MONITORING PEN-LEVEL DATA WITH INDIVIDUAL COW MONITORS USING STATISTICAL PROCESS CONTROL

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DEDICATION

I would like to dedicate my thesis to my family: my parents, Randy and Joan, my brother, Ross, and my fiancé, Christine. Growing up on a small dairy farm has taught me countless values and ideals that shape my life every day. You have instilled in me a passion for the dairy and agriculture industry that fills me with pride and joy. Your guidance, support, and love have continued to bless me throughout my education. There is no doubt in my mind that without your continued support and encouragement for my educational and personal decisions, I would not have completed this journey. Thank you for always being there for me and for providing a respite from the busy city life of Madison.

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ABSTRACT

The advent of new technologies has allowed commercial dairy producers the opportunity to collect a vast amount of data on individual cows. Although herd management software has been used on farm in a historical context to view cow records, little research in real-time monitoring tools for a commercial dairy farm has been conducted.

The first objective of this thesis was to characterize the variability in data streams on dairy farms exposed to different management strategies. In the first two experiments conducted in an experimental research setting, tall fescue hay replaced corn silage or alfalfa silage in a lactating cow TMR, with effects on fiber digestibility, animal performance and animal behavior observed. Tall fescue hay incorporation improved fiber digestibility with similar cow performance. Cow behavior was similar across most parameters, including rumination, physical activity, and meal patterns, but meal frequency and DMI was decreased in cows consuming TMR's with tall fescue incorporated.

The next studies sought to characterize variability in milk production, rumination, physical activity, DMI, and bodyweight on two commercial dairies with different management styles. For both dairies and most data streams, standard deviation among cows was greater than standard deviation among days within cow, and among day within pen.

A secondary objective of this thesis was to begin to use statistical process control (SPC) techniques to determine the frequency of out-of-control data points. Using the two commercial dairies from above, univariate SPC analyses were performed to determine the frequency of out-of-control data on each farm.

The last objective was to use multivariate SPC techniques to begin to integrate data streams and create a real-time analysis on commercial dairy farms. Data from a commercial

dairy was analyzed via the MVP procedures in SAS to integrate data via a Hotelling's T^2 control chart.

Integration of nutritional data streams on a commercial data could improve profitability on a commercial dairy by early recognition of an out-of-control process. Statistical process control, particularly multivariate SPC techniques, could integrate data streams and create a real-time analysis of a process. More research on events triggering an SPC alert and sensitivity analyses of the control charts should be conducted to improve the procedure.

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Chapter 1. Literature Review

General Introduction

Traditionally, milk quality and quantity measurements have been the common means of analyzing cow and herd performance on a commercial dairy farm, often via monthly milk testing. Modern technologies offer the ability to collect a vast amount of data on commercial dairy farms, with the potential to receive data in a real-time analysis. Sensors placed on the head, neck or in the rumen offer measurements of rumination, rumen pH, temperature, and respiration. Meal patterns, feed intake, and chewing activity can be measured by sensors placed on the cow's ear or via feeding gates. Characteristics of the milk including production, electrical conductivity, components, and somatic cell count can be measured with sensors in the parlor or in a robotic system. Sensors placed in the milking parlor or on the cow's legs can measure hoof health, mobility, lying behavior, animal location, body condition score, and body weight. However, data at both the individual animal and pen/herd level are often inadequate and underutilized. The objective of this literature review is to define and explore statistical process control (SPC) as a method to evaluate variability, learn how SPC is used in agricultural systems in a univariate analysis and multivariate analysis, and explain the rationale for the research studies presented in the remainder of this thesis.

Evaluating Variability of Data with Statistical Process Control

What is Statistical Process Control?

Statistical process control (SPC) is a method of achieving the highest quality process by analyzing the cause of and reducing variability (Montgomery, 2009). Variability is

inherent in every production process, whether biological or manufactured. However variability can be attributed to two main causes: natural cause and assignable cause. Natural or common cause variability is that which is inherent in every process and is unavoidable – essentially variability that is innate to the production practice and is completely random. In an animal production setting, an example of common cause variability is the biological variation in the performance of genetically-similar animals exposed to similar management (De Vries, 2001). Unnecessary intervention in a process that undergoes only common cause variability may induce extra variation without improvement in the underlying process (De Vries, 2001). A process that is only affected by common causes is said to be statistically "in-control".

In contrast, assignable cause or special cause variability are disturbances in a process that lead to deviations from the normal variability in a process (Montgomery, 2009). These disturbances arise periodically, and cause widespread deviations from the mean that result in significant economic losses. Assignable causes can be clearly identified and removed to improve the process performance. A process experiencing disturbances due to assignable causes is said to be statistically "out-of-control". Examples of assignable causes in agricultural systems include disease pressure and management changes (De Vries, 2001).

Statistical process control is often performed in two phases, with distinct objectives in each phase and control charts that reflect the differences in phases (Montgomery, 2009). Phase I is considered the establishment phase of the process, where the data from a process is typically gathered and retrospectively analyzed to determine if the process has been in control for a set period of time. Often phase I is used to attempt to eliminate existing assignable cause variation. Phase II is a monitoring phase, where new data from the process

in analyzed as each successive sample is added to quickly discover when a process is deviant from the mean.

One of the most important tools in SPC is the control chart. Statistical process control charts contain a center line representing the average value of the characteristic being measured. Two other horizontal lines are added, one above and one below the center line, called the control limits. When a process is in control, most points will fall within the control limits. A point plotted outside the control limits represents an out-of-control process (Montgomery, 2009). In SPC, a type I error (false positive) can be made when a process is detected as out-of-control, when it is, in fact, in-control. A type II error (false negative) is when the process is detected as in control, when it is in fact, out-of-control. Although both types of errors can cause economic loss, properly adjusting control charts can minimize errors through the use of calculating control limits.

Perhaps the most straight-forward and most well-known SPC chart is the Shewhart control chart (Shewhart, 1931). An example of a Shewhart control chart is illustrated in Figure 1 from De Vries and Conlin (2005). Walter A. Shewhart (1931) pioneered the use of the control chart to identify and distinguish between normal (common cause) variability and abnormal (special cause) variability (Reneau and Lukas, 2006). Shewhart charts are designed to detect large shifts (often $>3\sigma$) in process performance. Thus, Shewhart charts are useful during phase I implementation of SPC, when the process is likely out of control and experiencing assignable causes with large shifts from the mean. As such, Shewhart charts are much less sensitive to small process shifts from the mean, as would be common in a phase II SPC analysis when large scale assignable cause shifts have been improved (Montgomery, 2009). Upper and lower control limits of Shewhart charts are typically set at

three standard deviations above and below the mean center line, as shown in Figure 1-1, and the process typically utilizes rules to determine when a process is statistically too variable or statistically "out-of-control". Examples of these rules are as follows (Reneau and Lukas, 2006):

1. A single point more than 3 σ away from the mean
2. At least nine successive points on the same side of the mean
3. At least two of three successive points 2 σ away and on the same side of the mean
4. At least four of five successive points 1 σ away and on the same side of the mean

When at least one of these conditions is met, the process is considered too variable, and it is noted that a real change has occurred in the process. Shewhart charts are used when the sample size in each graphed data point is n=1 (Montgomery, 2009). In addition, Shewhart charts have no "memory", that is, they only use information from the current measurement, in contrast to charts discussed later.

What are the Applications of Statistical Process Control in Other Industries?

Manufacturing was the first industry to apply statistical process control, due to extensive variation in manufactured products. Parmar and Deshpande (2014) reviewed 12 published studies of use of SPC in a wide array of manufacturing industries including glass, plastic, paper, automotive, software, soap, tile, steel, and tobacco. Examples of some of the benefits and results of utilizing SPC were the reduction in defect rate from 13.49% to 7.4% (plastics industry; Rohani and KokTeng, 2001), increase in the capability index of the process (soap manufacturing; Mahesh and Prabuswamy, 2010), decrease by 50% in the number of nonconformities resulting in a more consistent product (automobile industry; Jadhav and Jadhav, 2013), and reduction in the frequency and time of machine breakdowns

(tobacco industry, Sultana et al., 2009). Other advancements of the use of SPC in manufacturing systems consist of multivariate SPC approaches (discussed in section 1.2.4) and manufacturing systems with multiple stages. The latter approach involves a multi-level SPC model focused on the different check-points in the manufacturing system (Tsung et al., 2008).

Although SPC control charts were primarily developed for use in a manufacturing setting, there have been multiple adaptations of SPC control charts by other industries, and can be broadly categorized into four main application areas outside of manufacturing, as reviewed by MacCarthy and Wasusri (2002): 1) engineering, industrial and environmental applications, 2) healthcare applications, 3) general service sector applications, and 4) statistical applications.

Engineering, industrial, and environmental applications are the most common uses for SPC control charts after manufacturing (MacCarthy and Wasusri, 2002). Many common applications of SPC charts include evaluating the performance of a particular piece of machinery or instrumentation, often through variables generated from the process. Planning of proper maintenance is another application of SPC (Cassady et al., 2000; Ben-Daya and Rahim, 2000), where maintenance intervals and schedules can be adjusted based on frequency of detection of statistically out-of-control data. Additionally, environmental variables such as concentration of pollutants in industrial processes (Ipek et al., 1999) or in the environment (Zimmerman et al., 1996; Maurer et al., 1998) can utilize control charts to avoid health hazards.

The healthcare industry has the second most published studies using SPC approaches with 31% of all studies in a review (MacCarthy and Wasusri, 2002). More recently, Thor et

al. (2007) reviewed SPC applications to the healthcare industry and noted that SPC in healthcare started as early as the 1960's (Fisher and Humphries, 1966) with use in laboratory settings, before incorporation into other areas of healthcare. The review concluded that SPC was applied to healthcare improvement in a wide array of settings and specialties, using many different types of variables (Thor et al., 2007). Of the reviewed publications, the hospital setting was the most common (40 studies), followed by non-hospital out-patient settings (primary care centers, specialty clinics, etc.) with 12 studies, and 6 studies in other areas. Statistical process control techniques were applied to 21 different medical specialties within healthcare, including the most common specialties of anaestheisa-intensive care (12 studies), family practice-primary care (7 studies) and emergency medicine (6 studies). The unit of analysis for the individual SPC studies was extremely diverse as well; they varied from the individual patient (9 studies) to the department process level (15 studies) to the entire hospital (6 studies), and many more levels in between. Finally, variables measured in the analyses were extremely diverse and included main categories such as biomedical variables, biomedical measurement variables, patient health variables, clinical management, and financial resources (Thor et al., 2007). Data on specific types of control charts incorporated were not included in this review.

The service sector also has many applications of SPC control charts, including in diverse fields such as banking, hospitals, financial services, airline industry, and utilities, among many others (Antony et al., 2007). Service processes including shipping, invoicing, billing, payroll, customer order entry, baggage handling processing, etc. perform at a poor quality level, with drastically reduced defect error rates, according to Yilmaz and Chatterjee

(2000). Thus, implementation of SPC techniques can reduce the error and improve efficiency in the service sector.

Lastly, statistical applications for SPC control charts also exist, with the main purpose of creating a model capable of accurate forecasts and monitoring model stability over time. These models are commonly created with residuals, which can be assumed to be approximately normally and independently distributed, with a constant variance (MacCarthy and Wasusri, 2002). Examples of SPC control charts in statistics include identifying (Atienza et al., 1997) and monitoring forecasts (Hill, 1996; Atienza et al., 1997), as well as developing appropriate forecasting models (Koksalan et al., 1999).

Overall, although wide applications of SPC exist for use across a wide array of industries, prevalence in the agricultural sector is relatively small, such that review papers often do not associate SPC with agricultural systems (MacCarthy and Wasusri, 2002).

How is Statistical Process Control Used in Agricultural Systems?

Despite the application of SPC to other industries as early as the 1920's, to our knowledge the earliest documentation of application of SPC techniques to agricultural systems began first in the 1970's (Wrathall, 1977; Sard, 1979; de Vries and Reneau, 2010). There are many examples of the use of SPC in an agricultural setting across a wide range of animal species, using many SPC techniques applied to many aspects of animal physiology, as previously reviewed (Reneau and Lukas, 2006; de Vries and Reneau, 2010; Mertens et al. 2011). This section serves to review the 46 published studies that we found that have used SPC methods in agricultural systems, as summarized in Table 1-1.

Of the 46 studies utilizing SPC techniques, 45 used SPC approaches in a univariate analysis. Thus to our knowledge, there is only one published study that used a multivariate

SPC approach in an agricultural system, as discussed below. These 45 studies used a variety of SPC techniques, with many studies using more than one SPC technique to complete the analysis.

Studies Incorporating SPC by Using Shewhart Charts

Shewhart control charts were used in 25 studies listed in Table 1-1. Of these 25 studies, eight were for use in swine, 15 in dairy cattle, one in poultry and one in beef. The eight swine studies incorporated Shewhart charts to measure characteristics at four levels: herd, individual animal, both herd and individual animal, and at an assay level. On the individual animal level, one study measured mortality, culling and average daily gain (Fraile et al., 2009). On the herd level, studies measured mortality rates (Bono et al., 2014), farrowing rates (Bono et al., 2013), number of piglets weaned (Deen, 1997), number of females mated per week (Koketsu et al., 1999), and return to service percentage, pigs per litter, and fetuses born dead (Wrathall, 1977). One study utilized a Shewhart chart of both the herd and individual levels to measure litter size (Bono et al., 2012). Finally, one study in swine also used a Shewhart chart to measure optical density percentage in an ELISA assay to measure the serologic responses of infected pigs (Baum et al., 2005).

Of the 15 studies using Shewhart charts in dairy cattle, eight studies conducted the analysis focusing on the individual cow, in which they analyzed milk yield (de Vries et al., 1997b; Lukas et al., 2009; Wallace, 2009; Huybrechts et al., 2014), milk electrical conductivity (Lukas et al., 2009), flow rate (Wallace, 2009), milking time (Wallace, 2009), insemination quantity (Cornou et al., 2014), insemination rate (Cornou et al., 2014), or estrous detection indices (de Vries and Conlin, 2003 a, b; 2005). One study conducted the Shewhart chart analysis on a pair of cows, where DMI and water consumption was measured

(Lukas et al., 2008b). There were two studies that analyzed data using a Shewhart chart on herd level measurements, both of which analyzed bulk tank somatic cell count (Reneau, 2000; Lukas et al., 2005). One set of two companion studies used Shewhart charts to analyze feed samples (St-Pierre and Cobanov, 2007a, b). Finally, two studies used both a herd level and individual cow level Shewhart chart analysis to look at bulk tank somatic cell count (de Vries et al., 1997), conception rate (de Vries et al., 1997), milk urea nitrogen (Reneau et al., 2007), milk fat percentage (Reneau et al., 2007), and physical activity (Reneau et al., 2007).

The two remaining studies using Shewhart charts were in the poultry industry, where the survival rate of turkeys was analyzed at the herd level (Cowen et al., 1994), and the beef industry, where sperm motility was investigated in bulls (Galli et al., 1998).

Studies Incorporating SPC by Using CUSUM Charts

The cumulative sum, or CUSUM, control chart is considered a more advanced technique compared to Shewhart charts, and as a result, there is performance improvement. Cumulative sum charts have been proposed as early as the 1950's (Page, 1954) and 1960's (Ewan, 1963), but have not been applied to agricultural processes until more recently. This chart is particularly useful in a phase II SPC analysis, when the process tends to be more in control, with more reliable estimates of the mean and standard deviation, and assignable causes that do not typically result in huge shifts from the mean (Montgomery, 2009). The reason for the performance improvement is that CUSUM charts incorporate information from a sequence of sample values by plotting the sums of the deviations of the data points from a target value. Thus, this chart is considered to have a "memory" of recent observations when calculating the current value, in contrast to Shewhart charts. Due to the combination of information from several data points, CUSUM charts are much more effective compared to

Shewhart charts in detecting small process shifts from the mean. The "V-mask" is an example of one method that CUSUM charts may use to detect significant shifts from the mean. Figure 1-2 is an example of a CUSUM control chart utilizing the V-mask technique, which originated from Kristensen and Cornou (2011). A V-mask is a visual detection method, when diagonal lines are drawn (in the shape of a sideways "V") from the current plotted point across the previous points to determine if any cross the line. The V-mask is applied to every successively added plotted point to determine if any points are out of control. The length and angle of the V-mask lines are adjusted based on desired specificity and sensitivity, as shown in Panel A of Figure 1-2. When plotting a new point, and adding the V-mask, if either "leg" of the V-mask crosses the previously established plotted points, the newly added point is considered statistically out-of-control (Hawkins and Olwell, 1998). For example, in Figure 1-2, the newest plotted point, sample#29, resulted in a V-mask that intersected sample #21, thus resulting in sample #29 being statistically out-of-control.

Cumulative sum charts were used in 26 of the 45 publications applying statistical process control techniques in agricultural systems. Of these 26 publications, nine publications were for use in swine, 12 publications for dairy, three publications for poultry, and two publications for beef production. The nine swine studies incorporated CUSUM charts to measure characteristics at four levels: individual animal (pig or sow), herd, both herd and individual animal, and pen level. On the individual animal level, three studies measured eating rank (Cornou et al., 2008), acceleration (Cornou et al., 2012), piglets born per sow (Krieter et al., 2009), and return to estrus rate (Krieter et al., 2009). On the herd level, four studies measured farrowing rate (Bono et al., 2013), mortality rate (Bono et al., 2014), pre-weaning mortality rate (Engler et al., 2009), number of piglets weaned (Engler et

al., 2009), and return to estrus rate (Wilson et al., 1980; Engler et al., 2009). One study measured litter size (Bono et al., 2012) at both the herd and individual animal level, and one study utilized a pen SPC analysis to measure water consumption (Madsen and Kristensen, 2005). In order to detect out-of-control data points, four swine studies utilized the V-mask technique within their CUSUM charts (Cornou et al., 2008; Bono et al., 2012, 2013, 2014).

The 12 publications using CUSUM for SPC analysis relevant to the dairy industry measured characteristics at four levels: individual animal (cow), pair of animals, herd or individual animal, and individual feed sample. On the individual cow level, nine studies measured variables including estrous detection indices (De Vries and Conlin, 2003 a, b, 2005), milk yield (Lukas et al., 2009; Huybrechts et al., 2014; Lukas et al., 2015), milk electrical conductivity (Greenstam, 2005; Lukas et al., 2009; Miekley et al., 2012), physical activity (Miekley et al., 2012), and weight distribution among legs (Pastell and Madsen, 2008). One publication measured DMI and water intake at the level of a pair of cows (Lukas et al., 2008b). Another study used a CUSUM chart to measure MUN, milk fat percentage, and physical activity at either the herd or individual cow level (Reneau et al., 2007), and finally, one study utilized CUSUM charts to measure forage composition at the level of a feed sample (St-Pierre and Cobanov, 2007b).

Only three publications were found that utilized SPC CUSUM charts to measure characteristics in the poultry industry. One study measured physical activity in the broiler chicken industry at the individual chicken level (Kristensen and Cornou, 2012), while two studies measured average egg weight (Mertens et al., 2008) and hen-day egg production (Mertens et al., 2009) at the flock level. One of the poultry studies utilized the V-mask technique to detect out-of-control data (Kristensen and Cornou, 2012).

Finally, in the beef industry, two publications conducted an SPC analysis using CUSUM charts. These two studies measured muscle pH (Pleasants et al., 1998) and feeding behavior (Quimby et al., 2001) at the individual animal level (steer).

