standing time of cows.



Tables

Table 2.1. Daily body temperature rhythm characteristics with respect to season.

Season	Mean ¹	SEM ²	Amplitude ³	Acrophase (hrs.)	<i>P</i> -Value ⁵
Fall	38.85	0.72	0.18 ^a	2047 ^a	0.0001
Winter	38.71	0.06	0.03 ^c	1856 ^a	0.12
Spring	38.78	0.06	0.05 ^c	2104 ^a	0.0001
Summer	39.02	0.05	0.14 ^b	0028 ^b	0.0001

¹Least-squared means of body temperature

²Standard Error of the Mean

³Difference from peak to mean

⁴Time at peak of 24 h rhythm

⁵*P*-value of the zero-amplitude test determining rhythm fit

3. EFFECTS OF AUTOMATED FEEDING SYSTEMS ON COW PERFORMANCE AND BEHAVIOR, AND TMR CONSISTENCY

Abstract

Automated feeding systems are increasingly used in North American dairy farms to reduce dependency on human labor and potentially improve feeding efficiency and cow health. Automated feeding robots (AFR) mix, deliver and push up feed to the cows 1 to 14 times throughout the day. The study objective was to investigate the impact of AFR (Vector, Lely Industries N.V., Maassluis, The Netherlands) on milk components and ration consistency across the day. The experiment was conducted on 16 commercial dairies with automated milking systems in the upper Midwest region of the United States. The experiment used a randomized complete block design with 8 blocks based on breed, herd size and geographic location. Each block had 2 herds, with each treatment (AFR vs non-AFR) represented once per block. The average herd size was 78 cows. Partially mixed ration (PMR) samples were collected at 4 equally spaced timepoints (0500, 1100, 1700, and 2300 h) for 3 consecutive days and analyzed for DM, CP, ADF, NDF, ash, and particle size distribution. The coefficient of variation (CV) of dietary nutrient composition among the 4 time points was determined for each day. Bulk tank milk samples were collected once per day for 3 days and analyzed for fat, protein, MUN and lactose. A linear mixed effects model in lme4 package of R (ver. 4.0.2) tested the fixed effects of feeding system, block, and the random effect of day nested within block. Significance was determined at $P < 0.05$ with a tendency declared at $0.05 < P < 0.10$. The CV of feed bunk DM, ADF, NDF and

lignin was lesser in AFR herds ($P < 0.05$). Milk fat, protein, and MUN ($P > 0.10$) were not different between AFR and non-AFR herds. Lactose concentration was greater in AFR herds ($P = 0.02$). The effect of treatment on the CV of particle size distribution was not significant. These data suggests that AFR systems can improve ration consistency throughout the day compared to conventional feeding systems.

Introduction

Dairy farm automation is growing worldwide as researchers and dairy producers embrace precision farming which is defined by the use of information and communication technology for real-time livestock monitoring (Berckmans, 2015; Henschion et al., 2022). The first commercial use of automated milking systems (AMS) was in 1992, while automated feeding systems (AFS) were first commercially used in 2004 (Hollander et al., 2005; de Koning, 2010; Bisaglia et al., 2012). Adoption of AMS and AFS has since expanded in Europe and North America as farm herd sizes increase, labor costs rise, while farmers make efforts to maximize efficiency, yields and profits (Bisaglia et al., 2012; Bach and Cabrera, 2017). These systems utilize robotic technology to milk cows, mix feed rations and deliver feed to cows, contrary to conventional ways that directly involve human operators (Karn et al., 2019).

Automated feeding systems have the advantage of increasing the frequency of feed delivery. The AFS deliver feed an average of 7 times a day while most farms deliver feed only 1 to 2 times each day with CFS (Bisaglia et al., 2012). Dairy cattle discriminate against long forages for smaller feed particles and the frequency of feed delivery is shown to influence feed sorting (Endres and Espejo, 2010; Feng et al., 2013). Feeding more than 1 x/d reduces feed sorting and decreases time for TMR sorting. Cows fed 3x/d reduced sorting against fine particles (DeVries et al., 2005; Hart et al., 2014). The total amounts of ration provided to cattle and the time period of feeding determine the extent of feed sorting (Miller-Cushon and DeVries, 2017). Frequent feed delivery by AFS may allow consistent particle ration distribution at the feed bunk and also avail freshly mixed TMR frequently throughout the day (Jordan, 2001). Particle size distribution has been evaluated to

determine the amount of feed sorting. The method of feed mixing may influence the uniformity among and within batch mixes and consistency of feed ration delivered every time to cows (Buckmaster, 2009). Manufacturers of mixing equipment provide recommendations and guidelines for how to achieve desired rations. Human operators can cause variability in mixing because of varied length of mixing times, forgetfulness, or distraction, change of order of adding ingredients. Automated mixer-feeders, however, follow programmed procedures and minimize errors (Schingoethe, 2017).

The feed ration composition, ration consistency and frequency of feeding are important for regulation of rumen pH. Frequent feeding leads to more consistent consumption of fresh TMR and subsequently more consistent rumen composition of fiber concentrates intake, which reduces fluctuations in rumen pH. More consistent rumen pH reduces milk fat depression and acidosis because of energy balance and reduced organ inflammation (Kaufmann, 1976; Macmillan et al., 2017; Benchaar and Hassanat, 2021). Macmillan et al. (2017) showed that 3 x/d feeding increased milk fat yield 1.22 kg/d compared to 1x/d feeding 1.08kg/dl, and that higher feeding frequency minimizes chance for sub-acute ruminal acidosis. Increased feeding frequency also increases milk yield by 2.7% and milk fat percent by 7.3% according to Gibson (1984). Cow milk production and milk nutrient composition is affected by nutritional factors as well as social behavior of cows.

The use of AFS in dairy barns affects the movement activity, flow to the AMS, and standing-lying time of dairy cows. Anecdotal evidence suggests that feed delivery by AFS stimulates cows to go to the feed bunk. These observations would be consistent with research demonstrating that delivery of fresh feed delivery stimulates cows to eat (Phillips

and Rind, 2001). The time of feed delivery affects length of feeding and distribution of lying time. The delivery of feed immediately after milking causes increased feeding time by 82% while feeding 6 hours after milking shows 4 peaks of cows' lying activity (DeVries and Von Keyserlingk, 2005). In the dairy barns with a free-flow system, frequency, and time of feeding by AFS affects time of visit and number cow visits to the AMS compared with CFS farms that utilize AMS. (Belle et al., 2012).