Studies Incorporating SPC by Using EWMA Charts

The exponentially weighted moving average (EWMA) control chart is another more advanced SPC procedure used to detect smaller shifts in a procedure, with a performance similar to that of the CUSUM (Montgomery, 2009). The EWMA design has been used as early as the 1950's (Roberts, 1959), with adaptations to agriculture systems not until much more recently (Krieter et al., 2005; Cavero et al., 2007; Linhares et al., 2014). Unlike the Shewhart chart, the EWMA has a "memory", meaning recent values are also considered in weighing the present value, thus providing detection of small shifts in the mean performance change. The magnitude of dependence on recent data versus older data is a parameter that can be adjusted according to the sum of squared forecast errors. One potential negative of this chart is that if the value of the EWMA is on one side of the center line when a mean shift in the other direction occurs, several periods would have to elapse prior to signaling an alert, since the present data are weighted less heavily (Montgomery, 2009). This phenomenon is known as the inertia effect, and it can reduce the effectiveness of the EWMA (Woodall and Mahmoud, 2005).

An example EWMA control chart is illustrated in Figure 1-3, which is derived from Engler et al. (2009). In Figure 1-3, the number of piglets weaned is monitored each week to determine if a relationship with a disease outbreak exists. The herd diagnosis of Porcine Reproductive and Respiratory Syndrome (PRS) is indicated on the graph, and the EWMA

chart performance deviation from the mean is noted prior to disease determination (Engler et al. 2009).

Exponentially weighted moving average SPC charts were used in three publications in agricultural systems, one in dairy and two in swine. The dairy study focused on somatic cell count, and used cow as the unit of measure (Cavero et al., 2007). Of the two swine studies, both used herd as the unit of measure, while one (Krieter et al., 2005) focused on number of litters, piglets born alive, and piglets weaned for variables and the other (Linhares et al., 2014) focused on time to baseline production following disease exposure as the variable measured.

Studies Incorporating SPC by Using Capability Analyses

The capability analysis or index is an SPC tool to measure how the variability inherent in a process differs from its specified limits (Bissell, 1994; Montgomery, 2009). In order to calculate this index, important assumptions must be met, including that the quality characteristic has a normal distribution, the process is in statistical control, and if using a two-sided specification, the process mean must be centered between the lower and upper specification limits. The index is calculated by subtracting the lower specification limit from the upper specification limit and dividing by six times the standard error. Depending on how stringent the guidelines are set, generally a value between 1 and 2 is considered a capable process (Lukas et al., 2008; Montgomery, 2009).

An example of a capability/consistency analysis is presented in Figure 1-4, which is derived from Lukas et al. (2008a). Based on the farms' bulk tank somatic cell count (BTSCC, presented on the x-axis), the consistency index indicated on the y-axis represents

the maximum sigma allowed in the BTSCC estimate in order for the herd to consistently produce the quality milk at the chosen SCC level.

Only two studies from Table 1-1 utilized capability analyses in their research. Both Lukas et al. (2008a) and Niza-Ribeiro et al. (2004) analyzed bulk tank somatic cell count on the herd level using capability analyses.

Integrating Data Streams with Multivariate Statistical Process Control

In many real-life biological and industrial settings, controlling variation in a process requires the monitoring of variables that are multivariate in nature and highly correlated (Yang and Trewn, 2004). Past efforts to monitor variation via SPC have focused largely on simplifying a multivariate process into a series of univariate control charts. However, not only does this create many control charts that are difficult to monitor, but also decreases the accuracy of detecting out-of-control data-points (Mason and Young, 2002; Yang and Trewn, 2004). For example, in Figure 1-5, obtained from Montgomery (2009), two independent variables are plotted in Panel A, one along the top, and one along the left side. Each is an example of a univariate control chart. All data points for both of these charts falls within the control limits, so no data are considered statistically out-of-control. However, in the upper left, the joint control region is also plotted. This represents the overlap of the two variables. Since the two data streams are independent, the shape of the joint control region is a circle, and the point in the upper left corner, while seemingly deviant from the other cloud of data, is still considered in control since it falls within both sets of control limits. In contrast, the data in Panel B shows the same data, but with two dependent variables. The joint control region is now in the shape of an ellipse, and despite falling within the control limits of both individual variables, the multivariate analysis shows that the point in the upper left corner is

in fact, statistically out-of-control. Thus the use of multivariate SPC techniques is warranted as a method to determine deviations from the mean values in dependent variables (Montgomery, 2009).

About two decades after the advent of univariate control charts, Hotelling (1947) introduced a statistic designed to manage multivariate observations and develop multivariate SPC charts, called Hotelling's T^2 (Yang and Trewn, 2004). An example of this is shown in Figure 6, which is derived from Montgomery (2009). Hotelling's T^2 is used to monitor the mean vector of a process, and is similar to the univariate version of the Shewhart chart (Montgomery, 2009). Two types of T^2 control charts are generally used: one for subgrouped data and another for individual observations. Subgrouped data are used to create control charts that are directionally invariant – meaning that a shift in the mean vector only depends on the magnitude of the shift and not the specific direction relative to the mean, as seen in Figure 6, where the lower control limit is actually non-existent. With data accumulated as individual observations, the subgroup size can be thought of as n=1, and different equations are used to calculate the control limits (Montgomery, 2009). Depending on the standard deviations used to calculate the upper control limit, the frequency of out-of-control data may change, as seen in Figure 1-6 with two different upper control limit values.

Similar to univariate analysis, multivariate SPC approaches also can utilize both an EWMA and a CUSUM chart. Multivariate EWMA and CUSUM charts, similar to their univariate counterparts, are much more sensitive to a smaller shift in the mean. Multivariate EWMA charts are also directionally invariant, and the weight of dependence of most recent data versus data more distant than the present is dependent on smoothing matrix determined by the control chart set-up (Montgomery, 2009).

Due to the complexity, initially industries had been slow to adapt multivariate SPC charts to their processes. More recently published accounts of multivariate SPC use in industry are becoming much more common, with countless recent publications utilizing multivariate SPC. For example, engineers have integrated multivariate SPC with engineering process control to improve fault detection in heating ventilation and air conditional systems (Siddiqui et al., 2015). The healthcare industry has utilized Hotelling's T^2 and multivariate EWMA charts to improve patient care (Jarrett, 2014). Additionally, manufacturing has incorporated Hotelling's T^2 to monitor the smelting process in aluminum production (Majid et al, 2011).

We were able to only find one published account of the use of multivariate SPC approaches in an animal production system (Table 1-1). Miekley et al. (2013) investigated the possibility of early detection of lameness and mastitis using a multivariate cumulative sum control chart and methodologies to obtain this detection. Variables included in the model were dry matter intake, feeding behaviors, milk conductivity, milk yield, and physical activity. Different preprocessing methods were used on the data to create different monitoring systems. The authors concluded that the potential exists to use these techniques in monitoring systems for disease detection, but the systems are not yet directly applicable (Miekley et al., 2013). In addition, the authors discussed the lack of publications utilizing multivariate SPC in livestock production, and noted the multiple benefits that they could provide.

Rationale of Current Research

Using Individual Cow Data to Evaluate Pen or Herd-Level Performance

Herd management currently focuses largely on utilizing historical data to make individual and herd-level decisions. Monthly individual cow testing (via DHI) has often been the source of this information in the past, but more recently, the advent of herd management software provides a readily available source of information for the producer or manager. In addition, new technologies have resulted in an abundance of data collected on farm with advanced sensors, however there is typically very poor analysis of the data, with little to no focus on variation, when presenting the data to the producer. The data is also still utilized in a reactive manner, with relatively little real-time analysis of incoming data contributing to the decision making on commercial dairies. Other industries have used more real-time analysis methods of data analysis for many years, and we believe that application to the dairy industry could prove useful in decision-making at the pen or herd level with real economic benefits. One primary method to achieve this goal is through the use of SPC control charts, which have the potential to predict future variance and make adjustments early, possibly reducing economic losses.

As previously discussed, use of SPC control charts in animal production systems has focused mainly on detecting individual animals that deviate from the mean to determine if a physiological change has occurred in an individual animal level, for example, diagnosis of illness, disease, or heat detection (Table 1-1). However, detection of deviant performance by a pen of animals, and fixing the source of the problem, has the potentially to drastically improve economics on a commercial dairy farm. Outside factors affecting performance of a pen of cows, such as forage quality or ambient temperature, could potentially be tied into the analysis to determine when changes need to be made. As seen in Table 1-1, relatively few research groups have conducted SPC studies in animal agriculture, and fewer still have

utilized more in-depth multivariate analyses. In addition, the variance structure of much of the data are unknown.

Hypothesis and Explanations of Present Studies

The first objective of this thesis was to characterize the variance structure in data recorded on an individual animal basis and averaged on a pen level to evaluate animal performance in both a controlled research environment (Chapters 2 and 3) as well as both a robotic and conventional commercial dairy (Chapter 4). Our second objective was to collect a wide array of data including feeding behavior (Chapter 2), intake (Chapters 2, 3, and 5), activity and rumination (Chapters 2 and 5), and production data (Chapter 2, 3, 4 and 5) to attempt to integrate diverse data streams on a dairy farm. Lastly, our final objective was to develop a model to utilize multivariate SPC methods to detect deviant pen performance on commercial dairies (Chapter 5). To conclude, we present a final chapter (Chapter 6) to explain further applications and research in this topic area.

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Table 1-1. Overview of publications applying statistical process control techniques to agricultural systems.

Reference	Control Chart[1]	Livestock	Unit of Measure	Variables Evaluated
Univariate Analyses				
Baum et al., 2005	Shewhart	Swine	ELISA assay	Optical density %
Bono et al., 2012	Shewhart, CUSUM (V-Mask)	Swine	Sow or Herd	Litter size
Bono et al., 2013	Shewhart, CUSUM (V-Mask)	Swine	Herd	Farrowing rate
Bono et al., 2014	Shewhart, CUSUM (V-Mask)	Swine	Herd	Mortality rate
Cavero et al., 2007	EWMA	Dairy	Cow	SCC
Cornou et al., 2008	CUSUM (V-Mask)	Swine	Pig	Eating rank
Cornou et al., 2012	CUSUM	Swine	Sow	Acceleration
Cornou et al., 2014	Shewhart	Dairy	Cow	Insemination quantity, insemination rate conception rate
Cowen et al., 1994	Shewhart	Poultry - Turkey	Flock	Survival rate
Deen, 1997	Shewhart	Swine	Herd	Number of piglets weaned
De Vries et al., 1997	Shewhart	Dairy	Herd or cow	Bulk tank SCC, conception rate
De Vries et al., 1997b	Shewhart	Dairy	Cow	Daily milk weights
De Vries and Conlin, 2003a	Shewhart, CUSUM	Dairy	Cow	Estrous detection ratio
De Vries and Conlin, 2003b	Shewhart, CUSUM	Dairy	Cow	Estrus detection
De Vries and Conlin, 2005	Shewhart, CUSUM	Dairy	Cow	Estrous detection index
Engler et al., 2009	CUSUM, EWMA	Swine	Herd	Number of piglets weaned, pre-weaning mortality, return to service rate
Fraile et al., 2009	Shewhart	Swine	Pig	Mortality, culling, average daily gain
Galli et al., 1998	Shewhart	Beef	Bull	Sperm motility
Greenstam, 2005	CUSUM	Dairy	Cow	Milk conductivity
Huybrechts et al., 2014	Shewhart, CUSUM	Dairy	Cow	Milk yield
Koketsu et al., 1999	Shewhart	Swine	Herd	Number of females mated per week
Krieter et al., 2005	EWMA	Swine	Herd	Litters, piglets born alive, piglets weaned
Krieter et al., 2009	CUSUM, EWMA	Swine	Sow	Piglets born in total, return to estrus rate

Table 1-1 (continued).

Kristensen and Cornou, 2012	CUSUM (V-Mask)	Poultry - Broiler	Chicken	Physical activity
Linhares et al., 2014	EWMA	Swine	Herd	Time to baseline production following disease exposure
Lukas et al., 2005	Shewhart	Dairy	Herd	Bulk tank SCC
Lukas et al., 2008a	Capability/Consistency Index	Dairy	Herd	Bulk tank SCC
Lukas et al., 2008b	CUSUM, Shewhart	Dairy	Pair of cows	DMI, water intake
Lukas et al., 2009	Shewhart, CUSUM	Dairy	Cow	Milk yield, milk electrical conductivity
Lukas et al., 2015	CUSUM	Dairy	Cow	Milk yield
Madsen and Kristensen, 2005	CUSUM	Swine	Pen	Water consumption
Mertens et al., 2008	CUSUM	Poultry	Flock	Average egg weight
Mertens et al., 2009	CUSUM	Poultry - Chicken	Flock	Hen-day egg production
Miekley et al., 2012	CUSUM	Dairy	Cow	Milk electrical conductivity, physical activity
Niza-Ribeiro et al., 2004	Capability Index	Dairy	Herd	Bulk tank SCC
Pastell and Madsen, 2008	CUSUM	Dairy	Cow	Weight distribution among legs
Pleasants et al., 1998	CUSUM	Beef	Steer	Muscle pH
Quimby et al., 2001	CUSUM	Beef	Steer	Feeding behavior
Reneau, 2000	Shewhart	Dairy	Herd	Bulk tank SCC
Reneau et al., 2007	Shewhart, CUSUM	Dairy	Herd or cow	MUN, milk fat %, physical activity
St-Pierre and Cobanov, 2007a	Shewhart	Dairy	Feed sample	Forage composition
St-Pierre and Cobanov, 2007b	Shewhart, CUSUM	Dairy	Feed sample	Forage composition
Wallace, 2009	Shewhart	Dairy	Cow	Milk yield, flow rate, milking time
Wilson et al., 1980	CUSUM	Swine	Herd	Return to estrus
Wrathall, 1977	Shewhart	Swine	Herd	Return to service %, pigs per litter, fetuses born dead
Multivariate Analyses				
Miekley et al., 2013	CUSUM	Dairy	Cow	Milk yield, milk electrical conductivity, physical activity, feed intake, time at feedbunk, number of feedbunk visits

[1]Column refers to method of statistical process control used in the analysis; CUSUM = cumulative sum chart

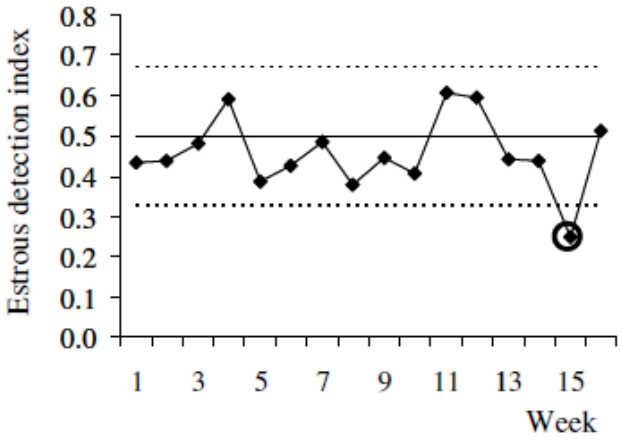

Figure 1-1. Example of a Shewhart chart, as presented by De Vries and Conlin (2005). A calculated estrous detection index (Y-axis) in dairy cattle is plotted against week number (X-axis). The Shewhart chart features a center line (solid straight line), surrounded by an upper and lower control limit (dotted lines). The control chart signals an out of control data point at Week 15.

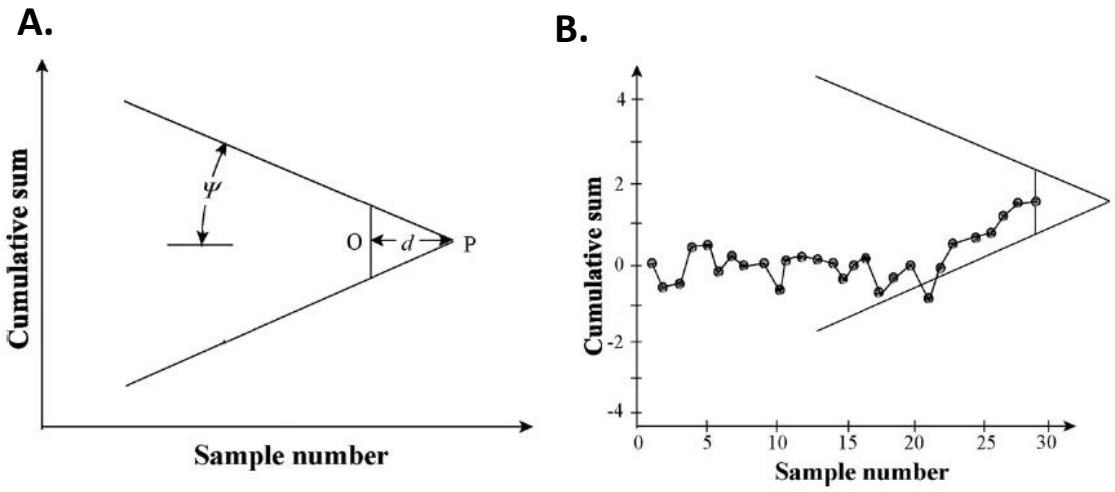

Figure 1-2. Example of a cumulative sum (CUSUM) control chart with a V-mask used to detect activity levels in pens of broiler chickens, as presented by Kristensen and Cornou (2011). Panel A shows the V-mask with parameters that can be altered in the analysis; Panel B is an illustration of the application of the V-mask to a control chart. Plotted points that cross the V-mask lines are considered out-of-control, as seen in sample#21.

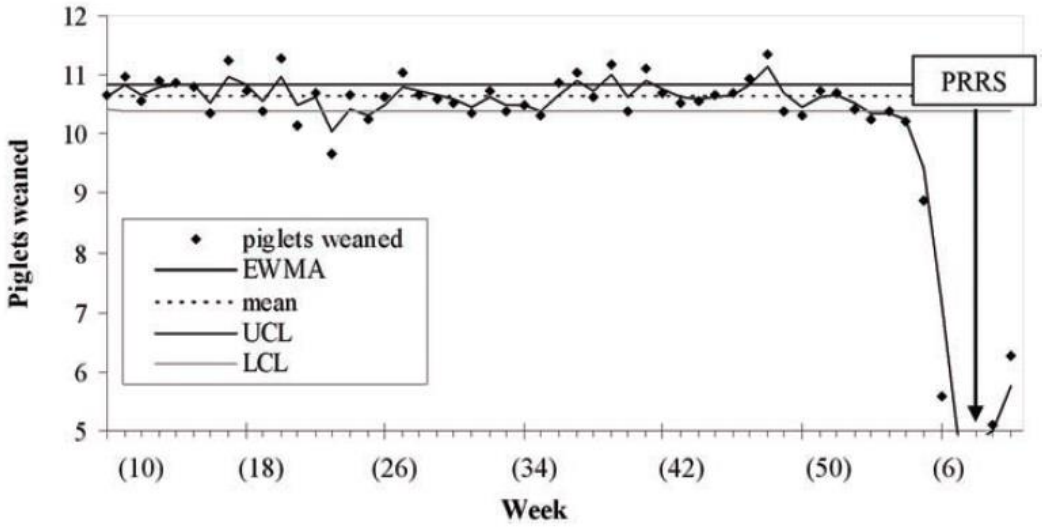

Figure 1-3. Example of a exponentially-weighted moving average (EWMA) control chart used to monitor the number of piglets weaned (Y-axis) each week (X-axis), as presented by Engler et al. (2009). The mean is represented by the dotted center line, with the upper control limit (UCL) and lower control limit (LCL) indicated by the solid lines above and below the mean. The raw data is plotted with dots, and the EWMA is plotted with the moving line. A herd diagnosis of Porcine Reproductive and Respiratory Syndrome (PRRS) is indicated near the end of the control chart, causing the EWMA to cross the LCL.

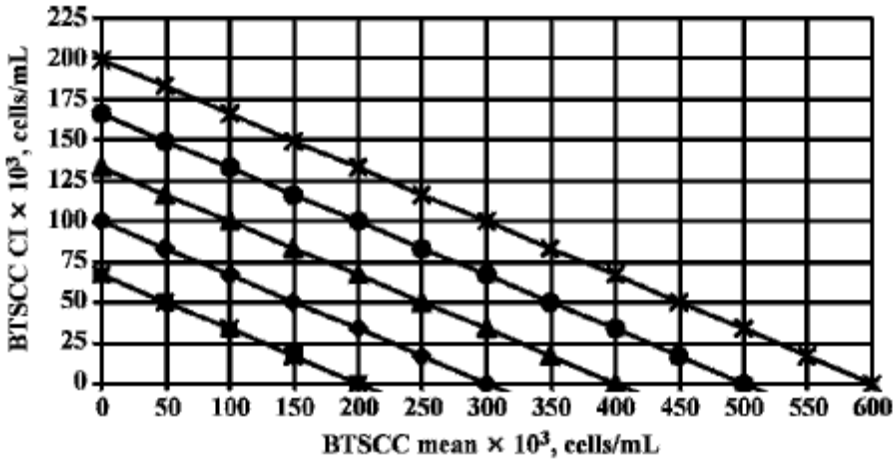

Figure 1-4. Example of a Consistency Index (CI) analyzing milk quality on a commercial dairy farm, as presented by Lukas et al. (2008a). Dairy managers and the milk plant can use the CI chart to assess a herds' capability of maintaining production of high quality milk. Based on the farms bulk tank somatic cell count (BTSCC; X-axis), the CI indicated on the Y-axis represents the maximum sigma that can be allowed in the BTSCC in order for the herd to consistently produce quality milk at the chosen SCC level.

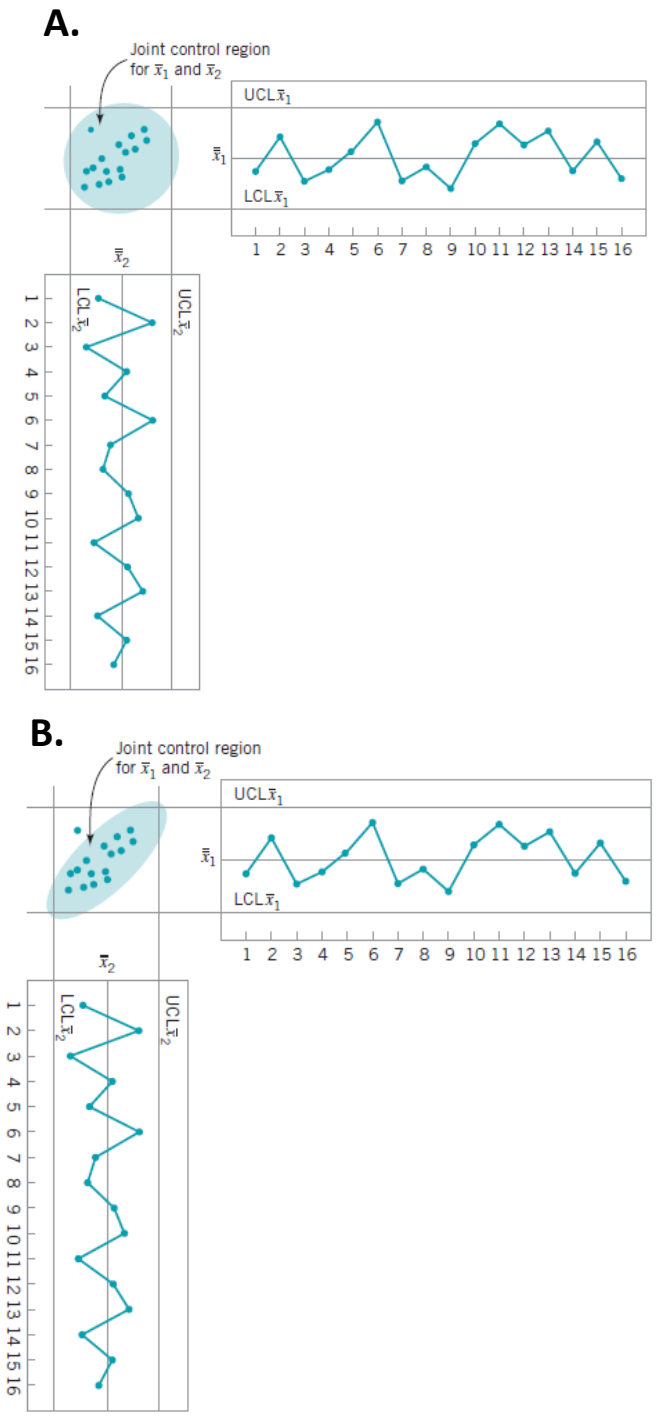

Figure 1-5. Examples of an ellipse for two independent (Panel A) or two dependent (Panel B) variables, as presented by Montgomery (2009). When two variables are independent (Panel A), the joint control region is in the shape of a circle, thus the point in the top left is consider statistically in control. When two variables are dependent (Panel B), the joint control region is in the shape of an ellipse, thus the point in the top left is considered statistically out-of-control, even though in each individual control chart, the point would fall within the control limits. This represents the basis for multivariate SPC approaches.

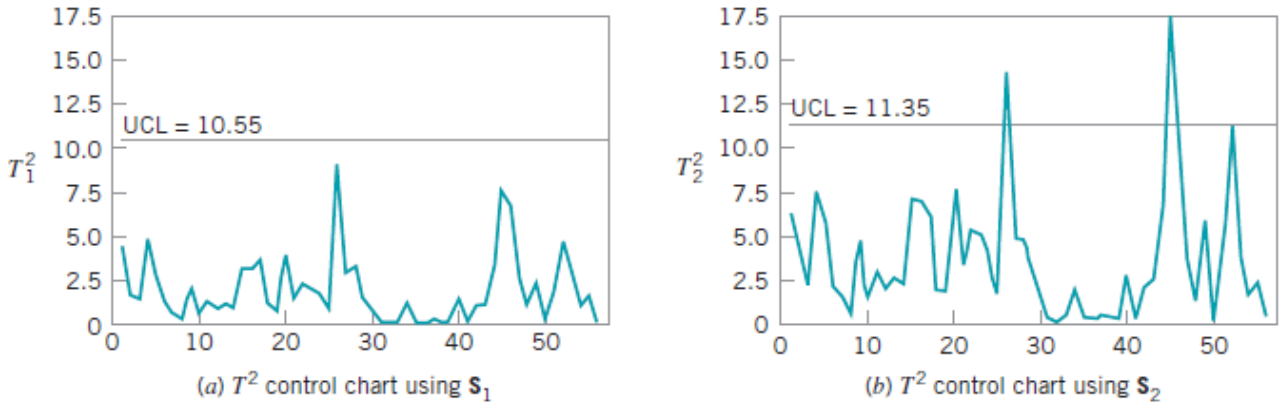

Figure 1-6. Example of a multivariate statistical process control using a Hotelling's T^2 control chart from Montgomery (2009). Very similar to the univariate Shewhart chart, the Hotelling's T^2 control chart monitors the mean vector of process with multiple variables.

Chapter 2. Behavioral Effects of Incorporation of Tall Fescue Hay into a Lactating Dairy Cow Ration: Feeding, Rumination, Physical Activity.

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Abstract

Our objective was to evaluate the effects of replacing either corn silage or alfalfa silage with tall fescue hay on cow behavior, including rumination, physical activity, eating, and meal patterns. Twenty-four primi- (75 ± 35 DIM) and 40 multi-parous (68 ± 19 DIM) Holstein cows were blocked by parity and randomly assigned to 1 of 4 treatment groups in a pen equipped with 32 feeding gates to record intake and eating patterns by cow. Each gate was randomly assigned to 1 treatment group, thus each cow had access to all 8 gates within the respective treatment and cow was the experimental unit. Collars were placed on the cows for the duration of the trial to record rumination and physical activity in 2 h intervals and presented as total per day and total per 2 h interval. Treatments were formulated to replace either corn silage (CS) or alfalfa silage (AS) with tall fescue hay (TF) as follows (DM basis): 67% CS and 33%AS (control), 60%TF and 40%AS (60TF40AS), 60%TF and 40%CS (60TF40CS), and 33%TF and 67%CS (33TF67CS). The experiment was a 7-week continuous lactation trial with a two week covariate period. Total rumination time, rumination per unit of NDF or DMI, total eating time, eating rate, meal duration, or meal size did not differ among treatments. Cows consuming diets incorporating tall fescue consumed fewer meals and tended to be less physically active. Multiparous cows had greater rumination, greater DMI, and tended to have an increased eating rate and increased meal size than primiparous cows. Rumination and physical activity also appeared to be divergent behaviors; when physical activity levels were high, rumination was relatively low. The present study concludes that consideration of the time budget constraints of dairy cows is important when implementing management changes such as diet alterations.

Keywords: tall fescue, feeding behavior, rumination, time budget, physical activity

Introduction

Recent technological advances have allowed commercial dairy farms to collect a wide array of data via sensors measuring cow variables at the individual animal level, including rumination (Schirmann et al. 2009), physical activity (Mattachini et al., 2014), and other eating behaviors (Kononoff et al., 2002; Bikker et al., 2014). However, this data is typically used at the animal-level to measure a change in physiological condition of an individual animal, such as notification of imminent calving (Schirmann et al., 2013; Buchel and Sundrum, 2014; Clark et al., 2015), heat detection (Reith and Hoy, 2012; Pahl et al., 2015), and early detection of ill cows (Calamari et al., 2014). Relatively few studies have looked at utilizing this data on a pen- or herd-level to look at the impact of management changes on dairy cattle behavior.

Consideration of the time budget of a dairy cow is important when determining management strategies. Dietary factors account for nearly 50% of the variation in milk yield on a dairy farm (Bach et al., 2008), thus management of nutrition, and recognition of cow responses to nutrition, is critical. Research has intensely focused on nutritional factors affecting the time budget in dairy cattle, including feed push-up schedule (Armstrong et al., 2009), TMR delivery frequency (DeVries et al., 2005; Sova et al., 2013) and timing (DeVries and von Keyserlingk, 2005), and bunk space (Hill et al., 2009; Rioja-Lang et al., 2012). However, with the recognition that cows spend about one-third of their daily time budget ruminating, or about eight to nine hours per day (Welch, 1982), and that cows will prioritize lying time over eating (Grant, 2015a), more research must be done on rumination and behavior changes during different time periods of the day (Pahl et al., 2015).

Dietary changes, such as varying the NDF level, affect rumination and eating behaviors. Previous data has shown that cows will ruminate anywhere from 25 to 80 min per kg of roughage consumed (Sjaastad et al., 2003). Grasses can contain higher NDF levels than corn silage and alfalfa silage (NRC, 2001), which could impact rumination and feeding behaviors. The objective of the present study was to evaluate the effects of replacing corn silage and alfalfa silage with tall fescue hay in a lactating dairy cow ration on cow behavior including rumination, physical activity, and rumination on both a daily basis and on time intervals within a day.

Materials and Methods

Tall Fescue Hay Production

Bariane Tall Fescue (Barenbrug USA, Tangent, OR) was planted in separate field plots at a rate of 22.42 kg/ha at the University of Wisconsin-Arlington Agricultural Research Station (UWAARS; Arlington, WI) on September 15, 2011 with a no-till drill. For this feeding trial, the forage was cut on June 18, 2013 (first cut) and baled in large square bales on June 21, 2013. The bales were stored in an enclosed shed for approximately eight months prior to trial initiation.

Description of Experiment

The experimental protocol was approved by the Animal Care and Use Committee of the College of Agriculture and Life Sciences at the University of Wisconsin–Madison. Twenty-four primiparous (75 ± 35 DIM) and 40 multiparous (68 ± 19 DIM) lactating Holstein dairy cows were housed in a pen equipped with Insentec Roughage Intake Control (**RIC**) system gates (Insentec BV, Marknesse, the Netherlands) in a freestall barn at the University of Wisconsin-Madison Emmons-Blaine Arlington freestall, milking parlor dairy

facility. The RIC feeding gates recorded number of bunk visits, duration of bunk visits, and feed intake during each bunk visit. Cows were equipped with SCR HR-TAG neck collars (SCR Engineers Ltd., Netanya, Israel) to record rumination and physical activity in two hour intervals and on a daily basis. Rumination per kg of NDF intake was based on NDF intake from weeks 5 and 7, as determined in the companion paper (Bender et al., 2015), and was calculated as total rumination minutes during each day divided by NDF intake for that day. Rumination per kg of DMI was based on DMI through the duration of the trial and was similarly calculated as total rumination minutes during each day divided by DMI for that day.

Cows were blocked by parity and randomly assigned to 1 of 4 treatment groups. Each of 32 feeding gates was randomly assigned to 1 treatment group, thus each cow had access to all 8 gates within the respective treatment group, and cow was the experimental unit. Treatments were formulated to replace either corn silage (CS) or alfalfa silage (AS) with tall fescue hay (TF) while maintaining similar diet CP and starch concentrations. Dietary NDF and iNDF levels were allowed to change. Treatments were as follows, with diet names abbreviated by the proportion that each forage contributes to the total forage component of each TMR: control, 60%TF and 40%AS (60TF40AS), 60%TF and 40%CS (60TF40CS), and 33%TF and 67%CS (33TF67CS). All cows received bovine somatotropin (Posilac, Elanco Animal Health) every 14 d through the duration of the trial, initiated during the covariate period. The experiment was a continuous lactation trial, beginning with a 2 week covariate period prior to 7 weeks of assignment to treatment diets. Cows were milked twice daily, at approximately 0330 and 1500.

Cows were fed a TMR with feed delivered 3 times daily at 0300, 1200, and 1500, and were offered free access to water. The TMR was mixed once daily before the mid-day

feeding and stored for delivery in the afternoon and the following morning. Cows were fed ad libitum for 10% refusals. Dry matter content of the forages was measured once weekly, and as fed ingredient proportions were modified accordingly to maintain near constant proportions of feeds on a DM basis.

Description of Feeding Behavior Analysis

Feeding behavior was analyzed similar to Ferraretto et al. (2012). Eating time (min/d) was defined as the time that a cow had her head in a gate feeder with the feeding gate down. Eating rate (kg of DM/min) was determined by dividing DMI by the time spent eating. De Vries et al. (2003) determined meal frequency (number of daily meals) by defining a new meal as an independent event when there was an intervening gap between gate visits of at least 27.7 minutes. A meal consisted of eating and interval times or intervals between feeding visits within a meal. Meal duration (min/meal) and size (kg of DM/meal) were determined by dividing eating time and DMI, respectively, by meal frequency.

Statistical Analyses

Data were analyzed as a randomized, complete-block design with a covariate using the MIXED procedure of SAS (version 9.4, SAS Institute, Inc., Cary, NC). The model included parity, treatment, week, and the relevant interactions as fixed effects, and cow within treatment as a random effect. Day of treatment was included as a repeated measure using the compound symmetry structure to account for autocorrelated errors. Means were determined using the least squares means statement, and treatment means were compared using the Bonferroni *t*-test option after a significant ($P < 0.05$) overall treatment *F*-test. Interactions were partitioned using the SLICE options of SAS (version 9.4, SAS Institute,

Inc., Cary, NC). Statistical significance and trends were considered at $P \leq 0.05$ and $P > 0.05$ to $P \leq 0.10$, respectively.

Results and Discussion

Nineteen cows were removed from the trial for health and behavior reasons. One cow could not be trained to the feeding gates. Two were removed for health reasons, one for severe mastitis and one for lameness. Sixteen cows were also removed because they habitually consumed feed from gates in which they were not assigned that totaled at least 5% of their feed intake. These nineteen cows were not used in the statistical analysis.

Dietary ingredients of the treatment TMR's are listed in Table 2-1. Treatment TMR's were formulated to replace corn silage (60TF40AS) or alfalfa silage (60TF40CS and 33TF67CS) with tall fescue hay while maintaining similar CP and starch levels, as detailed in the companion paper (Bender et al., 2015).

Treatment Effects on Cow Behavior

Effects of tall fescue hay incorporation on rumination, physical activity, and feeding behavior is presented in Table 2-2. Dry matter intake was greater ($P < 0.05$) for the control diet compared to the 60TF40CS and 33TF67CS diets, and tended ($P = 0.07$) to be greater than the 60TF40AS diets. There was no effect of tall fescue hay incorporation on rumination measurements including total rumination minutes ($P = 0.42$), rumination time per kg of NDF intake ($P = 0.93$), or rumination time per kg of DMI ($P = 0.29$), and averaged 481.0 min/d, 75.4 min/kg NDF intake, and 21.5 min/kg DMI, respectively, among all treatments. Figure 2-1 Panel B displays the effects of treatment on rumination per 2 hour time interval over all 24 hours of an average day. Rumination was similar among treatments throughout most time intervals, however, the 60TF40AS treatment had greater ($P < 0.05$) rumination than all other

treatments in the 10th time interval (1800 – 2000 h) and 60TF40CS had greater ($P < 0.05$) rumination than 33TF67CS in the 11th time interval (2000 – 2200 h).

Grant (2015b) observed differences in total rumination time among four treatments comparing higher forage diets (and therefore higher NDF) to lower forages diets as well as comparing conventional corn silage to brown midrib (BMR; higher NDF digestibility) corn silage at each forage level. Total rumination time was significantly ($P < 0.01$) greater in the high forage diets compared to the low forage diet with BMR corn silage. In the present study, despite diets ranging in NDF from 25.3% to 30.5% (% of DM) and formulated from different forage sources (corn silage, alfalfa silage, and tall fescue grass hay), rumination was similar across treatments. Cows in the present study spent approximately 30 fewer min per day ruminating among all treatments compared to Grant (2015b). Although rumen fill was not measured in the present study, this suggests that highly digestible grasses provide an alternate source of NDF with no effects on rumination. Rinne et al. (2002) evaluated dairy cows fed silages at four different maturities (increasing NDF content with increasing maturity) and agreed with the present study; eating time and rumination time per kg of NDF intake did not differ among maturities. However, contrary to the present study, total rumination time and rumination time per kg DMI increased as maturity increased (Rinne et al. 2002). Finally, in companion studies by Oba and Allen (2000, b), brown midrib (BMR) corn silage fed at two levels of NDF were fed to dairy cows. Dry matter intake was lower in the high NDF diets, while total rumination time and rumination time per kg of DMI was greater compared to the low NDF diets.