While the use of automated feeding robots is expected to increase feed bunk nutrient consistency and are expected to increase number of meals consumed by cows per day which may result in decreased incidence of milk fat depression, to our knowledge, these effects have not been studied. The objective of our study was to compare farms using AFS or CFS based on feed bunk nutrient and particle distribution consistency, and milk yield and milk components. We also examined total feed intake, rumination, and AMS visit patterns of the cows. We hypothesized that the use of automated feeding system would reduce feed ration variability, improve rumen health, milk components and milk productivity.

Materials and Methods

Farms and Data Collection

The experiment was conducted from September 2021 through January 2022. Sixteen dairy farms within the Midwest region, in Minnesota, Iowa and Wisconsin were assigned to one of 8 blocks based on geographical location, breed, and herd size. Within each block 1 herd utilized used AFS while the other 8 used CFS. The AFS used in all the

farms was the vector robot (Vector, Lely Industries N.V., Maassluis, The Netherlands). All farms had used automated milking systems (AMS; Lely Astronaut V3 or V4; Lely North America; Pella, IA). All the farms had a free flow system to the AMS. Average herd size was 78 cows, and all herds had Holstein as the predominant breed (**Table 3.2**). The main characteristic of experimental herds in this study.

Feed bunk samples of PMR were collected at the two farms within each block at four equally spaced time points each day (0500, 1100, 1700, 2300), for three consecutive days. Subsamples were collected along the length of the feed bunk in an evenly spaced zigzag pattern. Particle size separation for each sample was performed using the 4-compartment Penn State Particle Size separator according to (Kononoff et al., 2003). Samples were then frozen at -20°F prior to nutrient analysis. Bulk tank milk samples were collected into 50 ml vials one time each day per herd for three consecutive days. Samples were frozen immediately after collection. Feed samples were analyzed at the DHIA laboratories (in Sauk Centre, Minnesota) for dry matter (DM), crude protein (CP), acid detergent fiber (ADF), neutral detergent fiber (NDF) and ash. Milk samples were analyzed for fat, protein, milk urea nitrogen (MUN) and lactose using Fourier-transform Infrared Spectroscopy (Minnesota DHIA, Sauk Centre). Data from the automated milking system and activity monitors including milking intervals, successful milkings, refusals, milk yield per cow per visit, feed intake at parlor, rumination time, fat, and protein concentration were obtained from the Time for Cows (T4C) management program (Lely North America, Pella, Iowa).

Statistical Analysis

Data from T4C were filtered for blank measurements and checked for outliers in Microsoft Excel version. The data were then analyzed using R software version 4.0.2. using the ‘nlme’ package. A linear mixed model (lme function) was used with the fixed effects of feeding system (AFS vs. CFS), block, and the random effect of day nested within block. The effect of feeding system on visits to the AMS, milk yield milk components and rumination were determined. The coefficient of variation (CV) of feed bunk particle size distribution and feed bunk nutrients were determined in Microsoft Excel®. Significance was determined at $P < 0.05$ and a tendency for significance declared at $0.05 < P < 0.10$.

Results and Discussion

Particle size distribution and nutrient composition

The coefficients of variation (CV) of feed bunk particle size distribution across the day for each sieve were compared between AFS and CFS **Figure 3.1**. The upper sieve content variability was slightly higher in AFS than in CFS across the day, but the difference was not significant ($P = 0.15$). The variation in content of the middle sieve were not different (8%; $P = 0.99$), as well as the lower sieve ($P = 0.32$) and the bottom pan ($P = 0.14$). A consistent AFS 10% particle size distribution in TMR is an important determinant of cow rumen health and milk production. Fiber facilitates chewing and production saliva that helps in rumen buffering, substrate for rumen microbe hence acetate to propionate ratio. Concentrates provide available energy and proteins and help prevent milk fat depression (Maekawa et al., 2002).

The variation of DM was greatly reduced in AFS (2%) compared to CFS (31%) ($P = 0.02$; **Figure 3.2**). Additionally, using AFS significantly lowered the variation of NDF (3.9%, $P = 0.05$) and ADF (5.0%, $P = 0.05$) across the day compared to herds with CFS (NDF 5.9%, ADF 7% variability: **Figure 3.3** and **Figure 3.4**). However, there was no observed differences in the variation of lignin, starch, crude protein, or total fatty acid in the feed bunk across the day between farms using AFS and CFS. This comparison of particle distribution and nutrient composition between robotic feeding and conventional feeding is novel and continued studies might provide a clearer picture on any differences.

Previous results suggest that increasing the frequency of feeding may reduce feed sorting on dairy farms (Feng et al., 2013). However, our results do not suggest a major difference in particle size distribution occurred through using an automated feeding robot. The lack of difference may be because of farm-to-farm variability in factors like diet composition, mixing times, methods of feed cutting among others.

Milk yield and milk components

Average daily milk yield did not vary between AFS and CFS farms ($P = 0.80$; **Figure 3.6**). Milk fat concentration did not differ between CFS (3.86%) and AFS (3.81%; $P = 0.16$) (**Figure 2.4**). Protein concentration was equal in both treatments 3.12% ($P = 0.96$; **Figure 3.8**). However, AFS was found to increase the lactose concentration (CFS = 4.793%, AFS = 4.82%, $P = 0.02$; **Figure 3.9**). Overall, the association between feeding system and milk production and milk quality was observed to be very limited. This may be because of high on-farm data variability and limited number of vector herds for optimal blocking.