A tendency ($P = 0.07$) for a treatment effect of tall fescue incorporation on physical activity occurred, however no significant differences were observed among treatments (Table

2-2). A treatment x week interaction for physical activity was also observed (Figure 2-2 Panel A), where the control treatment was greater ($P < 0.05$) than the 33TF67CS treatment in weeks 3, 4, 6, and 7, and greater ($P < 0.05$) than the 60TF40AS treatment in weeks 3, 4, and 6. Effects of tall fescue incorporation on physical activity per 2 h time interval over the course of an average day are displayed in Figure 2-1 Panel A. Physical activity was greater ($P < 0.05$) in time interval 8 (1400 – 1600 h) and 10 (1800 – 2000 h) for the control treatment compared to all other treatments, and greater ($P < 0.05$) for control than 60TF40CS and 33TF67CS in time interval 9 (1600 – 1800 h).

Activity has previously been reported to be used along with rumination as a heat detection aid (Silper et al., 2015) and investigated as a possible early indicator of calving time (Schirmann et al., 2013; Clark et al., 2015) and animal health, but the authors found no studies relating physical activity to feeding behaviors and dairy cattle nutrition. It is possible that changing inclination of the head during eating could be counted as physical activity in the algorithm of the sensor, since activity is recorded by an accelerometer on the collar measuring changes in three planes of motion (Silper et al., 2015). Thus, the difference in physical activity could be partially explained by the numerical difference in eating time, however no significant difference in eating time was observed among treatments.

Effects of tall fescue hay on eating and meal patterns are also presented in Table 2-2. Tall fescue incorporation had no effect on eating time (min/d) or meal size (kg of DM/meal), which averaged 222.2 min/d and 4.83 kg of DM/meal, respectively. Eating rate (kg/h) tended ($P = 0.09$) to be higher for 60TF40AS compared to 60TF40AS, but no significant differences were observed among all treatments. Meal frequency (number of daily meals) was significantly greater ($P < 0.05$) for the control treatment compared to all treatments

incorporating tall fescue. In addition, there was also a treatment by week interaction ($P < 0.05$) for meal frequency (Figure 2-2 Panel B), where the control treatment was higher ($P < 0.05$) than the 33TF67CS treatment in week 4 and the 60TF40CS treatment in week 6, but was numerically higher than all treatments during the duration of the trial. Finally, meal duration (min/meal) was not significantly different among all treatments, but tended ($P = 0.05$) to be greater for 60TF40CS compared to control.

The increase in DMI observed in the control treatments appears to be largely due to increasing the meal frequency. Compared to treatments incorporating tall tescue, the control treatment consumed nearly one full meal more per day. The definition of a meal in the present study was derived from De Vries et al. (2003) and utilized in Ferraretto et al. (2012), where a new meal was initiated after an intervening gap of at least 27.7 min without frequenting the feed trough. Modification of this intervening gap could change the meal frequency, and this may contribute to the differences observed in the present study. Fisher et al. (1993) compared feeding orchardgrass silage to tall fescue silage to lactating dairy cows, and reported that DMI was greater for tall fescue-fed cows, and in addition both eating time (141.4 vs. 68.3 min; $P < 0.05$) and eating rate (69.6 vs. 54.0 g/min; $P < 0.05$) were higher for tall-fescue fed cows as well. Rumination and other eating parameters were not reported in Fischer et al. (1993). Companion studies by Oba and Allen (2000a, b) also measured feeding activity and reported that higher NDF diets had greater eating time with no difference in number of meals consumed or dry matter meal size.

Primiparous vs. Multiparous Cows

Effect of parity on rumination and physical activity is shown in Table 2-3. Total rumination time (min/d) was greater ($P < 0.01$) for multiparous compared to primiparous

cows (543.8 vs. 418.2 minutes/d), with multiparous cows spending approximately 2 hour more per day ruminating than primiparous cows. No effect of parity on rumination either per kg of NDF intake or per kg of DMI was observed, and averaged 75.4 and 21.6, respectively. Physical activity was similar across parities and averaged 560.2 units per day.

Parity effects on eating and meal patterns is also shown in Table 2-3. Dry matter intake was greater ($P < 0.01$) for multiparous compared to primiparous cows (24.2 vs. 22.1 kg/d). No effect of parity on eating time (min/d), meal frequency (number of daily meals), or meal duration (min/meal) was observed, and averaged 222.2 min/d, 5.1 meals, and 46.0 min/meal, respectively. Eating rate ($P = 0.08$; 6.69 vs. 6.19 kg/h) and meal size ($P = 0.07$; 5.04 vs. 4.61 kg of DM/meal) tended to be greater for multiparous compared to primiparous cows.

Time Budget of a Dairy Cow

Figure 2-3 details rumination and physical activity broken down by 2 h time intervals over the course of a day. Rumination and physical activity appeared to have an inversely related. For example, rumination was lowest (and significantly lower than all other intervals, $P < 0.05$) during time interval 8 (1400 – 1600), while physical activity was highest during the same time interval (and significantly higher than all other intervals, $P < 0.05$). This data suggests that cows do not ruminate, or ruminate infrequently, during physical activity. Cow ruminated less than 20 min/2 h interval during time interval 8 (1400 – 1600), likely due to the fact that new feed was delivered at 1500 h and milking also occurred during this time period. Physiologically, cows cannot ruminate at the same time as they eat, so while the eating event may contribute to physical activity due to the motion of the head, as previously discussed,

cows reduce rumination concurrently. Cows likely also ruminate less during events such as milking, which may slightly elevate stress levels.

Lying time was not measured in the present study, and is an important consideration in the time budget analysis of a dairy cow, as cows prefer to ruminate while lying down (Cooper et al., 2007). Grant (2015a) reported that since lying time is prioritized over eating, cows may sacrifice eating time in order to increase lying time. In fact, Grant (2015a) reported that with every 3.5 min of lost rest, cows will sacrifice 1 min of eating time. Thus, consideration of the whole time budget of a dairy cow, including lying time, rumination, physical activity, and eating behaviors are important when considering management changes to dairy cows.

Conclusions

Replacement of corn silage and alfalfa silage with tall fescue hay had no effect on rumination time, rumination time per unit of DMI or NDF intake, eating time, eating rate, meal duration, or meal size. Tall fescue hay incorporation tended for cows to be less physically active, and meal frequency was reduced. Multiparous cows ruminate more per day, consume more feed, and tend to eat bigger meals at a faster eating rate than primiparous cows. Rumination and physical activity appear to be largely divergent behaviors, with rumination times increased during periods of rest, and physical actively elevated near meal times and milking times. The present study concludes that time budget consideration of dairy cows is important when making management changes such as diet alterations.

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Table 2-1. Dietary ingredients of treatment TMR's (% of DM unless otherwise noted)

	Covariate	Control	60TF40AS	60TF40CS	33TF67CS
Alfalfa silage	11.0	18.3	18.4	----	----
Corn silage	36.7	36.6	----	20.0	33.1
Tall fescue hay	9.2	----	27.7	30.0	16.6
H.M.C.	22.1	23.8	38.7	30.0	26.7
Soybean meal (48% CP)	14.7	14.7	9.8	14.0	16.94
Energy Booster 100[1]	2.02	2.01	2.03	2.21	2.03
Calcium carbonate	1.28	1.28	1.29	1.40	1.66
Sodium bicarbonate	0.73	0.73	0.81	0.88	0.74
Potassium carbonate	0.37	0.37	----	----	0.55
Monocalcium phosphate	0.37	0.37	0.07	0.08	0.37
Trace mineral salt[2]	0.37	0.37	0.41	0.44	0.41
Magnesium-potassium-sulfate[3]	0.73	0.73	0.18	0.20	0.18
Magnesium Oxide	----	0.33	0.18	0.20	0.28
Vitamin E	0.02	0.02	0.02	0.02	0.04
UW ADE[4]	0.37	0.37	0.37	0.40	0.37
Smartamine M[5]	0.05	0.05	0.06	0.06	0.04

[1]Minimum 98% total fatty acids (MSC Company, Dundee, IL).
[2]Contained 88% NaCl; 0.002% Co; 0.2% Cu; 0.012% I; 0.18% Fe; 0.8% Mn; 0.006% Se; 1.4% Zn.
[3]Dynamate (11% Mg, 18% K, 22% S; The Mosaic Co., Plymouth, MN).
[4]Vitamin A 3,300,000 IU/kg; Vitamin D 1,100,000 IU/kg; Vitamin E 11,000 IU/kg.
[5]Smartamine M, 70% Methionine (Adisseo, SAS, France).

Table 2-2. Effects of tall fescue hay incorporation on rumination, physical activity, and feeding behavior.

	Treatment					*P* – value[1]		
	Control	60TF40AS	60TF40CS	33TF67CS	SEM	Trt	Week	Trt x Week
DMI, kg/d	24.5[a]	23.2[ab]	22.1[b]	22.7[b]	0.4	<0.01	<0.01	<0.01
Rumination, min/d	465.6	492.9	480.5	485.1	35.0	0.42	<0.01	<0.01
Rumination, min/kg DMI	20.3	22.1	22.6	21.1	1.0	0.29	<0.01	<0.01
Rumination, min/kg NDF intake	77.7	72.3	75.6	76.0	5.1	0.93	<0.01	<0.01
Physical activity, units/d	594.8	540.8	564.9	540.5	23.0	0.07	<0.01	<0.01
Eating time, min/d	228.8	212.8	231.9	215.3	8.1	0.20	<0.01	0.03
Eating rate, kg/h	6.61	6.72[*]	5.96[*]	6.46	0.23	0.09	<0.01	<0.01
Meal frequency (number of daily meals)	5.6[a]	4.9[b]	4.9[b]	4.9[b]	0.2	<0.01	0.10	<0.01
Meal duration, min/meal	42.5[*]	46.2	49.8*	45.5	1.9	0.06	0.08	<0.01
Meal size, kg of DM/meal	4.45	5.06	4.89	4.91	0.19	0.13	0.37	0.04

[1]Trt = treatment; Trt x Week = Treatment by week interaction

Table 2-3. Effects of parity on rumination, physical activity, and feeding behavior.

	Primiparous	Multiparous	SEM	*P* – Value
Rumination time, min/d	418.2	543.8	25.7	<0.01
Physical activity, units/d	553.5	566.9	16.9	0.54
DMI, kg/d	22.1	24.2	0.4	<0.01
Eating time, min/d	223.8	220.6	5.5	0.67
Eating rate, kg/h	6.19	6.69	0.36	0.08
Meal frequency (number of daily meals)	5.1	5.1	0.1	0.74
Meal duration (min/meal)	46.4	45.5	1.3	0.61
Meal size (kg of DM/meal)	4.61	5.04	0.15	0.07
Rumination (min/kg NDF intake)	74.6	76.2	8.0	0.88
Rumination (min/kg DMI)	21.2	21.8	0.7	0.51

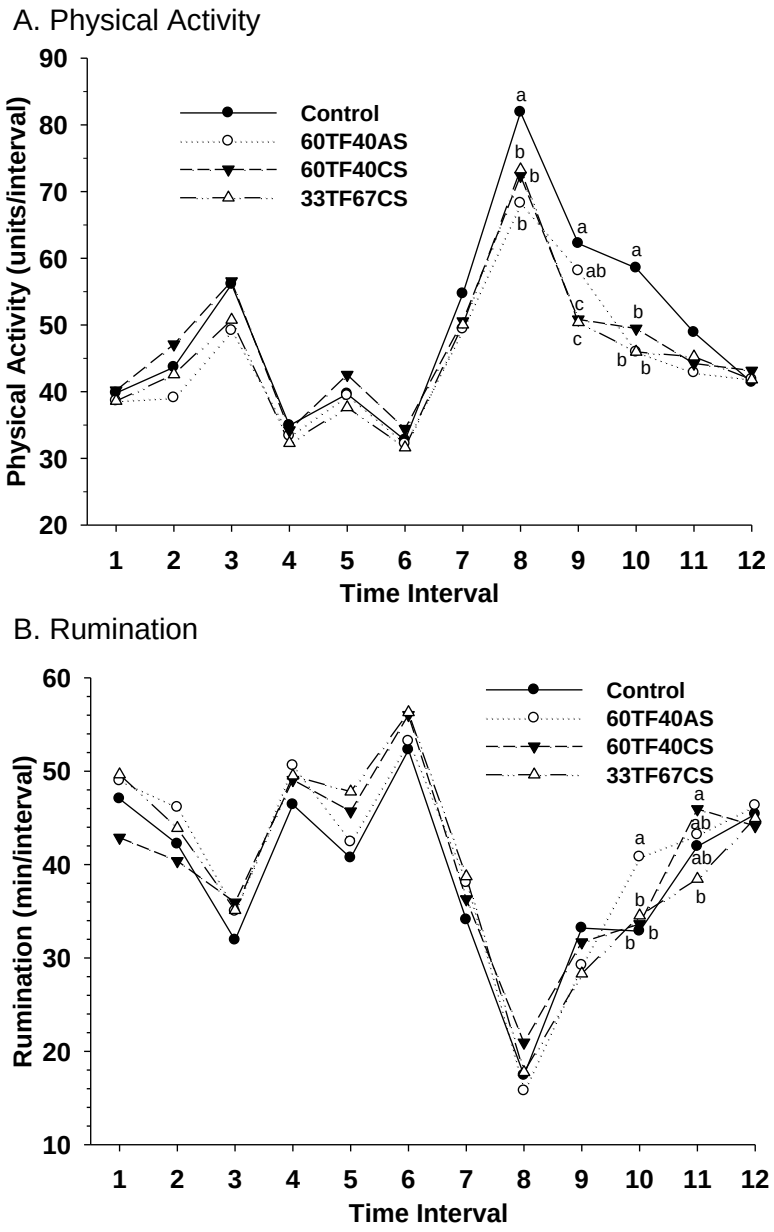

Figure 2-1. Least squared means of activity (units/interval; Panel A) and rumination (min/interval; Panel B) by time interval for cows consuming TMR with the forage portion composed (DM basis) of 33% alfalfa silage and 67% corn silage (Control), 60% tall fescue hay and 40% alfalfa silage (60TF40AS), 60% tall fescue hay and 40% corn silage (60TF40CS), and 33% tall fescue hay and 67% corn silage (33TF67CS). Time interval refers to consecutive 2 h segments over the course of a day, beginning with 2400 h (interval 1 = 2400 h to 0200 h; interval 2 = 0200 h to 0400 h, etc.). Within time interval, means with different superscripts differ ($P < 0.05$). Effects for physical activity were evaluated for treatment ($P = 0.06$), time interval ($P < 0.01$), and treatment by time interval interaction ($P < 0.01$). Effects for rumination were evaluated for treatment ($P = 0.47$), time interval ($P < 0.01$), and treatment by time interval interaction ($P = 0.73$).

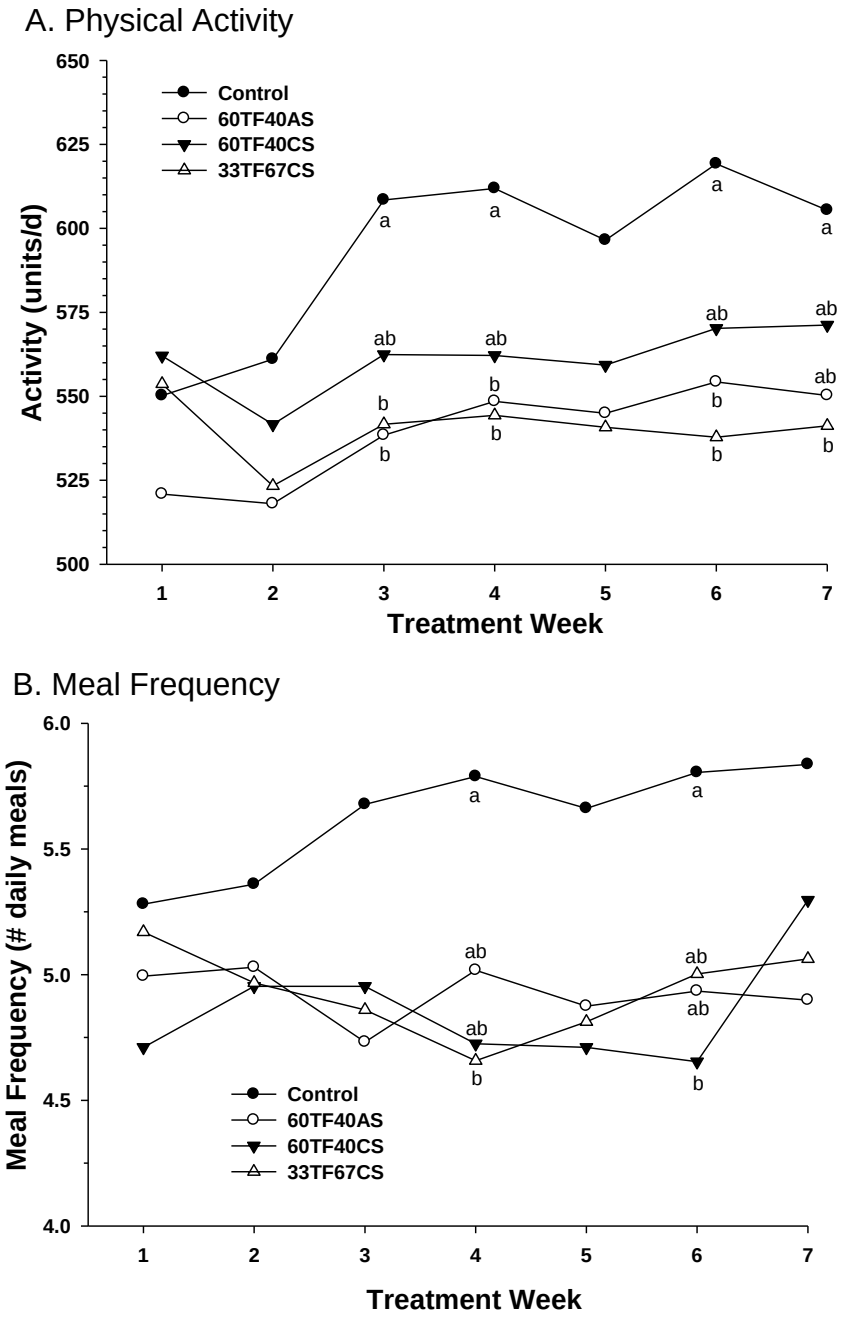

Figure 2-2. Least squared means of activity (units/d; Panel A) and meal frequency (number of daily meals) by week for cows consuming TMR with the forage portion composed (DM basis) of 33% alfalfa silage and 67% corn silage (Control), 60% tall fescue hay and 40% alfalfa silage (60TF40AS), 60% tall fescue hay and 40% corn silage (60TF40CS), and 33% tall fescue hay and 67% corn silage (33TF67CS). Within week, means with different superscripts differ ($P < 0.05$).

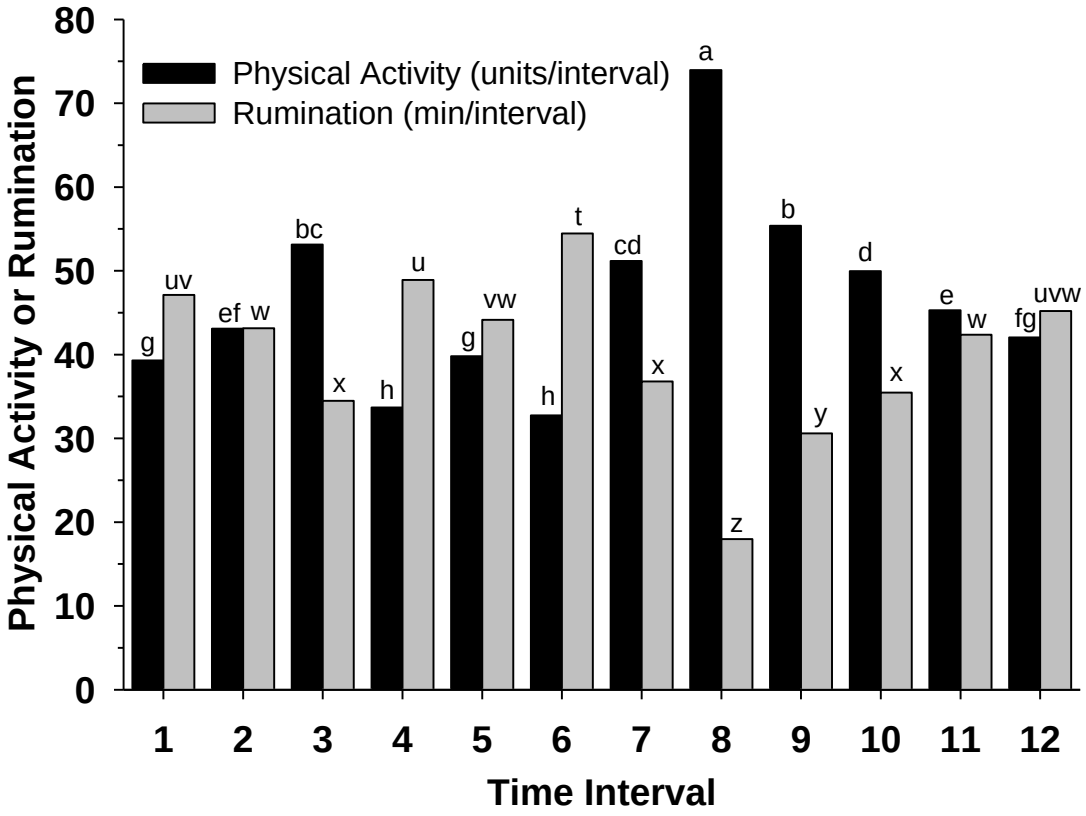

Figure 2-3. Least squared means of activity (units/interval; black bars) and rumination (min/interval; grey bars) by time interval. Time interval refers to consecutive 2 h segments over the course of a day, beginning with 2400 h (interval 1 = 2400 h to 0200 h; interval 2 = 0200 h to 0400 h, etc.). Within variable (physical activity or rumination), means with different superscripts differ ($P < 0.05$) among time intervals.

Chapter 3. Effects of Partial Replacement of Corn and Alfalfa Silage with Tall Fescue Hay on Total-Tract Digestibility and Lactation Performance in Dairy Cows.

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Abstract

Our objective was to evaluate the effects of replacing either corn or alfalfa silage with tall fescue hay on total-tract NDF digestibility and lactation performance in dairy cows. Twenty-four primi- (75 ± 35 DIM) and 40 multi-parous (68 ± 19 DIM) Holstein cows were blocked by parity and randomly assigned to 1 of 4 treatment groups in a pen equipped with 32 feeding gates to record intake by cow. Each gate was randomly assigned to 1 treatment group, thus each cow had access to all 8 gates within the respective treatment and cow was the experimental unit. Treatments were formulated to replace either corn silage (CS) or alfalfa silage (AS) with tall fescue hay (TF) as follows (DM basis): 67% CS and 33%AS (control), 60%TF and 40%AS (60TF40AS), 60%TF and 40%CS (60TF40CS), and 33%TF and 67%CS (33TF67CS). The experiment was a 7-week continuous lactation trial with a two week covariate period. Milk production did not differ among treatments and averaged 40.4 kg/d. Fat yield and concentration and protein yield and concentration did not differ among treatments and averaged 1.58 kg/d, 3.94%, 1.28 kg/d, and 3.15%, respectively. Dry matter intake was greater for the control treatment (24.5 kg/d) compared to 60TF40CS (22.1 kg/d) and 33TF67CS (22.7 kg/d) and tended to be greater than 60TF40AS (23.2 kg/d). In vivo total-tract dry matter digestibility did not differ among treatments and averaged 66.2%. In vivo total-tract NDF digestibility was lower for control (37.8%) compared to 60TF40AS (44.4%) and 33TF67CS (45.3%) and similar to 60TF40CS (42.4%). In vivo total tract NDF digestibility and an estimate of in situ total tract NDF digestibility were similar between techniques across all treatment diets (42.3% vs. 42.6%, respectively). Inclusion of tall fescue grass hay increased the total-tract NDF digestibility of the diet and has the potential to replace corn silage and alfalfa silage and maintain milk production.

Keywords: NDF, fiber digestion, tall fescue, dairy cow

Introduction

Lactating dairy cow DMI is limited by rumen distension as NDF levels in the ration increase (Allen, 1996), particularly when energy requirements are high (Allen, 2014). Grasses typically have a higher NDF content compared to alfalfa and corn silages (NRC, 2001), and alfalfa and corn silages have become commonplace in high producing dairy cow rations due to superior DM digestibility (Hoffman, et al., 1993). Improvements in NDF digestibility in corn silage (Ferraretto et al., 2015a; Ferraretto et al., 2015b) and alfalfa silage (Turnbull et al., 1982) have improved DMI and milk production. Similarly, incorporation of highly digestible NDF in grasses in the ration has the potential to increase DMI and milk production (Rinne et al., 2002; Cherney et al., 2004; Kuoppala et al., 2008) or maintain DMI and increase milk fat (Kendall et al., 2009).

Modern varieties of cool season grasses are bred to have greater NDF digestibility; tall fescue grass can contain greater than 50% NDF and have an in vitro 48-h NDF digestibility of up to 75% of NDF (Brink et al., 2010; Pelletier et al., 2010). Fiber digestibility of grasses can be influenced by many factors. Agronomic practices such as harvesting earlier (Hoffman et al., 1993; Rinne et al., 2002) or harvesting from spring growth (Rinne et al., 2002; Cherney et al., 2004; Pelletier et al., 2010) increases NDF digestibility. Although varieties of tall fescue with endophytes have been associated with toxicosis and poor cattle performance (Foote et al., 2013), modern varieties of tall fescue have been bred to be free of endophytes and have been shown to improve milk production compared to their infected counterparts (Strahan et al., 1987). Thus, incorporating a cool season, perennial grass, such as endophyte-free tall fescue, may allow for increased digestible NDF in a ration fed to lactating dairy cattle.

A novel tool to predict total-tract digestibility of NDF has recently been developed (Lopes et al., 2015a) using an in vitro fermentation assay to measure the proportion pdNDF and rate of digestion of NDF. Although the model has been validated in high producing cows at different intake levels (Lopes et al., 2015a), with different alfalfa to corn silage ratios (Lopes et al. 2015, accepted JDS), and across several TMR's incorporating a diverse array of forage silages (Lopes et al., 2015b), the model has not been validated to in vivo NDF digestion data in grass hay.

The objectives of the present study were to evaluate the effects of replacing either corn silage or alfalfa silage with tall fescue hay on lactation performance and to compare estimates of total-tract fiber digestibility as predicted by the TTNDFD model to in vivo measurements.

Materials and Methods

Description of the Experiment

The experimental protocol was approved by the Animal Care and Use Committee of the College of Agriculture and Life Sciences at the University of Wisconsin–Madison. Twenty-four primiparous (75 ± 35 DIM) and 40 multiparous (68 ± 19 DIM) lactating Holstein dairy cows were housed in a pen equipped with Insentec Roughage Intake Control (**RIC**) system gates (Insentec BV, Marknesse, the Netherlands) in a freestall barn at the University of Wisconsin-Madison Emmons-Blaine Arlington freestall, milking parlor dairy facility. The RIC feeding gates recorded individual cow feed intake continuously. Cows were blocked by parity and randomly assigned to 1 of 4 treatment groups. Each of 32 feeding gates was randomly assigned to 1 treatment group, thus each cow had access to all 8 gates within the respective treatment group, and cow was the experimental unit.

Treatments were formulated to replace either corn silage (CS) or alfalfa silage (AS) with tall fescue hay (TF; Table 3-1). Bariane Tall Fescue (Barenbrug USA, Tangent, OR) was planted in separate field plots at a rate of 22.42 kg/ha at the University of Wisconsin-Arlington Agricultural Research Station (UWAARS; Arlington, WI) on September 15, 2011 with a no-till drill. For this feeding trial, the forage was cut on June 18, 2013 (first cut) and baled in large square bales on June 21, 2013. The bales were stored in an enclosed shed for approximately eight months prior to trial initiation.

Diets were formulated to contain similar CP and starch concentrations while dietary NDF and iNDF levels were allowed to change (Table 3-2). Treatments were as follows, with diet names abbreviated by the proportion that each forage contributes to the total forage component of each TMR: control, 60%TF and 40%AS (60TF40AS), 60%TF and 40%CS (60TF40CS), and 33%TF and 67%CS (33TF67CS). All cows received bovine somatotropin (Posilac, Elanco Animal Health, Greenfield, IN) every 14 d through the duration of the trial, initiated during the covariate period. The experiment was a continuous lactation trial, beginning with a 2 week covariate period prior to 7 weeks of assignment to treatment diets.

Cows were fed a TMR with feed delivered 3 times daily at 0300, 1200, and 1500, and were offered free access to water. The TMR was mixed once daily before the mid-day feeding and stored for delivery in the afternoon and the following morning. Cows were fed ad libitum for 10% refusals. Dry matter content of the forages was measured once weekly, and as fed ingredient proportions were modified accordingly to maintain near constant proportions of feeds on a DM basis.

Data Collection and Laboratory Analyses

Cows were milked twice daily and milk yield was recorded at each milking. Milk samples were collected from all cows weekly on the p.m. milking of one day and the subsequent a.m. milking of the following day throughout the duration of the trial. Samples were composited by cow by week and analyzed for fat, true protein, lactose, MUN concentrations, and SCC by infrared analysis (AgSource Milk Analysis Laboratory, Menomonie, WI) using a Foss FT6000 near infrared spectrophotometer (Foss Electric, Hillerod, Denmark). Average daily yields of fat and protein were calculated from these data for each week. Yields of FCM were calculated according to National Research Council (2001) equations.

Body weight (BW) was recorded on an individual basis weekly. Body condition score (BCS; 1 to 5 in 0.25-unit increments; Wildman et al., 1982) was recorded every other week. For both BW and BCS, within each cow, a linear regression using all data points was calculated to determine the change in BW and BCS over time.

Samples of TMRs, corn silage, alfalfa silage, tall fescue hay, high moisture corn, and concentrate mixes were obtained during the covariate period, wk 5, and wk 7 of the treatment period. Samples were frozen at -20ºC until analysis was conducted. All samples were dried at 60ºC for 48 h in a forced-air oven to determine DM content. Dried samples were ground to pass a 1-mm Wiley mill (Arthur H. Thomas, Philadelphia, PA) screen and sent to Rock River Laboratory, Inc. (Watertown, WI) for further analysis. Absolute DM was determined by oven-drying at 105ºC for 3 h (method 2.2.2.5; NFTA, 2001). All samples were analyzed for DM, CP (method 990.03; AOAC, 2006), ether extract (method 920.39; AOAC, 2006), and starch (Hall, 2009). Acid detergent lignin was determined via method 973.18 (AOAC, 2006), modified to use 1.0 grams per sample in each Ankom F57 bag (Ankom Technology, Macedon, NY). All Ankom bags were washed in acetone, dried in a fume hood, and further dried in a forced-air oven at

60°C for 1 h prior to weighing the sample. Neutral detergent fiber was determined using α-amylase and sodium sulfite (Van Soest et al., 1991). Indigestible NDF (iNDF) was determined by in situ rumen incubation for 288 h (NorFor, 2011). Total tract NDF digestibility was determined using the procedures described in Lopes et al. (2015a) modified to use the in situ 288 h iNDF estimates to determine the potentially digestible NDF fraction. In situ NDFD was determined via incubation of 0.5 grams of dried and ground TMR constituents from wk 5 and wk 7 for 18, 24, 30 and 48 h, then subsequently analyzed for NDF as indicated above. Particle size distribution of treatment diets were determined as described by Kononoff et al. (2003).

Apparent total-tract digestibilities of DM, OM, and NDF were determined using iNDF as an internal marker. Six fecal grab samples were collected from each cow at 8- to 12-h intervals covering every 4-h clock period over 2 consecutive days during wk 5 and 7 of the treatment period. Ort samples were collected daily during the fecal sampling period. Treatment TMR, fecal, and ort samples were composited by cow (fecal samples), week (TMR samples) or treatment within week (ort samples). The composited treatment TMR and ort samples were analyzed for DM, OM, NDF, starch, and iNDF as defined above. The composited fecal samples were analyzed for NDF and iNDF. In vivo total-tract nutrient digestibilities were calculated from iNDF and nutrient concentrations in the orts-adjusted diet and feces.

Statistical Analyses

Data were analyzed as a randomized, complete-block design with a covariate using the MIXED procedure of SAS (version 9.4, SAS Institue, Inc., Cary, NC). The model included parity, treatment, week, and the relevant interactions as fixed effects, and cow within treatment as a random effect. Week of treatment was included as a repeated measure using the compound symmetry structure to account for autocorrelated errors. Means were determined using the least

squares means statement, and treatment means were compared using the Bonferroni *t*-test option after a significant ($P < 0.05$) overall treatment *F*-test. Interaction effects were partitioned using the SLICE options of SAS (version 9.4, SAS Institute, Inc., Cary, NC).

Comparison of in vivo and in situ TTNDFD was conducted using the MIXED procedure of SAS (version 9.4, SAS Institute, Inc., Cary, NC). The model included method of TTNDFD determination (in vivo and in situ) as a fixed effect. Means were determined using the least squares statement, and treatment means were compared using the Bonferroni t-test option. Statistical significance and trends were considered at $P \leq 0.05$ and $P > 0.05$ to $P \leq 0.10$., respectively.

Results and Discussion

Nineteen cows were removed from the trial for health and behavior reasons. One cow could not be trained to the feeding gates. Two were removed for health reasons, one for severe mastitis and one for lameness. Sixteen cows were also removed because they habitually consumed feed from gates in which they were not assigned that totaled at least 5% of their feed intake. These nineteen cows were not used in the statistical analysis.

Forage and Diet Composition

Nutrient composition and in situ fiber digestibility of the forages used in the treatment diets are in Table 3-1. Nutrient composition was typical of good quality forages used in lactating rations in the upper Midwest of the United States. Tall fescue hay had the highest concentration of NDF, following by alfalfa silage, and corn silage. Indigestible NDF (iNDF, % of DM) was higher for alfalfa silage and tall fescue hay, compared to corn silage. The rate of digestion of the potentially digestible NDF fraction was greatest for alfalfa silage (5.1%/h), lowest for corn silage (2.1%/h), and intermediate for tall fescue hay (2.9%/h). As a result, the TTNDFD (% of total

NDF) was similar between alfalfa silage and tall fescue hay (42.8% and 42.4%, respectively) and lower for corn silage (36.2%). Tall fescue has previously been reported as having a greater NDF content and greater NDF digestibility when compared to corn and alfalfa silages (Cherney et al., 2004), but similar to other perennial grasses (Cherney et al., 1993) harvested at a similar maturity.

Dietary ingredients, nutrient composition, and particle sizing of the treatment and covariate diets are listed in Table 3-2. Treatment TMR's were formulated to replace corn silage (60TF40AS) or alfalfa silage (60TF40CS and 33TF67CS) with tall fescue hay while maintaining similar CP and starch levels based on initial forage test results taken prior to initiation of the trial. Neutral detergent fiber was higher for the diets incorporating tall fescue at a high rate (60TF40AS and 60TF40CS; averaged 30.2%) compared to the control diet (25.3%) and the diet with a moderate amount of tall fescue incorporation (33TF67CS; 26.7%). The proportion of TMR retained in the top pan of the Penn State Particle Separator increased with inclusion of tall fescue hay as expected due to the longer particles of the hay. The second screen had a higher proportion of TMR in the control compared to the tall fescue diets likely due to the higher silage inclusion rate, which had smaller particles than the hay.

DMI, Milk Yield, and Milk Components

Treatment effects on lactation performance are in Table 3-3. Milk production did not differ among treatments and averaged 40.4 kg/d. Dry matter intake was greater ($P < 0.05$) for the control diet compared to the 60TF40CS and 33TF67CS diets, and tended ($P = 0.07$) to be greater than the 60TF40AS diets. The three diets incorporating tall fescue hay had similar DMI and averaged 22.7 kg/d. There was also a treatment x week interaction for DMI that is shown in Figure 3-1, where DMI was similar among all treatments for treatment weeks 1, 2, 3, and 6, but

was greater ($P < 0.05$) for the control treatment compared to 33TF67CS treatment in weeks 4, 5, and 7, greater for the control treatment compared to the 60TF40CS treatment in weeks 5 and 7, and greater for the control treatment compared to the 60TF40AS in week 7.

The present study differs from Verbeten (2012), which reported that DMI and milk production did not differ when meadow fescue silage, tall fescue silage, or wheat straw was added to a low (23.4%) NDF diet to supplement digestible NDF (meadow fescue diet, 26.0% NDF and tall fescue silage diet, 26.5% NDF). However the diets in the present study incorporating tall fescue grass are all higher in NDF than Verbeten (2012), suggesting that gut fill could potentially be limiting DMI (Allen, 1996) in the diets incorporating tall fescue. Similarly, two Finnish studies (Kuoppala et al. 2008; Kuoppala et al., 2010) fed a combination of timothy-meadow fescue silages to lactating cows and observed that the silages with higher NDF limited DMI as well, however these studies saw a corresponding decrease in milk production concomitant with the decreased DMI. Cherney et al. (2004) evaluated the effects of feeding alfalfa silage compared to orchardgrass or tall fescue silage on lactation performance in high producing cows, and found that the tall fescue diet, which was greatest in NDF among the diets, had significantly lower DMI compared to the lower NDF diets. Milk production followed a similar pattern and was decreased compared to the lower NDF diets. The week x treatment interaction for DMI in the present study was not observed to our knowledge in other studies feeding grasses, however many of these are Latin square design (Broderick et al., 2002; Fisher et al., 1993; Kuoppala et al., 2008; Kuoppala et al., 2010; Rinne et al., 2002) or other trial designs of shorter periods (Cherney et al., 2004; Hoffman et al., 1998), which do not allow for a pronounced interaction over time. Strahan et al. (1987; 4 or 8 weeks) and Verbeten (2012; 98 d) conducted trials of longer duration and did not report an interaction over time.

The production of 4.0% fat corrected milk (FCM; Table 3-3) did not differ among treatments, and averaged 40.1 kg/d. Feed efficiency, measured as 4% FCM/DMI (kg/kg) in the present study, did not differ among treatments and averaged 1.72. A tendency ($P = 0.09$) for a treatment x week interaction for feed efficiency was observed, likely due to the treatment x week interaction observed for DMI as previously discussed.

The effects of tall fescue hay incorporation on milk composition are also in Table 3-3. There was no effect of treatment on milk fat, protein, or lactose yield or composition. However there was a significant treatment x week interaction for both protein ($P < 0.01$) and lactose ($P < 0.01$) composition. The lack of an effect on milk fat was consistent with the companion study (Bender et al. 2015, unpublished data) that reported no difference in rumination between the treatments. Similarly, Cherney et al. (2004) reported no change in yield or composition of fat, protein, or lactose when tall fescue replaced alfalfa. However, Verbeten (2012) reported higher milk fat composition and yield when supplementing tall fescue and meadow fescue silage, but this was compared to a control diet with lower NDF levels than the control in the present study (23.4 vs. 25.3%, respectively). Rumination was not reported in any of these studies.