Milking Intervals, Visits, Refusals and Milkings

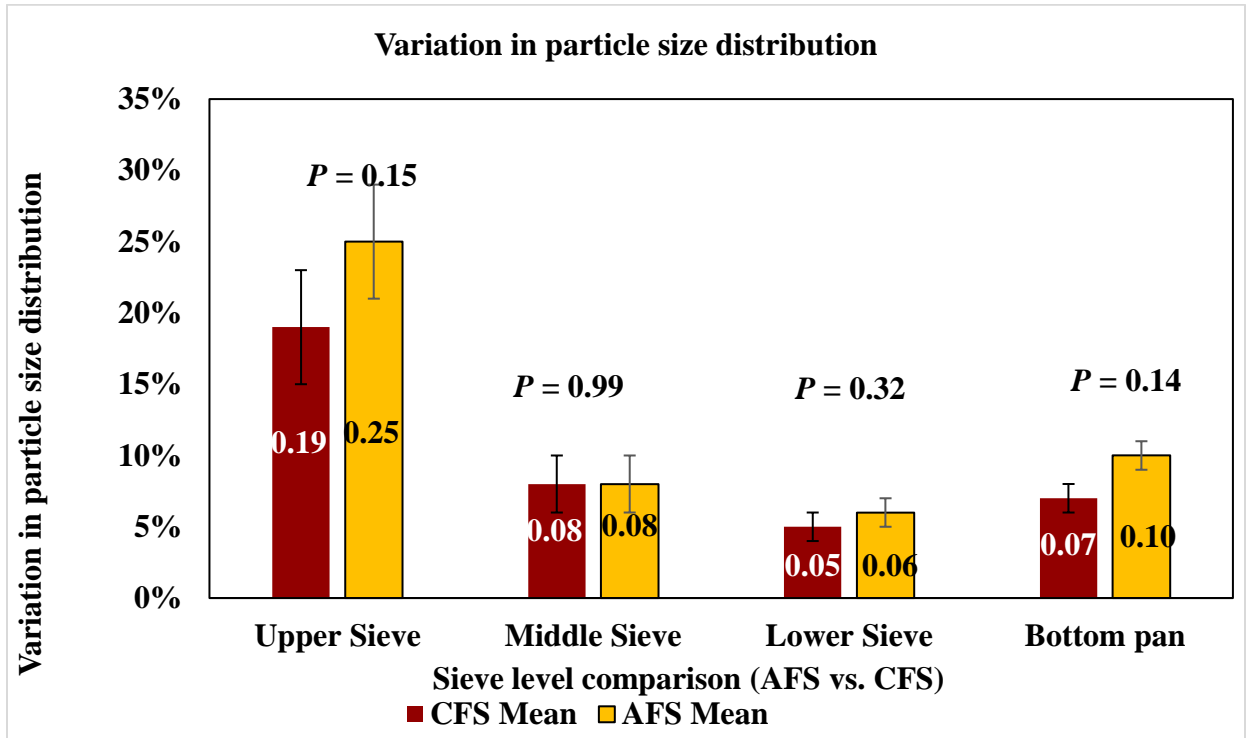
The intervals of visits to the AMS each day did not vary between AFS (9.09 h) and CFS (9.02 h; $P = 0.85$). This findings are consistent with previous studies that did not find differences in visits to the AMS between CFS and AFS (Belle et al., 2012). However, in this study, the average daily refusals at the AMS in this study were increased from 0.813 cow⁻¹ day⁻¹ in CFS to 1.048 cow⁻¹ day⁻¹ in AFS herds ($P = 0.003$). In contrast, Belle et al. (2012) found no significant differences in refusals. The increase in refusals we observed may indicate that cows are visiting the AMS more, suggesting that increased feeding frequency due to AFS may stimulate greater cow activity, which may lead to additional visits to the AMS. Additionally, the daily average milkings per cow in this study was not different ($P = 0.7537$, CFS 2.69 and AFS 2.73 milkings). There were no significant differences observed with rumination time and fat and protein ($P > 0.10$; **Table 3.1**). The Lely vector feeding system is new in the dairy industry and therefore it is likely that high variability among farms might have influenced the overall findings of this experiment. This variability also affected the data collected from T4C of farms utilizing AFS mainly because farmer preferences and practices play a major role in the type and consistency of data collected. Under a controlled experimental facility for example, this type of study can yield significant insights on the impact of feeding systems on the cows.

Conclusion

This study found that AFS reduces the feed bunk variation DM, ADF and NDF. The AFS systems can improve ration consistency throughout the day compared with CFS. Milk fat and milk protein were not different between AFS and CFS ($P > 0.10$). Lactose concentrations was higher in AFS than in CFS ($P < 0.005$), a factor that needs to be studied further to understand the cause. The refusals at the AMS were significantly higher with AFS than CFS ($P < 0.005$) thus supporting the findings that frequent feeding increases cow activity. High variability among farms likely impacted results. Future work using a more controlled model to compare AFS versus CFS herds should be conducted. Randomized controlled trials where the same cows are used throughout the study may reveal clearer effects of feeding system on cow productivity and feeding and milking patterns.