Milk SCC did not differ among treatments. To our knowledge, no other studies reported significant effects of tall fescue hay incorporation on milk SCC. Milk urea nitrogen was greater ($P = 0.03$) for the control treatment compared to the 60TF40AS, but similar among other treatments. However, this was likely due to higher level of crude protein creating an excess of RDP in the control diet (Broderick and Clayton, 1997; Huhtanen and Hristov, 2009) compared to the other treatments.

Change in body weight and body condition score did not differ among treatments, and averaged 0.60 kg/d and 0.01units/wk, respectively. Across all treatments, body weight for cows

averaged 648 ± 87 kg (primiparous 583 ± 65 kg; multiparous 688 ± 73 kg) at trial initiation and 690 ± 91 kg (primiparous 631 ± 67 kg; multiparous 727 ± 84 kg) at completion of the trial. Similarly, other studies feeding grass forages reported no difference in body weight change or body condition score change (Verbeten, 2012; Cherney et al., 2004).

Nutrient Digestibility

Milk production, intake, and digestibility data is presented in Table 3-4. There were no treatment x parity interactions for any of the milk production, intake, nutrient digestibility, or feed efficiency data. Milk production from weeks 5 and 7 were similar to the whole trial averages and did not differ among treatments. Dry matter intake during weeks 5 and 7 was greater ($P < 0.01$) in the control treatment compared to the 60TF40CS treatment and tended ($P = 0.08$) to be greater than the 33TF67CS treatment. Apparent total-tract dry matter digestibility tended ($P = 0.07$) to be greater for the 33TF67CS treatment compared to the 60TF40CS treatment, but was similar among all treatments and averaged 66.2%. As a result, the control treatment had greater ($P < 0.01$) total-tract dry matter digested compared to the 60TF40CS treatment.

Organic matter intake followed a similar pattern; control had a greater ($P < 0.01$) intake than 60TF40CS, and 60TF40AS tended ($P = 0.10$) to have a greater intake compared to 60TF40CS. Apparent total-tract organic matter digestibility was greater for 33TF67CS compared to 60TF40AS ($P = 0.02$) and 60TF40CS ($P = 0.02$) treatments. As a result, the control treatment had greater ($P < 0.01$) and 33TF67CS tended ($P = 0.09$) to have greater total-tract organic matter digested compared to the 60TF40CS treatment.

Intake of NDF was greater ($P < 0.05$) for 60TF40AS compared to all other treatments, likely due to the elevated NDF level in the diet and high DMI compared to the other treatments

(Tables 3-2 and 3-4). As a percentage of bodyweight, NDF intake tended to be greater for 60TF40AS than both control ($P = 0.07$) and 33TF67CS ($P = 0.08$). Apparent total-tract NDF digestibility was lower for the Control treatment compared to the 40TF60AS ($P = 0.01$) and 33TF67CS ($P < 0.01$) treatments. As a result, the Control treatment had less NDF digested compared to 60TF40AS ($P < 0.01$) and 33TF67CS ($P = 0.02$). The 60TF40AS treatment tended ($P = 0.05$) to increase the amount of NDF digested compared to the 60TF40CS treatment.

Indigestible NDF intake was significantly ($P < 0.05$) greater for the Control and 60TF40AS treatments compared to the 60TF40CS and 33TF67CS treatments, likely due to the elevated iNDF concentration of the alfalfa silage combined with the higher intake from the Control and 60TF40As treatments. Indigestible NDF intake as a percentage of bodyweight was significantly ($P < 0.05$) lower for 33TF67CS compared to all other treatments, and the 60TF40CS treatment was significantly lower than both the Control ($P < 0.01$) and 60TF40AS ($P < 0.01$) treatments.

As DMI decreased between treatments, dry matter and organic matter digested decreased. However, the quantity of NDF digested increased despite the decline in DMI between treatments. This is because a greater proportion of digested organic matter is coming from NDF when tall fescue hay is incorporated into the diets. Additionally, as DMI decreased between treatments, NDF intake both in kg/d and as a percentage of bodyweight increased relative to the control, while iNDF intake both in kg/d and as a percentage of bodyweight decreased for 60TF40CS and 33TF67CS, and only slightly increased for 60TF40AS relative to the control (Table 3-4). This can likely be attributed to the fact that although alfalfa silage has the highest digestion rate of pdNDF, tall fescue hay still has a higher digestion rate of pdNDF compared to

corn silage, while having a much higher level of NDF relative to either alfalfa or corn silage (Table 1).

Grant (2015) suggests that grasses have less fragile NDF compared to legumes, and therefore grasses tend to increase rumen gut fill and result in slower passage from the rumen. In addition, Grant (2015) observed that feeding a high forage diet with either conventional corn silage or BMR corn silage increases both rumination and time spent eating. In contrast, Kammes and Allen (2012) suggest that when feeding TMR diets composed of orchardgrass compared to alfalfa, rumination time was similar and time spent eating tended ($P = 0.10$) to be greater for the alfalfa diet. Similar to the latter study, the present study and companion study (Bender et al., 2015, unpublished data) report that despite diets ranging in amount of NDF (ranging from 25.3% to 30.5% NDF) and forage source (alfalfa silage, corn silage, tall fescue grass hay), no effect ($P > 0.05$) on rumination (averaged 481.0 minutes among all treatments) or eating time (averaged 222.2 minutes among all treatments) was observed. In addition, no treatment effect ($P > 0.05$) on rumination time per unit of NDF (averaged 75.4 minutes/kg NDF intake among all treatments) or DM (averaged 21.5 minutes/kg DMI among all treatments) was observed (Bender et al., 2015, unpublished data), suggesting that highly digestible grasses could provide an alternate source of NDF with no effect on rumen fill and rumination.

Milk production per DMI (kg/kg) and milk production per organic matter intake (kg/kg) were similar across all treatments and averaged 1.72 and 1.88 kg/kg, respectively. Milk production per digested NDF (kg/kg) was significantly greater ($P = 0.02$) for the Control treatment compared to the 60TF40AS treatment and tended ($P = 0.05$) to be greater than the 33TF67CS treatment.

Cherney et al. (1993) reported that dry matter digestibility of cool season grasses declined as maturity increased, however that was in an in vitro setting. Rinne et al. (2002), Kuoppala et al. (2008), and Kuoppala et al. (2010) evaluated apparent nutrient digestibilities by dairy cows of DM, OM, and NDF and agreed, reporting that as maturity of grass silage increased, digestibility of the respective nutrients decreased. Cherney et al. (2004) compared cool season grasses as replacement for alfalfa in lactating cows. Although in vivo NDF digestiblities were not evaluated, in vitro results indicated that incorporating tall fescue into the TMR improved NDF digestibility by nearly 20% compared to the alfalfa-based TMR (76.3 vs. 58.1, $P < 0.05$). Collectively, these results, along with similar results by Verbeten (2012) and the present study indicate that feeding a highly digestible source of NDF from a cool season immature grass could be a viable replacement for corn silage and alfalfa silage in a high producing dairy cow ration.

In Situ Versus In Vivo NDF Digestibility

In situ total tract NDF digestibility is also reported in Table 3-4. Treatment had no effect of in situ TTNDFD and averaged 42.6% of NDF. A comparison of in situ versus in vivo TTNDFD is reported in Table 3-5. Across all treatments, method of TTNDFD determination was similar and averaged 42.5%.

The higher standard error of the mean associated with the in situ TTNDFD values is likely due to the low number of replicates compared to the in vivo data. The in vivo data used each cow as an experimental unit, and thus each treatment had 16 cows initially assigned to determine in vivo TTNDFD in two different weeks of the trial (5 and 7). Since the in situ data was calculated using forage samples from these same two weeks and not individual cow data, the power is reduced. Thus, the comparison between in situ and in vivo data was conducted using

one value from each of two weeks for each diet for both in vivo and in situ data in order to conduct a fair analysis.

Lopes et al. (2015b) validated an in vitro model to predict in vivo TTNDFD. The present study measured in situ TTNDFD, but applying these data to the same equation from Lopes et al. (2015b) to predict in vivo TTNDFD from in vitro TTNDFD, our results follow a very similar pattern as the relationship among the 21 diets from Lopes et al. (2015b).

Conclusions

Replacing corn silage and alfalfa silage with tall fescue hay decreased DMI but maintained milk production and milk components compared to the control treatment in high producing dairy cows, with no effect on rumination and eating time. Apparent total-tract DM digestibility did not differ from the control diet, but apparent TTNDFD was greater in treatments incorporating tall fescue hay. Thus, despite the depression in feed intake, the higher NDF digestibility in diets incorporating tall fescue hay resulted in similar milk production. In addition, in situ TTNDFD's were similar to in vivo TTNDFD data. The present study concludes that inclusion of tall fescue grass hay increased the TTNDFD of the diet and has the potential to replace corn silage and alfalfa silage and maintain milk production in lactating dairy cow rations.

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Table 3-1. Nutrient composition of forages

	Alfalfa silage	Corn silage	Tall fescue hay
DM, % as fed	34.4 ± 2.6	37.3 ± 6.2	83.5 ± 0.6
OM, % of DM	89.6 ± 1.6	96.4 ± 0.2	89.3 ± 1.6
CP, % of DM	21.9 ± 1.3	7.7 ± 1.6	14.3 ± 0.8
Starch, % of DM	0.4 ± 0.1	35.6 ± 3.9	1.6 ± 0.2
Lignin, % of DM	9.4 ± 0.5	3.6 ± 0.3	8.1 ± 1.0
Ether extract, % of DM	3.2 ± 0.7	3.8 ± 0.5	3.0 ± 0.5
NDF, % of DM	44.9 ± 2.5	38.0 ± 1.5	64.4 ± 1.7
iNDF,[1] % of DM	18.9 ± 2.0	9.2 ± 0.7	16.9 ± 1.4
iNDF,[1] % of NDF	41.2 ± 1.2	25.8 ± 1.2	26.6 ± 0.3
pdNDF kd,[2] %/h	5.1 ± 0.2	2.1 ± 0.1	2.9 ± 0.3
TTNDFD,[3] % of total NDF	42.8 ± 1.4	36.2 ± 0.3	42.4 ± 2.2

[1]iNDF = indigestible NDF determined by in situ incubation for 288 h.
[2]pdNDF kd = potentially digestible NDF fraction digestion rate calculated from TTNDFD model.
[3]TTNDFD = predicted total-tract NDF digestibility using in situ TTNDFD model.

Table 3-2. Dietary ingredients and TMR nutrient composition (% of DM unless otherwise noted)

	Covariate	Control	60TF40AS	60TF40CS	33TF67CS
Ingredient					
Alfalfa silage	11.0	18.3	18.4	----	----
Corn silage	36.7	36.6	----	20.0	33.1
Tall fescue hay	9.2	----	27.7	30.0	16.6
H.M.C.	22.1	23.8	38.7	30.0	26.7
Soybean meal (48% CP)	14.7	14.7	9.8	14.0	16.94
Energy Booster 100[1]	2.02	2.01	2.03	2.21	2.03
Calcium carbonate	1.28	1.28	1.29	1.40	1.66
Sodium bicarbonate	0.73	0.73	0.81	0.88	0.74
Potassium carbonate	0.37	0.37	----	----	0.55
Monocalcium phosphate	0.37	0.37	0.07	0.08	0.37
Trace mineral salt[2]	0.37	0.37	0.41	0.44	0.41
Magnesium-potassium-sulfate[3]	0.73	0.73	0.18	0.20	0.18
Magnesium Oxide	----	0.33	0.18	0.20	0.28
Vitamin E	0.02	0.02	0.02	0.02	0.04
UW ADE[4]	0.37	0.37	0.37	0.40	0.37
Smartamine M[5]	0.05	0.05	0.06	0.06	0.04
Nutrient					
DM, % of as fed	47.5	43.2	52.7	53.2	52.7
OM	92.7	90.2	89.2	89.0	89.8
CP	18.0	17.9	17.5	17.1	17.3
Starch	31.3	31.2	29.3	30.1	32.4
NDF	27.8	25.3	29.8	30.5	26.7
iNDF	7.3	7.0	8.0	6.9	6.0
Ether extract	5.1	6.3	5.5	5.6	5.9
Penn State Separator Sieves,[6] % of as fed retained					
19 mm	6.9 ± 0.4	7.9 ± 0.2	9.9 ± 3.0	12.2 ± 4.5	9.3 ± 3.1
8 mm	42.5 ± 1.1	45.5 ± 1.4	30.7 ± 2.5	30.0 ± 1.5	24.6 ± 0.3
1.18 mm	37.9 ± 0.8	36.3 ± 0.6	42.4 ± 1.0	41.0 ± 1.3	46.9 ± 1.6
Bottom pan	12.7 ± 0.8	10.3 ± 0.7	16.9 ± 0.5	16.9 ± 1.8	19.2 ± 1.7

[1]Minimum 98% total fatty acids (MSC Company, Dundee, IL).
[2]Contained 88% NaCl; 0.002% Co; 0.2% Cu; 0.012% I; 0.18% Fe; 0.8% Mn; 0.006% Se; 1.4% Zn.
[3]Dynamate (11% Mg, 18% K, 22% S; The Mosaic Co., Plymouth, MN).
[4]Vitamin A 3,300,000 IU/kg; Vitamin D 1,100,000 IU/kg; Vitamin E 11,000 IU/kg.
[5]Smartamine M, 70% Methionine (Adisseo, SAS, France).
[6]Particle size was measured using the Penn State Particle Size Separator as described by Kononoff et al. (2003).

Table 3-3. Effects of tall fescue (TF) hay incorporation on lactation performance

	Treatment[1]					*P* – value[2]		
	Control	60TF40AS	60TF40CS	33TF67CS	SEM	Trt	Week	Trt x Week
Milk, kg/d	42.3	39.2	39.1	41.0	1.2	0.19	0.03	0.83
4.0% FCM[3], kg/d	41.2	39.8	38.2	41.0	1.1	0.15	0.01	0.49
DMI, kg/d	24.5[a]	23.2[ab]	22.1[b]	22.7[b]	0.38	<0.01	<0.01	<0.01
4%FCM/DMI, kg/kg	1.69	1.71	1.71	1.78	0.04	0.37	<0.01	0.09
Fat, %	3.88	3.94	3.83	4.09	0.13	0.33	0.35	0.11
Protein, %	3.18	3.08	3.14	3.19	0.05	0.26	<0.01	<0.01
Lactose, %	4.84	4.90	4.92	4.91	0.02	0.18	0.04	<0.01
SCC	67.8	50.5	90.0	84.0	25.7	0.57	0.67	0.63
MUN, mg/dL	14.0[a]	12.7[b]	13.4[ab]	13.6[ab]	0.4	0.03	<0.01	<0.01
BW Change, kg/day	0.66	0.60	0.55	0.57	0.09	0.86		
BCS Change, units/week	0.02	0.01	0.00	0.01	0.01	0.65		

[a-b]Means within a row with different superscripts differ ($P < 0.05$).

[1]Treatments: TMR with the forage portion composed (DM basis) of 33% alfalfa silage (AS) and 67% corn silage (CS; Control), 60% TF and 40% AS (60TF40AS), 60% TF and 40% CS (60TF40CS), and 33% TF and 67% CS (33TF67CS).

[2]Trt = treatment; Trt x Week = Treatment by week interaction.

[3]4.0% FCM yield = (0.4 x kg of milk) + (15.0 x kg of fat).

Table 3-4. Effects of tall fescue hay incorporation on milk production, intake and apparent total-tract digestibility of DM, OM, and NDF during weeks 5 and 7

	Treatment[1]					
	Control	60TF40AS	60TF40CS	33TF67CS	SEM	*P*-value
Milk, kg/d	42.2	40.3	39.0	39.6	1.43	0.39
Dry Matter						
Intake, kg/d	24.9^{a}	23.5^{ab}	21.4^{b}	22.6^{ab}	0.68	<0.01
Total tract digestibility, %	66.3	65.3	65.0	68.0	0.87	0.04
Total tract digested, kg	16.5^{a}	15.3^{ab}	13.9^{b}	15.3^{ab}	0.48	<0.01
Organic Matter						
Intake, kg/d	22.8^{a}	21.5^{ab}	19.4^{b}	20.7^{ab}	0.62	<0.01
Total tract digestibility, %	68.7^{ab}	67.4^{b}	67.4^{b}	70.6^{a}	0.81	<0.01
Total tract digested, kg	15.7^{a}	14.4^{ab}	13.1^{b}	14.6^{ab}	0.44	<0.01
NDF						
Intake, kg/d	6.25^{b}	7.11^{a}	6.36^{b}	6.33^{b}	0.19	<0.01
Intake, % of bodyweight	0.96	1.05	1.00	0.97	0.03	0.04
Total tract digestibility, %	37.8^{b}	44.4^{a}	42.4^{ab}	45.3^{a}	1.50	<0.01
Total tract digested, kg	2.35^{b}	3.17^{a}	2.74^{ab}	2.86^{a}	0.12	<0.01
iNDF						
Intake, kg/d	1.75^{a}	1.89^{a}	1.48^{b}	1.35^{b}	0.05	<0.01
Intake, % of bodyweight	0.27^{a}	0.28^{a}	0.23^{b}	0.20^{c}	0.01	<0.01
Milk/DMI, kg/kg	1.64	1.71	1.84	1.67	0.09	0.43
Milk/OMI, kg/kg	1.80	1.87	2.03	1.82	0.10	0.36
Milk/digested NDF, kg/kg	17.7^{a}	13.1^{b}	15.5^{ab}	13.7^{ab}	1.11	0.01
In situ TTNDFD[2], %	41.6	44.0	42.5	42.4	1.51	0.53

$^{a-c}$Means within a row with different superscripts differ ($P < 0.05$).

[1]Treatments: TMR with the forage portion composed (DM basis) of 33% alfalfa silage (AS) and 67% corn silage (CS; Control), 60% TF and 40% AS (60TF40AS), 60% TF and 40% CS (60TF40CS), and 33% TF and 67% CS (33TF67CS).

[2]TTNDFD = predicted total-tract NDF digestibility using in situ TTNDFD model.

Table 3-5. Comparison of in situ to in vivo TTNDFD[1]

	Method			
	In Vivo	In Situ[2]	SEM	*P*-value
TTNDFD[1], %	42.3	42.6	1.2	0.86

[1]Total-tract NDF digestibility.

[2]Predicted total-tract NDF digestibility using in situ TTNDFD model.