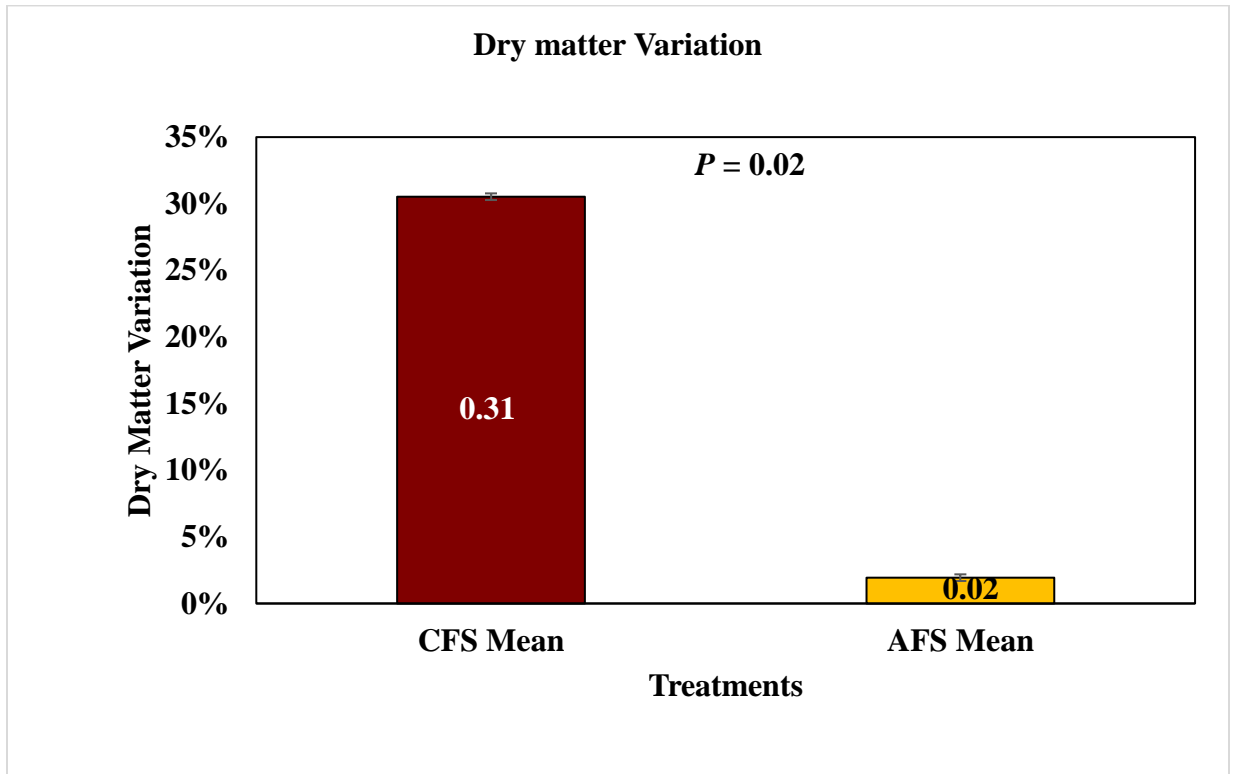
Figures

Figure 3.1 The effect of automated feeding systems on daily variation in feed bunk particle size distribution¹.



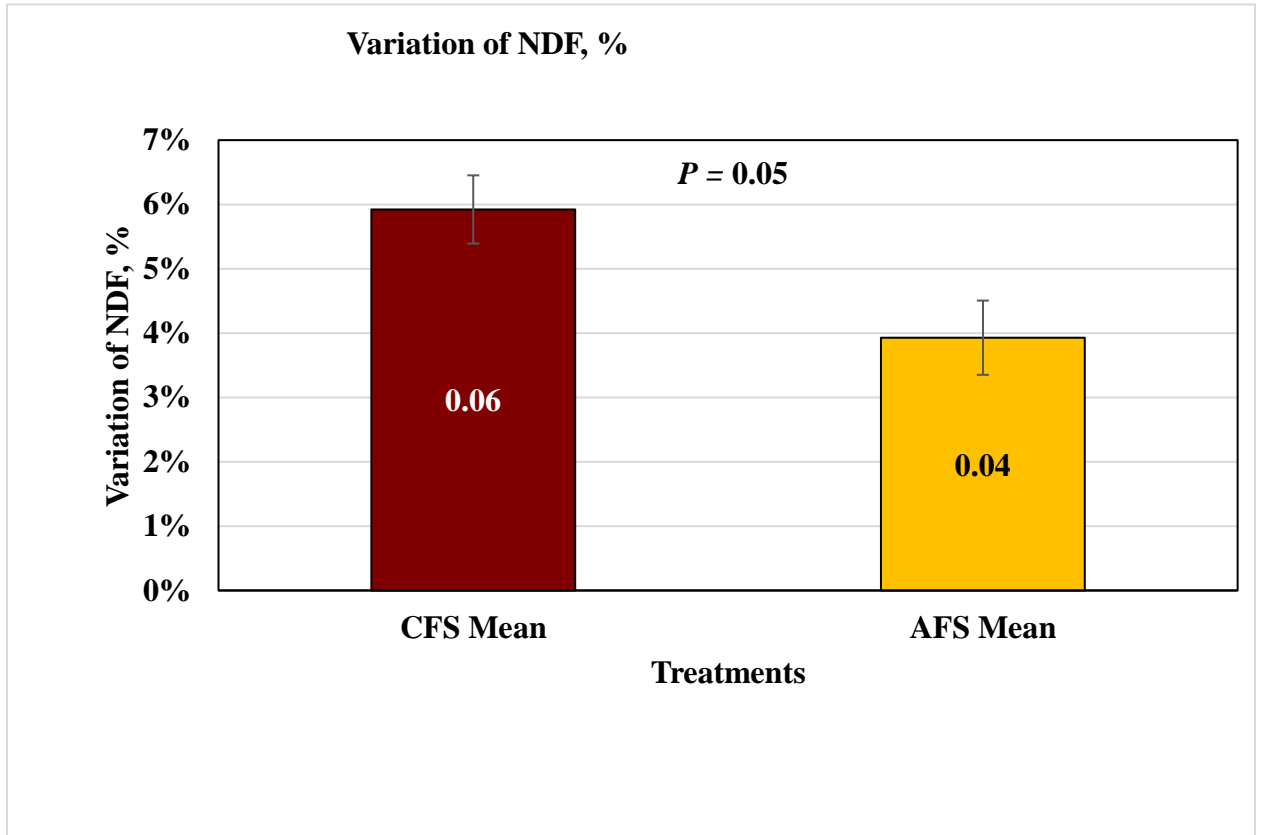
¹CFS: Conventional feeding systems with herds being fed 1x/d with a farmer-operated total mixed ration mixer. AFS: Automated feeding system (Lely Vector).

Figure 3.2 The effect of automated feeding systems on daily variation in feed bunk dry matter concentration¹.



¹CFS: Conventional feeding systems with herds being fed 1x/d with a farmer-operated total mixed ration mixer. AFS: Automated feeding system (Lely Vector).

Figure 3.3 Coefficients of daily variation of NDF¹ between farms using AFS² versus CFS³.

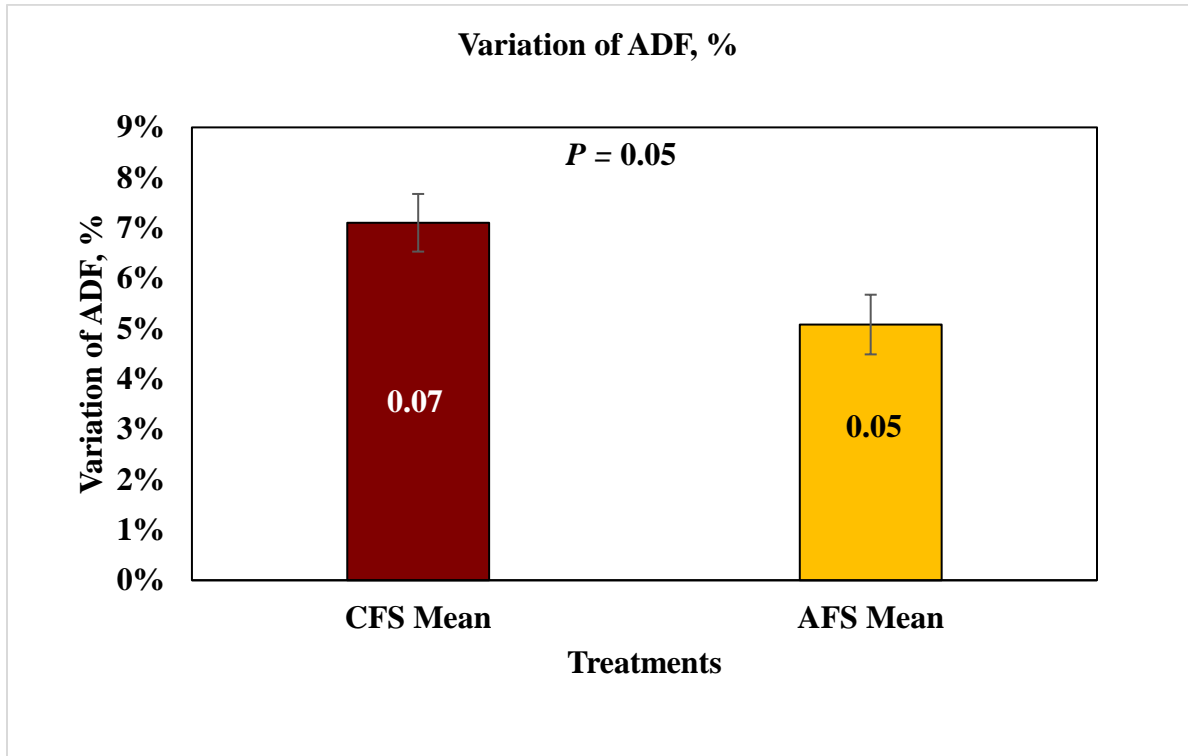


¹NDF – Neutral detergent fiber

²AFS – Automated feeding systems (Lely Vector).

³CFS – Conventional feeding systems with herds being fed 1x/d with a farmer-operated total mixed ration mixer.

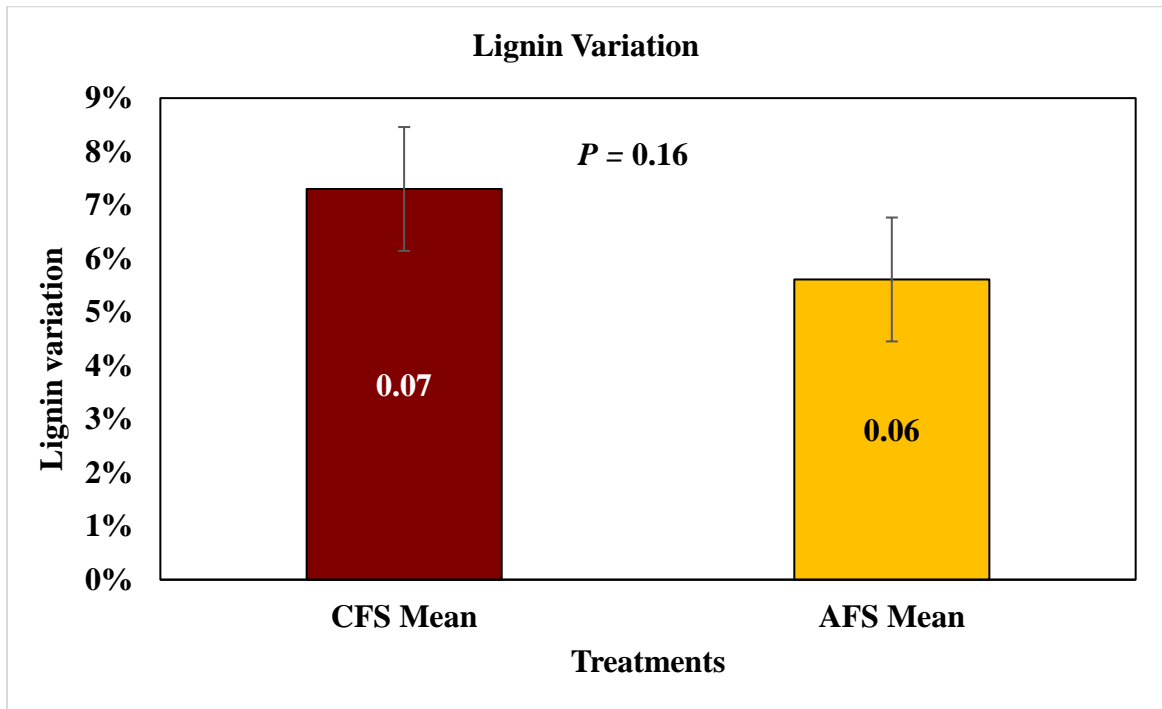
Figure 3.4 Coefficients of daily variation of ADF¹ between farms using AFS² versus CFS³.



¹ADF- Acid detergent fiber

²AFS – Automated feeding systems (Lely Vector).³CFS – Conventional feeding systems with herds being fed 1x/d with a farmer-operated total mixed ration mixer.

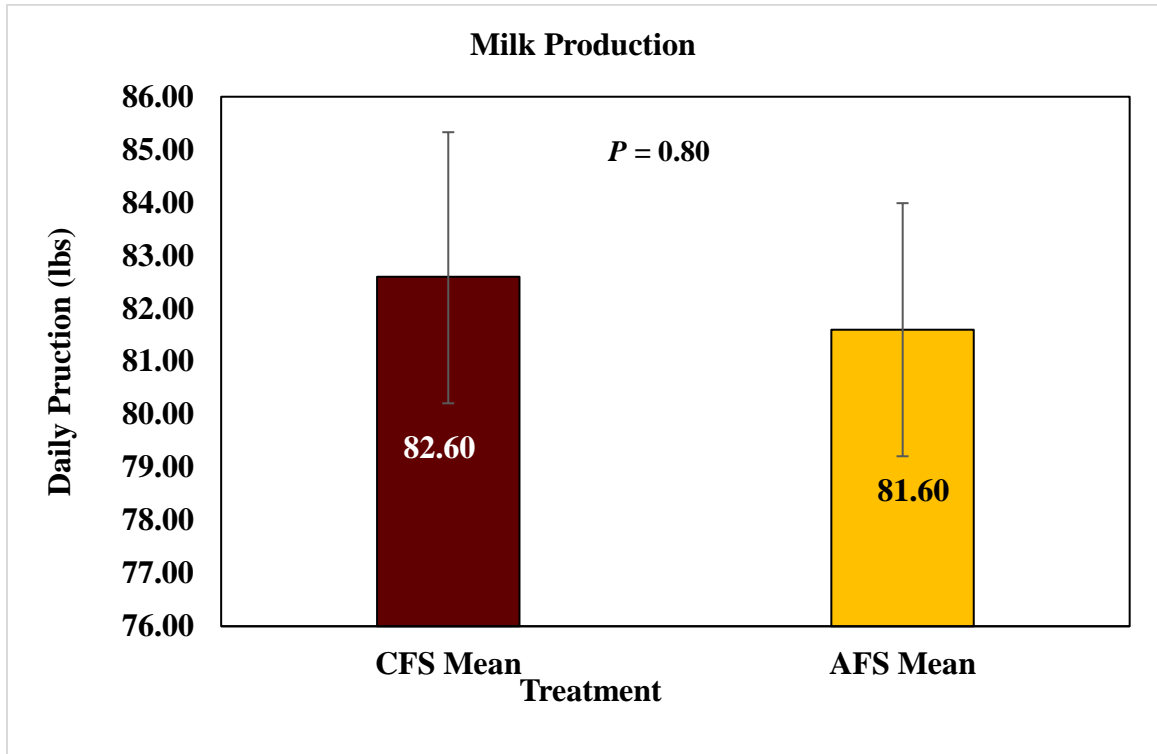
Figure 3.5 Variation of lignin between CFS¹ and AFS².