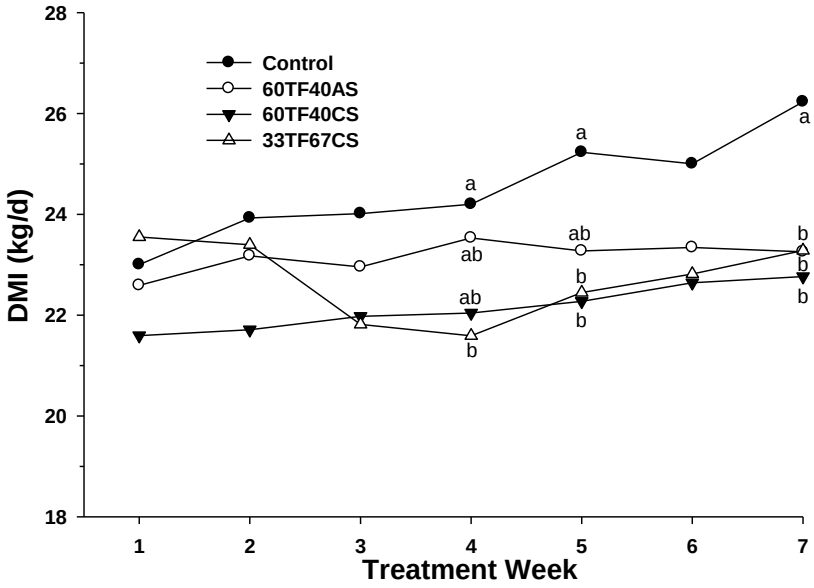

Figure 3-1. Least squared means of DMI by week for cows consuming TMR with the forage portion composed (DM basis) of 33% alfalfa silage and 67% corn silage (Control), 60% tall fescue hay and 40% alfalfa silage (60TF40AS), 60% tall fescue hay and 40% corn silage (60TF40CS), and 33% tall fescue hay and 67% corn silage (33TF67CS). Within week, means with different superscripts differ ($P < 0.05$).

Chapter 4. Characterizing Variability in Data Streams From Two Commercial Dairy Farms.

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Abstract

The objective of the study was to characterize variability in both individual animal and pen data on two commercial dairy farms with different management techniques. An additional objective was to explore statistical process control (SPC) tools as a method to determine out-of-control data points. Case study #1 was a commercial robotic dairy farm in Melrose, WI. The Lely T4C© herd management system was used to collected data including milk production, rumination, and body weight for a year. Data from approximately 500 cows in seven pens (1 pen per robot, approximately 70 cows per pen) were utilized. In case study #1, milk production averaged 37.4 kg, with a standard deviation of 1.0 kg among days within a pen, 14.4 kg among individual cows within a pen, and 21.2 kg among days within individual cows. Body weight averaged 689.2 kg, with a standard deviation of 4.5 kg among days within a pen, 75.6 kg among individual cows within a pen, and 34.6 kg among days within individual cows. Rumination (min per day) averaged 461.1 min, with a standard deviation of 6.1 min among days within a pen, 128.0 min among individual cows within a pen, and 43.6 min among days within individual cows. Case study #2 was a 1,400 cow dairy in Eden, WI, which was equipped with milk meters and rumination/activity collars to record individual cow milk production, rumination, physical activity, and pen-based feed intake. Data were collected over a three month period. On this dairy, milk production averaged 45.1 kg, with a standard deviation of 1.3 kg among days within a pen, 11.5 kg among individual cows within a pen, and 20.1 kg among days within individual cows. Rumination (min per day) averaged 441.6 min, with a standard deviation of 14.0 among days within a pen and 120.7 min among individual cows within a pen. Physical activity (measured in arbitrary units) averaged 489.2, with a standard deviation of 17.1 among days within a pen, 103.4

among individual cows within a pen. Feed intake (DMI kg/cow/d) averaged 26.6 kg, with a standard deviation of 1.8 kg among days within pens. Shewhart and exponentially weighted moving average control charts were constructed via univariate SPC techniques and determined the presence of out-of-control data points. Understanding the magnitude of the variability among these parameters could be a powerful tool to monitor pen level changes over a short period of time and implement immediate managerial modifications.

Keywords: dairy cow, statistical process control, milk production, rumination, physical activity

Introduction

Early signaling of an unexpected deviation in process performance is advantageous in order to quickly return the process to normal performance and prevent severe economic losses (De Vries, 2001). The collective set of problem-solving tools to achieve stability in a production process by eliminating or reducing the amount of variability is termed statistical process control (SPC; De Vries and Reneau, 2010). Graphical representations of these data are commonly used as visual aids in SPC analyses. Thus control charts are considered the primary tool in SPC techniques (Mertens et al., 2011). Although used in industrial engineering and other industries for nearly a century (Chandra, 2001), application of SPC to the agriculture production systems occurred much more recently (De Vries and Reneau, 2010; Mertens et al., 2011).

A recent review (Mertens et al., 2011) of published papers utilizing SPC techniques in production agriculture noted that about half of the 42 reviewed papers were published within the decade prior to the publication of the review, indicating the increasing interest in

application of SPC techniques to agricultural production systems. However, while many of the studies were applied to dairy cattle, the predominant focus was to apply SPC techniques to deviations in performance of individual dairy cows in milk quality (Greenstam, 2005; Cavero et al., 2007; Lukas et al., 2009; Miekley et al., 2012), reproduction (De Vries and Conlin, 2003a, b; De Vries and Conlin, 2005; Cornou et al, 2014), production parameters (De Vries et al., 1997b; Lukas et al., 2009; Huybrechts et al., 2014; Lukas et al., 2015), or other cow-specific variables (Reneau et al., 2007; Pastell and Madsen, 2008; Wallace, 2009; Miekley et al., 2012). Few studies have investigated deviations in performance of groups of dairy cows (De Vries et al., 1997a; Niza-Ribeiro et al., 2004; Lukas et al., 2005; Lukas et al., 2008a), and to our knowledge no published studies have investigated deviations in pen-level nutritional variables in dairy cattle using SPC.

The objectives of the present research were to characterize variability in pen-based data in commercial dairies differing in management strategies and explore SPC tools as a method of determining out-of-control data points.

Materials and Methods

Data was collected at two different commercial dairy farms and presented as separate case studies in data variability.

Case Study 1: Robotic Commercial Robotic Dairy

A 500-cow, robotic commercial milking herd in Melrose, WI was selected for case study #1. The dairy was equipped with seven robotic milking units (Lely Astronaut A4, Lely Group, The Netherlands), one each per pen of approximately 70 cows. Cows were assigned to a pen post-calving and remained there until dry-off, resulting in seven pens with a similar

DIM and parity profile. The robotic system was set-up as a "free flow" system; cows were milked on a voluntary basis and manually brought by the farm staff once per day to the robot to be milked when time since prior milking exceeded 6 – 8 h for early lactation cows, 24 h for late lactation cows, and 12 – 16 h for all other cows.

Values for milk production, body weight, and rumination activity were obtained on an individual cow basis from the herd management software (Lely T4C Herd Management System, Lely Group, The Netherlands) for a full year, from June 1st, 2013 to May 31st, 2014. Daily milk production was calculated as the sum of milk production from all individual milkings in a calendar day on an individual cow basis. Body weight for all cows was determined at every milking in the robotic milking unit, and averaged among all milkings in one day to create an average daily body weight per cow. Cows were equipped with rumination collars (Lely Qwes-HR; Lely Group, The Netherlands) to measure rumination activity in minutes of rumination per cow per day.

Case Study 2: Conventional Freestall Commercial Dairy

A 1,400 cow commercial dairy herd, housed in a conventional freestall design was used for case study #2. Data was collected from eight pens of approximately 180 cows each from Dec. 23rd, 2014 to March 31st, 2015. Cows were partitioned by parity (1 vs. >1) and pregnancy status (confirmed pregnant vs. non-pregnant) into pens. Milk production, rumination, and physical activity was obtained on an individual cow basis and averaged by pen. Cows were milked three times daily in a GEA milking parlor (GEA Group, Germany), with milk weight data collected via management software (Dairy Plan, GEA Group, Germany). Each cow was fitted with a neck collar that monitored physical activity and rumination (SCR HR-TAG; SCR Engineers Ltd., Netanya, Israel) continuously. Dry matter

intake was determined on a pen-basis and was recorded with feed management software (Feed Watch, Valley Agricultural Software, Tulare, CA). Refusals were weighed approximately 24 hours after delivery of feed, and subtracted from the amount of feed delivered to determine DMI for the pen, and tracked with the feed software program.

Statistical Analysis

Mean and standard deviations of the data in two individual case studies was determined via Microsoft Excel 2010 (Microsoft Corporation, Redmond, Washington). Data were analyzed in two individual case studies by the SHEWHART procedure of SAS (version 9.4, SAS Institue, Inc., Cary, NC), and analyzed by day (Figures 1 – 3) or by pen (Figure 4). Shewhart charts were constructed using upper and lower control limits that deviate 3 times the standard deviation of the mean, calculated over the entire 365 day period (Figure 1 and Figure 4), or calculated using the exponentially weighted moving average (EWMA; Figure 2 and Figure 3).

Results and Discussion

The mean and standard deviation of milk production, rumination, physical activity, DMI, and bodyweight from the two case studies is presented in Table 4-1. Only two data streams were measured in both case studies: milk production and rumination. Although mean milk production was greater in case study 2 than case study 1, the standard deviation of milk production among day within pen and among day within cow were very close between case studies. The mean rumination time was also comparable between case studies. Dry matter intake data was available only at the pen level for case study 2, thus, standard deviations among and within cow are not available. All data streams (except for milk

production for both case studies) had the greatest standard deviation occur among cows within pen, following by among days within cow. Variation among days within pen was the lowest level of variation for each data stream. Due to different management techniques, statistics comparing case studies were not valid.

Figure 4-1 Panel A displays a Shewhart chart constructed from case study #1. The mean and control limits were calculated by taking the average and 3 times the standard deviation over the full year of data, respectively. Bodyweight and rumination time are also presented in Figure 4-1, Panels B and C, respectively, and are similarly calculated. Control limits can be calculated based on several different criteria. Although the present study sought to use the most traditional methodology (three times the standard deviation), other methodologies are available, as detailed by Reneau and Lukas (2006). Shewhart charts are particularly useful when the process is likely out of control and experiencing large shifts from the mean that can be attributed to specific causes (Montgomery, 2009). Other control charts are more sensitive to detect small shifts from the mean (Montgomery, 2009).

Figure 4-2 Panel A presents an example of a control chart for milk production from case study #1 with much greater sensitivity to small shifts in the mean, termed an exponentially weighted moving average (EWMA; Montgomery, 2009). Unlike the Shewhart chart from Figure 4-1, the EWMA has a memory, resulting in recent values contributing to the weighted present plotted point. Thus, smaller shifts in the mean performance are more readily detected compared to the Shewhart chart (Montgomery, 2009), and therefore more indicative of a real change from the mean. Figure 4-2 Panel B shows the residual analysis of the data from Figure 4-2 Panel A, and control limits are straight. Thus, out-of-control data points can be more readily distinguished compared to Panel A. Although fewer data points

are outside the control limits in Figure 4-2 versus Figure 4-1, Figure 4-2 is a weighted averaged with more of a focus on current data, so it is likely more applicable to a process such as a dairy farm that constantly changes management strategies.

Similarly, Figure 4-3 represents the EWMA control chart from case study #2 for milk production. There are no data points that cross the upper or lower control limits, indicating that this process is operating in control.

Figure 4-4 presents an example of using SPC control charts to monitor variables at the pen level. Rumination time is displayed for pens 102 (Panel A) and 105 (Panel B). Although the pens occur on the same farm, in the same barn, deviant pen behavior has occurred. An out-of-control data point around day 50 occurs in Panel B, compared to in control data around the same time period in Panel A. The scope of the present study did not allow for links between the out-of-control data to specific events on farm.

Control charts constructed via SPC techniques could provide a valuable method to monitor variability on commercial dairy farms. Although the present study displayed control charts monitoring univariate data, future research should focus on integration of pen-level data using multivariate control charts to increase sensitivity (Montgomery, 2009; Miekley et al., 2013). In addition, cause and effect relationships should be evaluated on a commercial dairy farm to determine the responsiveness to a control chart to specific events.

Conclusions

Characterizing the variability in data streams such as milk production, rumination, physical activity, DMI, and bodyweight is important to understand the variance structure in pen-based data. Statistical process control charts such as the Shewhart or EWMA chart

could be a viable method to quantify and eliminate the variability in a process. Further research on integrating pen-based data streams with statistical process control is warranted.

Acknowledgements

The authors acknowledge USDA Hatch NEW-1044 Multi-State Research Formula fund #WIS01635 for the funding of this study. Many thanks go to Steve Abel and the staff at Abel Dairy (Eden, WI), as well as Pfaff Prairie Dairy (Melrose, WI) for participating in the research study.

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Table 4-1. Mean and standard deviation of milk production, rumination, physical activity, bodyweight, and DMI from two commercial dairies (Case Study 1 and 2).

		- - - - - - - - - - Standard Deviation - - - - - - - - - -		
	Mean	Day within Pen	Cow within Pen	Day within Cow
Case Study 1				
Milk (kg/d)	36.6	1.0	14.4	21.2
Bodyweight (kg)	695.2	4.5	75.6	34.6
Rumination (min/d)	461.1	6.1	128.0	43.6
Case Study 2				
Milk (kg/d)	45.1	1.3	11.5	20.1
Rumination (min/d)	441.6	14.0	120.7	75.6
Physical Activity (units/d)	489.2	17.1	103.4	85.2
DMI (kg/d)	26.6	1.8	-----	-----

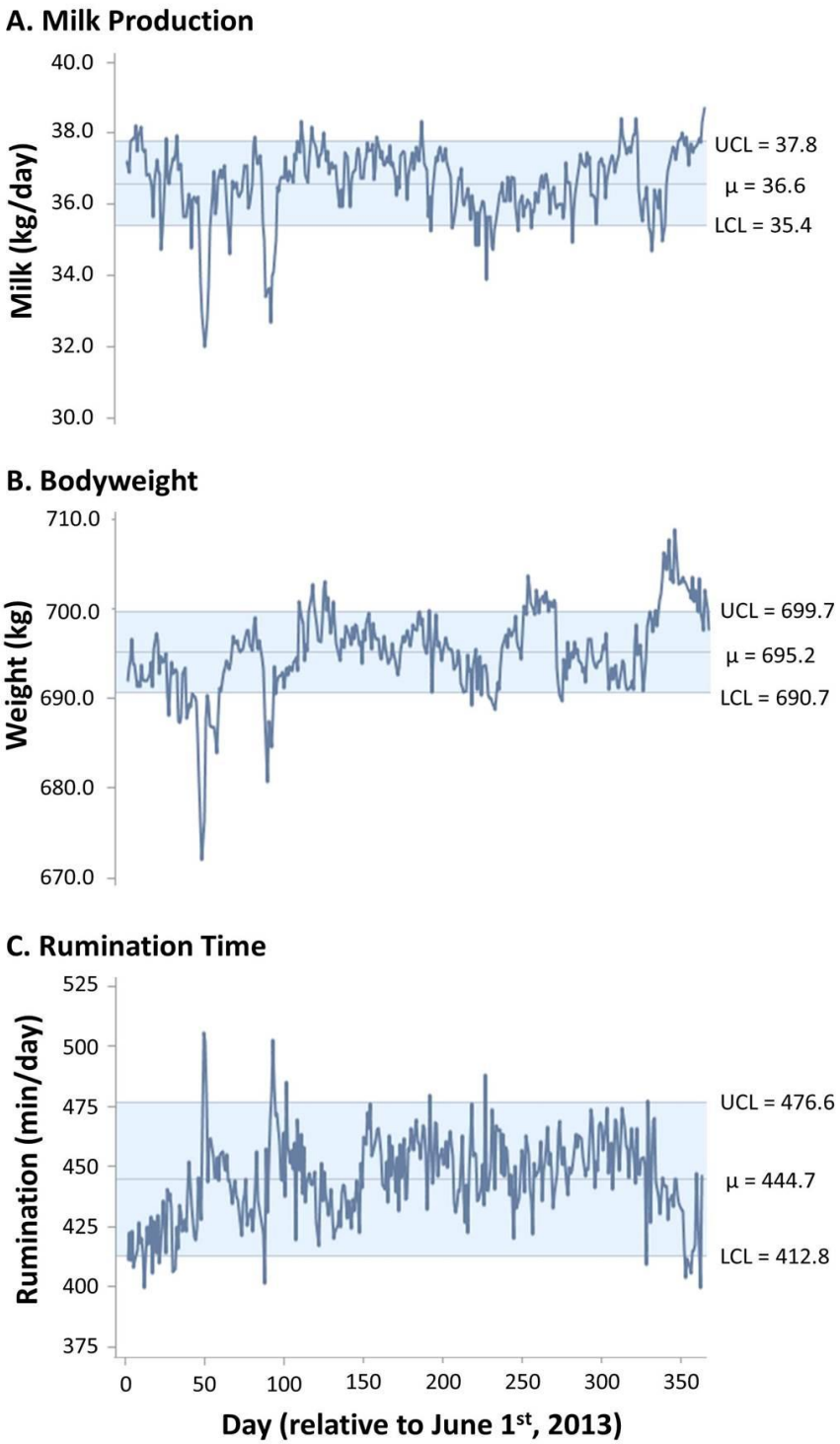

Figure 4-1. Statistical process control chart for milk production (Panel A), bodyweight (Panel B), and rumination (Panel C) from Case Study #1 using the mean and standard deviation over the full year to determine the upper (UCL) and lower (LCL) control limits.

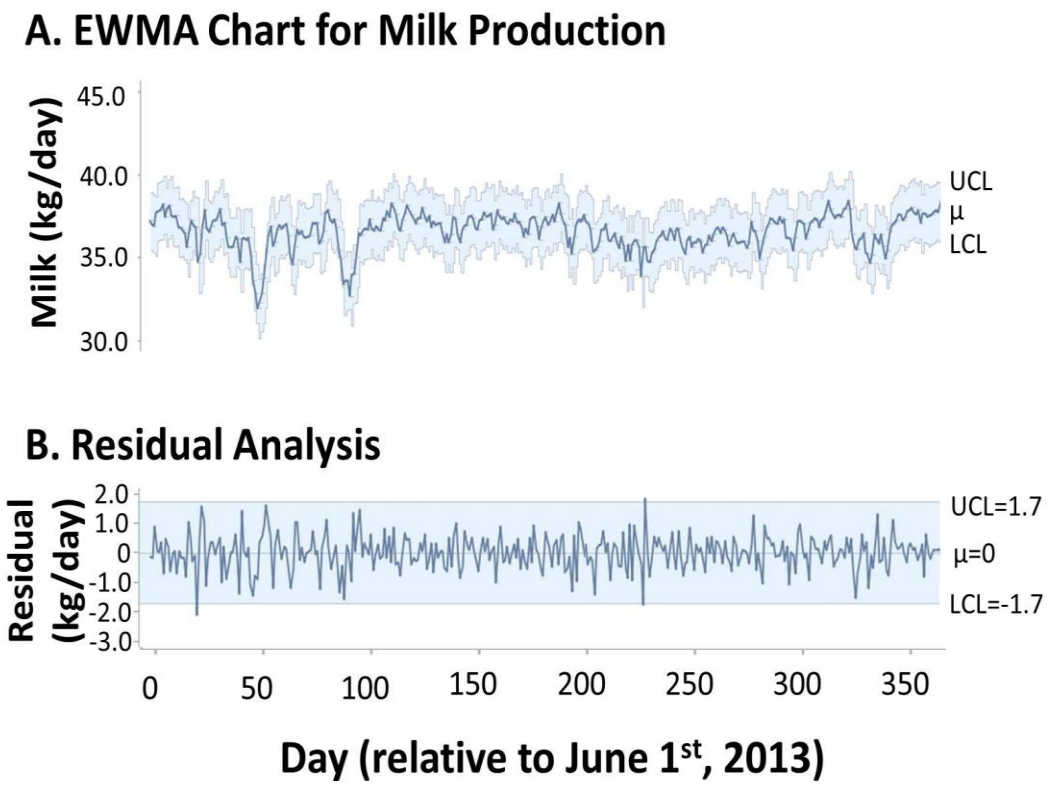

Figure 4-2. Statistical process control chart for milk production from Case Study #1 using a mean and standard deviation calculated via the estimated weighted moving average (EWMA; Panel A) to determine the mean and upper and lower control limits. The residual (actual - forecast), is shown in Panel B.