¹CFS – Conventional feeding systems with herds being fed 1x/d with a farmer-operated total mixed ration mixer.

²AFS – Automated feeding systems (Lely Vector).

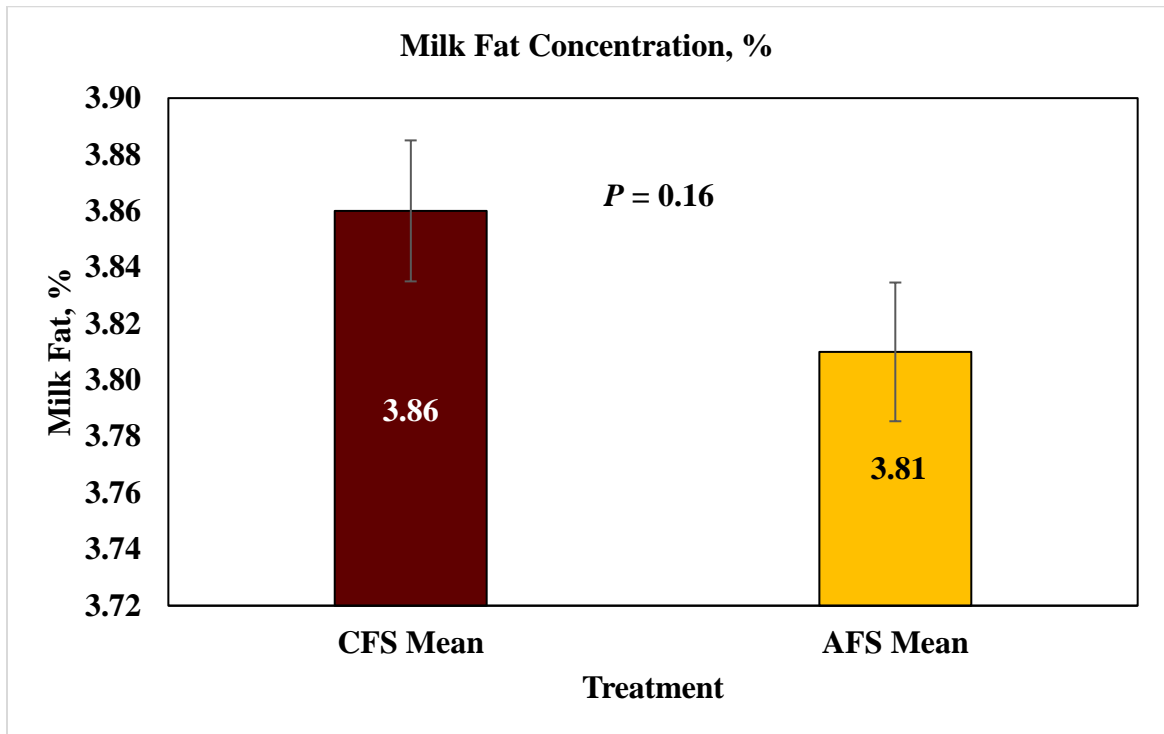
Figure 3.6 .Daily total milk yield of farms that use CFS¹ compared to farms that use AFS².



¹CFS – Conventional feeding systems with herds being fed 1x/d with a farmer-operated total mixed ration mixer.

²AFS – Automated feeding systems (Lely Vector).

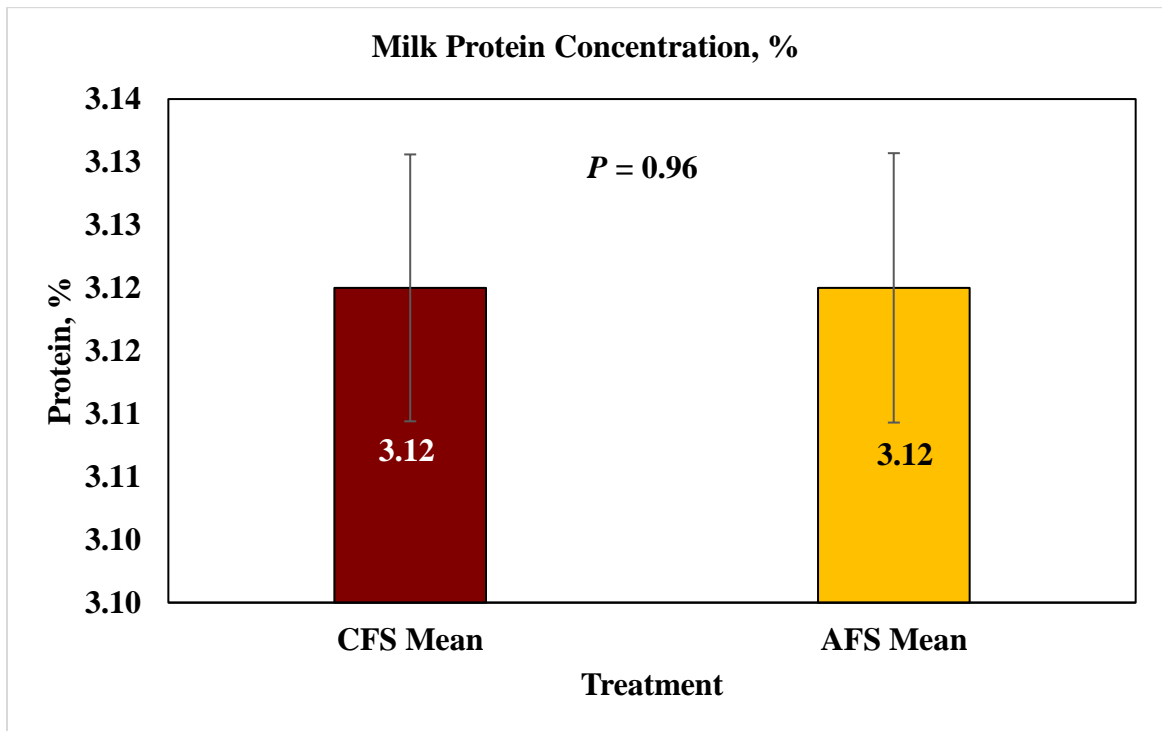
Figure 3.7 Milk fat concentration comparison between CFS¹ and AFS².



¹CFS – Conventional feeding systems with herds being fed 1x/d with a farmer-operated total mixed ration mixer.

²AFS – Automated feeding systems (Lely Vector).

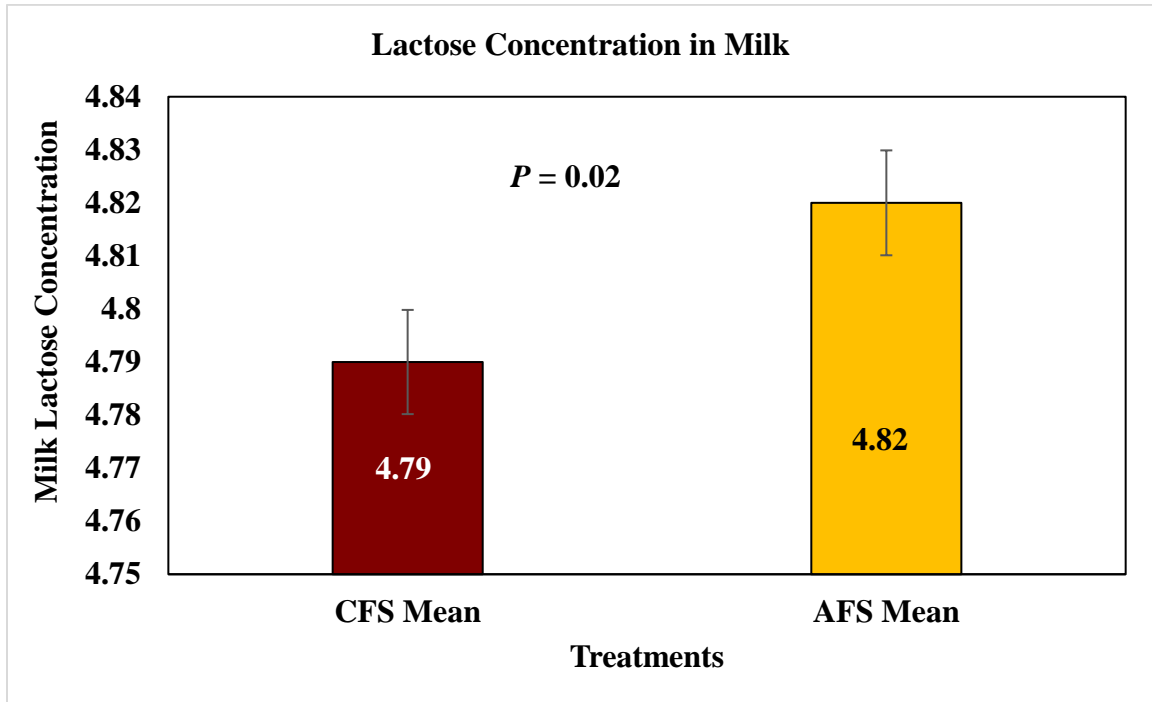
Figure 3.8 Effect of Bulk tank Milk protein concentration comparison between CFS¹ and AFS².



¹CFS – Conventional feeding systems with herds being fed 1x/d with a farmer-operated total mixed ration mixer.

²AFS – Automated feeding systems (Lely Vector).

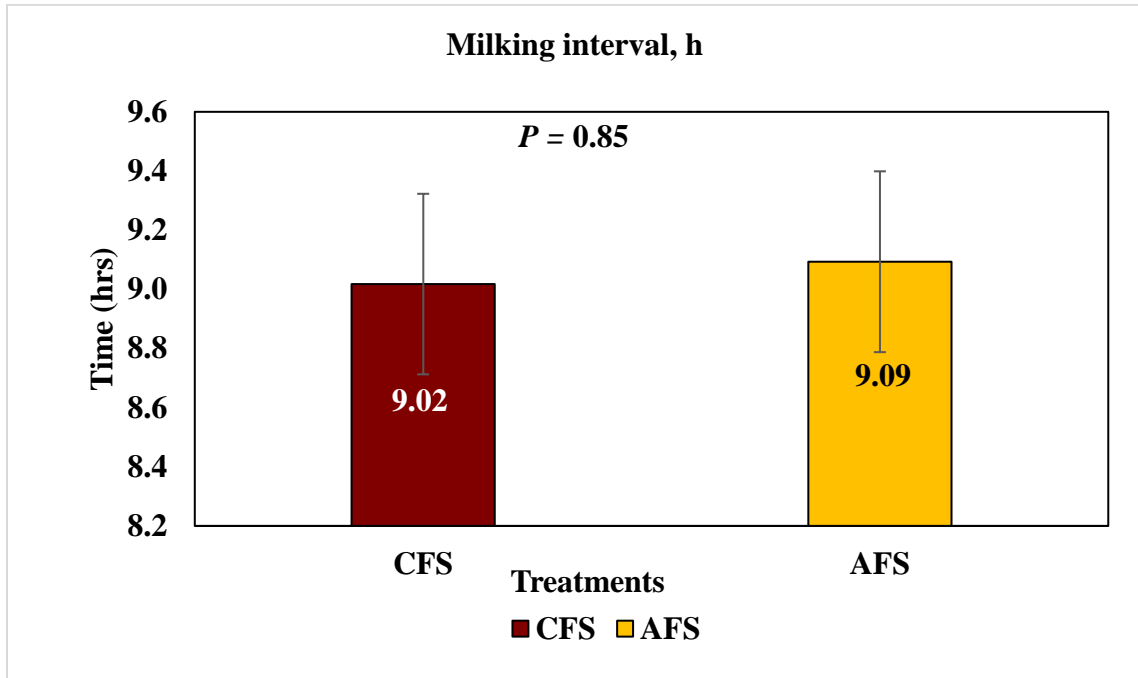
Figure 3.9 Comparison of bulk tanks milk lactose concentration between the control herd (CFS)¹ and the treatment (AFS)².



¹CFS – Conventional feeding systems with herds being fed 1x/d with a farmer-operated total mixed ration mixer.

²AFS – Automated feeding systems (Lely Vector).

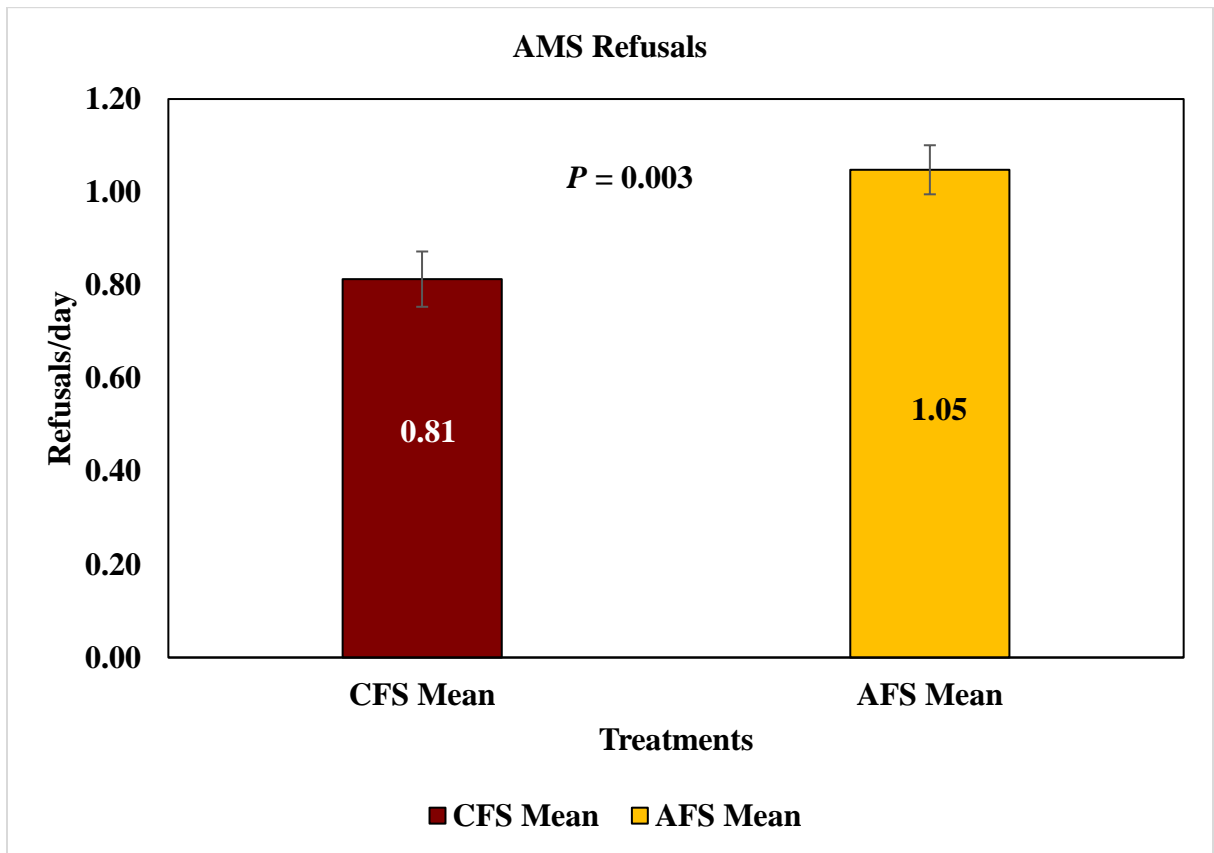
Figure 3.10 The average time between cow visits to the AMS on farms that use CFS¹ versus farms utilizing AFS²



¹CFS – Conventional feeding systems with herds being fed 1x/d with a farmer-operated total mixed ration mixer.

²AFS – Automated feeding systems (Lely Vector).

Figure 3.11 The average number of daily refusals by the AMS between herds with CFS¹ and AFS².



¹CFS – Conventional feeding systems with herds being fed 1x/d with a farmer-operated total mixed ration mixer.

²AFS – Automated feeding systems (Lely Vector).

Tables

Table 3.1. Effect of automated feeding system on feed intake, rumination, milking frequency, milk production, and milk components of individual cows.

Item ¹	CFS ²		AFS ³		P-Value
	Mean	SEM ⁴	Mean	SEM	
Rumination, min/d	479.00	19.50	506.00	17.10	0.31
Intake, lbs/d	11.50	0.93	9.89	0.82	0.19
Milk Production, lbs/d	82.60	2.73	81.60	2.39	0.80
Concentrates	13.90	0.80	12.80	0.70	0.31
Milkings/d	2.69	0.08	2.73	0.07	0.75
Fat Indication, %	3.98	0.05	3.93	0.04	0.42
Fat yield, lbs	3.22	0.10	3.11	0.08	0.40
Fat: Protein Ratio	1.28	0.02	1.25	0.01	0.22
Protein Indication, %	3.10	0.02	3.13	0.02	0.37
Protein, lbs	2.56	0.08	2.54	0.07	0.86
Fat +Protein, lbs	5.81	0.17	5.71	0.15	0.67

¹Data were obtained from the Time for Cows (T4C, Lely) software from all 16 dairy herds.

²CFS - Conventional feeding systems

³AFS - Automated feeding systems

⁴SEM- Standard error mean

Table 3.2. The main characteristic of experimental herds in this study

Item	CFS¹		AFS²	
	Mean	SEM³	Mean	SEM
Herd average per farm	78.1	1.70	77.4	1.59
AMS average per farm	2.3	0.19	2.3	0.19
Milk production average (lbs)	82.2	2.30	81.8	2.24
Feed delivery average per day	2.0	0.00	7.0	0.00
Average no AFS per farm	1.0	0.00	1.0	0.00

¹CFS - Conventional feeding systems

²AFS - Automated feeding systems

³SEM – Standard error means

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