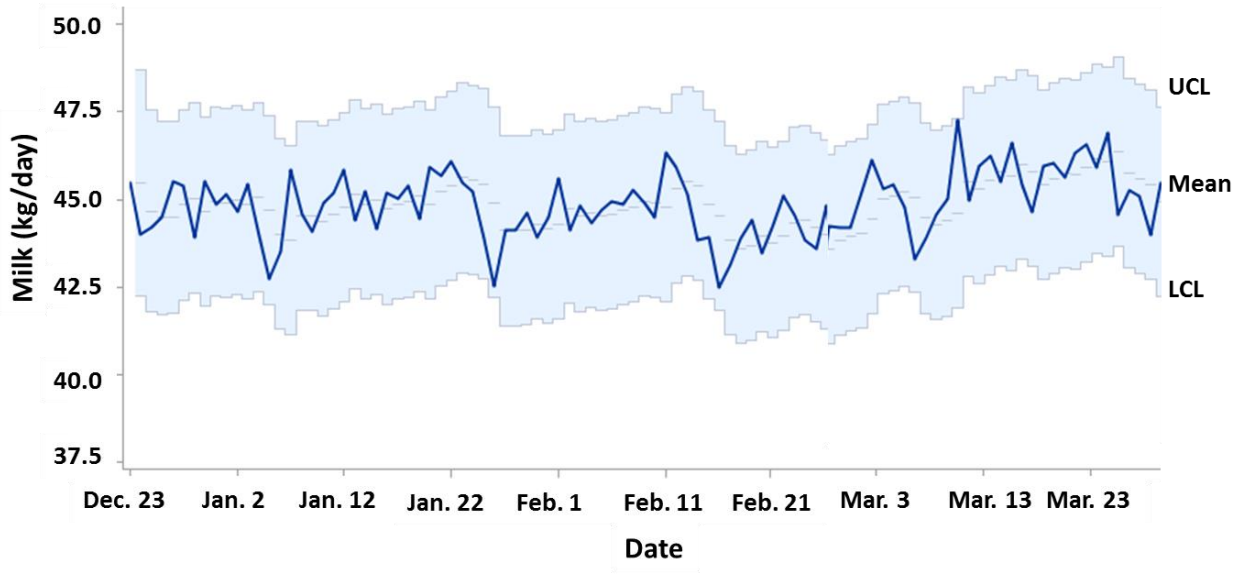

Figure 4-3. Statistical process control chart for milk production from Case Study #2 using a mean and standard deviation calculated via the estimated weighted moving average (EWMA; Panel A) to determine the mean and upper and lower control limits.

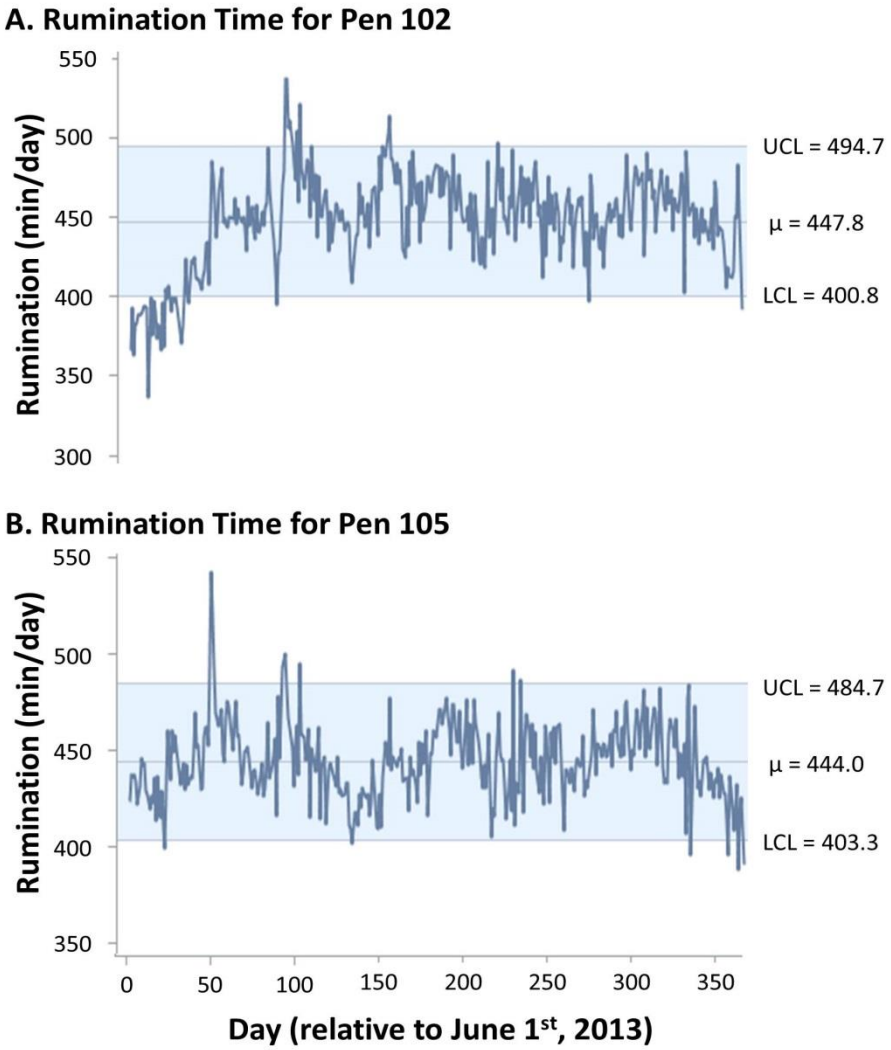

Figure 4-4. Example of statistical process control charts for rumination time for two pens (Panel A, Pen 102; Panel B, Pen 105) using the mean and standard deviation over the full year to determine the upper (UCL) and lower (LCL) control limits.

Chapter 5. Technical Note: Monitoring Variability on a Commercial Dairy Farm with Multivariate Statistical Process Control.

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Abstract

Statistical process control (SPC) has been used in other industries for decades as a way to identify and reduce variation in a process. The objective of the present study was to investigate the use of multivariate SPC techniques to detect variability at the pen-level on a commercial dairy farm. Eight pens of 180 cows housed in a freestall barn at a 1,400 cow commercial dairy farm were utilized. Data streams collected from the cows and incorporated into the multivariate models included rumination time, physical activity, and dry matter intake. The multivariate SPC procedures in SAS used in the present study include MVPMODEL to set up the principal components model for multivariate SPC, MVPMONITOR to create a Hotelling's T^2 and squared prediction error control charts, and MVPDIAGNOSE to diagnose and interpret the variation exceeding the control limits. Integrating variables on a commercial farm using multivariate SPC could provide a tool for determining variation exceeding control limits at the pen level, and preemptively correct extreme variability before affecting animal performance.

Keywords: dairy cow, multivariate, statistical process control, variability

Technical Note

New technologies have the potential to collect a large amount of physiological data on dairy cows that could improve management, milk production, and profitability on a commercial dairy farm. Herd managers extensively utilize herd management software to record data and create reports to make individual cow and herd-level decisions. However, analysis of the collected data often is presented in a historical context, with little effort to focus on real-time data streams or on the variability of the data stream.

Statistical process control (SPC) is a set of problem-solving tools used to identify causes of variability and improve process stability through reduction in variation (Montgomery, 2009). Statistical process control has been successfully used in other industries to improve performance since the 1920s (Chandra, 2001), and more recently has been applied to animal agriculture. Reviews by Mertens et al. (2011) and De Vries and Reneau (2010) have detailed the applications of SPC across production agriculture, including the poultry, beef, swine, and dairy industries. The studies cited in these reviews utilized a variety of univariate SPC techniques, focusing on variation in only one variable at a time. However, technologies on a commercial dairy farm collect many different data streams that are highly dependent on one another, suggesting that a multivariate analysis would be more powerful and appropriate to detect changes.

More recently, Miekley et al. (2013) utilized multivariate SPC techniques to detect mastitis and lameness by evaluating variables including DMI, feeding patterns, milk conductivity, milk yield, and physical activity. The authors found promising applications of multivariate SPC charts in lameness and mastitis detection, with possible further applications. However, Miekley et al. (2013) focused on a multivariate analysis on an individual animal, without analyzing data on a pen or herd level. Analyzing variability at the pen-level could have the potential to reduce causes of variability and prevent drops in milk production. The objective of the present study is to investigate the use of multivariate SPC techniques to detect extreme variability on the pen-level on commercial dairy farms.

A 1,400 cow commercial dairy herd, housed in a conventional freestall design was used for this study. Data were collected from eight pens of approximately 180 cows each from Dec. 23rd, 2014 to March 31st, 2015. Cows were partitioned by parity (1 vs. >1) and

pregnancy status (confirmed pregnant vs. non-pregnant) into pens. Milk production, rumination, and physical activity was obtained on an individual cow basis and averaged by pen. Cows were milked three times daily in a GEA milking parlor (GEA Group, Germany), with milk weight data collected via management software (Dairy Plan, GEA Group, Germany). Each cow was fitted with a neck collar that monitored physical activity and rumination (SCR HR-TAG; SCR Engineers Ltd., Netanya, Israel) continuously. Dry matter intake was determined on a pen-basis and was recorded with feed management software (Feed Watch, Valley Agricultural Software, Tulare, CA). Refusals were weighed approximately 24 hours after delivery of feed, and subtracted from the amount of feed delivered to determine DMI for the pen, and tracked with the feed software program.

Dry matter intake, rumination, and physical activity was analyzed via the multivariate statistical process control set of procedures (Christian and Ransell, 2012) in SAS (version 9.4, SAS Institue, Inc., Cary, NC) to create a Hotelling's T^2 multivariate control chart, including MVPMODEL (to set up the principal components model for multivariate SPC), MVPMONITOR (to create multivariate control charts of the T^2 and squared prediction error), and MVPDIAGNOSE (to diagnose and interpret the variation).

Statistical process control analyses are often constructed in two phases. In phase I, the process is not assumed to be stable, and the goal of a phase I analysis is to identify and remove extreme causes of variation. Thus, a control chart is created using a limited, initial set of data to determine the presence of out-of-control data. In phase II, new data are added to the control chart, using control limits established from the previously established model for stable variation from phase I.

MVPMODEL: Creating a Principal Components Model

A principal components model was created using daily pen averages for three data streams: DMI, rumination (min/cow/d), and physical activity (units/cow/d). The MVPMODEL procedure of SAS was used first to create the principal components model as the first step of multivariate SPC phase I analysis. Table 5-1 displays the correlation matrix among pen averages for the three variables. A positive correlation between rumination and physical activity (0.49) was observed, and a negative correlation between DMI and physical activity (-0.46) and DMI and rumination (-0.55). Eigenvalues of the correlation matrix are presented in Table 5-2, and display the amount of variation explained by the addition of each principal component. Figure 5-1 displays a scree plot of both the proportion and cumulative sum of variation explained by each principal component. Previous work has suggested that the location of the major bend in the proportion plot of the eigenvalues represents the adequate number of principal components to use in the model (Mardia et al., 1979). In this scenario, as presented in Figure 5-1 and Table 5-2, we used 2 principal components, explaining 85% of the variation.

MVPMONITOR: Creating Multivariate Control Charts

Figure 5-2 presents the phase 1 control chart created using the MVPMONITOR procedure of SAS, using the principal components model discussed above and the first 20 data points in the data set. Panel A displays the Hotelling's T^2 control chart (Hotelling, 1947), which is a multivariate SPC chart that displays the variation in the model plane defined by the number of principal components (in the present model, 2 principal components were used; Christian et al., 2012). Only 1 point exceeded the upper control limit, which is not too variable since the control limits were set to α=0.05. One additional

point was below the lower control limit, however this represents a point that is very close to origin of the model plane and thus is not considered too extreme of variation.

The squared prediction error control chart (SPE) is displayed in Figure 5-2, Panel B. This chart can also be used to detect shifts from the mean, but is based on the error between the raw variables and the principal component analysis-derived variables. Since no data points exceeded the upper control limit, the entirety of the 20 data points are considered in control, and the phase I analysis is complete. Since the variation in the initial time period is considered stable, the model used to construct these control charts will be used to continue analysis of new data (phase II).

Figure 5-3 displays the T^2 (Panel A) and SPE (Panel B) control charts for the remainder of the data (remaining 80 data points), using the same model as above. Several points in both control charts display data that were considered statistically out-of-control. In particular, beginning around the March 13th time period, the T^2 and SPE charts indicate that variation exceeds the control limits frequently, suggesting that an event on farm could possibly be traced as a cause. However, the present study did not investigate casual relationships of events on farm causing extreme variation.

MVPDIAGNOSE: Diagnosing the Variation

One advantage of using a multivariate SPC analysis is the ability to diagnose the cause of variation. Figure 5-4 presents a contribution plot developed from the MVPDIAGNOSE procedure of SAS for the out-of-control data on Jan. 24th. This plot indicates that physical activity was the most deviant variable from the predicted level of variation. Although the present study did not address causality of out-of-control data points,

the contribution plot serves as a way to further investigate specific events that cause the extreme variation.

Conclusions

Multivariate SPC charts provide a tool for determining extreme variability at the pen level. Incorporating this analysis into a real-time decision-making management plan could provide an early indication of deviant pen performance and preemptively correct extreme variability before affecting animal performance. The present study offered two control charts as one multivariate SPC methodology (Hotelling's T^2 and SPE control chart). Further research should focus on utilizing more sensitive multivariate methods and causal relationships between events on farm and variability of data.

Acknowledgements

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Miekley, B., E. Stamer, I. Traulsen, and J. Krieter. 2013. Implementation of multivariate cumulative sum control charts in mastitis and lameness monitoring. *J. Dairy Sci.* 96:5723-5733.

Montgomery, D. C. 2009. Introduction to Statistical Quality Control. 6th ed. John Wiley & Sons. Inc. Hoboken, NJ.

Table 5-1. Correlation matrix[1] displaying correlations among pen-averages for physical activity, rumination, and DMI at a commercial dairy farm.

	Physical Activity	Rumination	DMI
Physical Activity	1.00	0.49	-0.46
Rumination	0.49	1.00	-0.55
DMI	-0.46	-0.55	1.00

[1]Correlation matrix was generated from the MVPMODEL PROCEDURE of SAS (version 9.4, SAS Institue, Inc., Cary, NC).

Table 5-2. Eigenvalues of the correlation matrix[1] presented in Table 1. Eigenvalues represent the amount of variation explained by each principal component.

Principal Component	Eigenvalue	Difference[2]	Proportion[3]	Cumulative[4]
1	2.00	1.45	0.67	0.67
2	0.55	0.11	0.18	0.85
3	0.44	----	0.15	1.00

[1]Eigenvalues and variance information was generated from the MVPMODEL PROCEDURE of SAS (version 9.4, SAS Institue, Inc., Cary, NC).

[2]Difference between the eigenvalue for the present principal component and the next principal component.

[3]Proportion of the total variance explained by each principal component.

[4]Cumulative sum of the proportion of the variance explained by the present principal component and those before it.

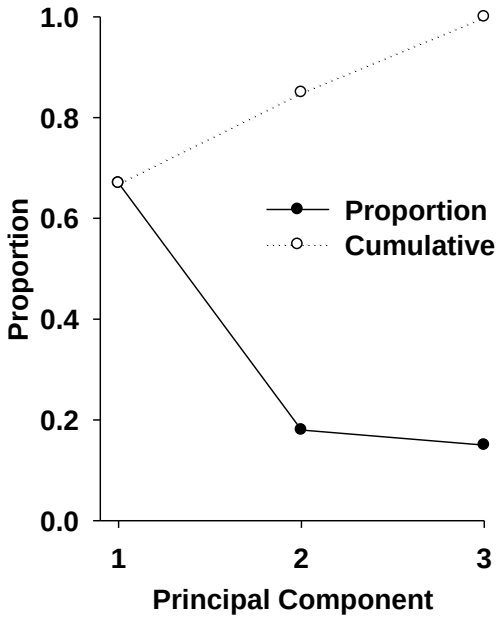

Figure 5-1. Principal component analysis using rumination, physical activity, and DMI. The Y-axis represents the portion or cumulative proportion of variance explained by each principal component.

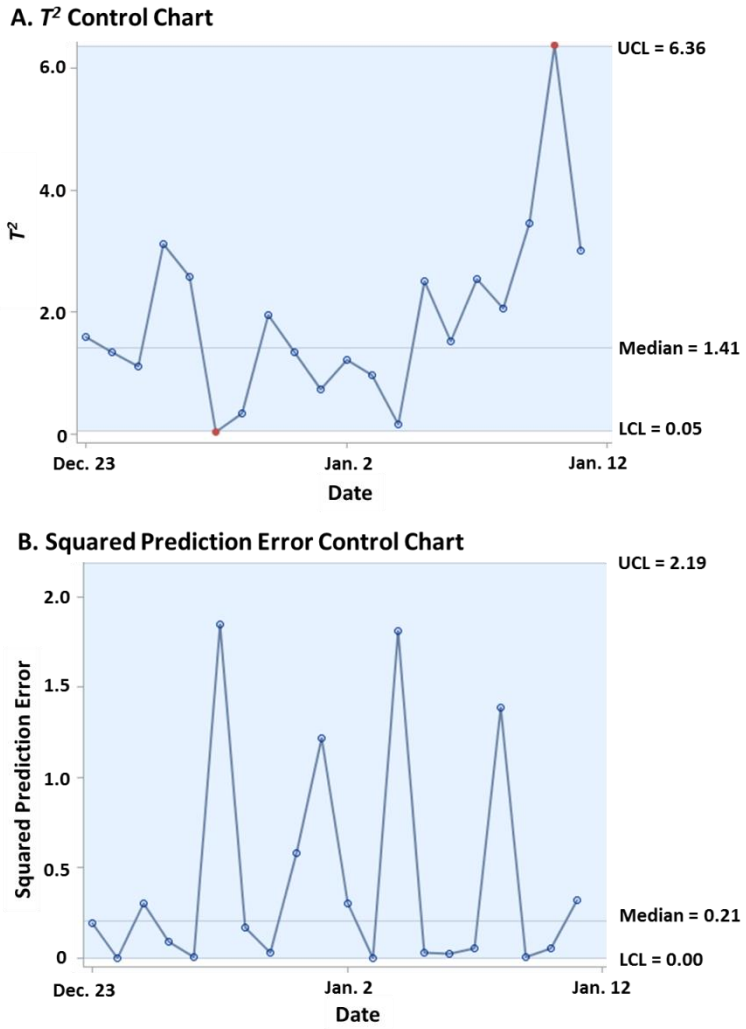

Figure 5-2. Phase I analysis of MVPMONITOR procedure using the first 20 data points of collected data with 2 principal components. Panel A is a Hotelling's T^2 control chart and Panel B represents a squared prediction error control chart. $\alpha = 0.05$ for control limits (upper, UCL; lower; LCL). Data points outside the shaded area indicate an out-of-control data point.

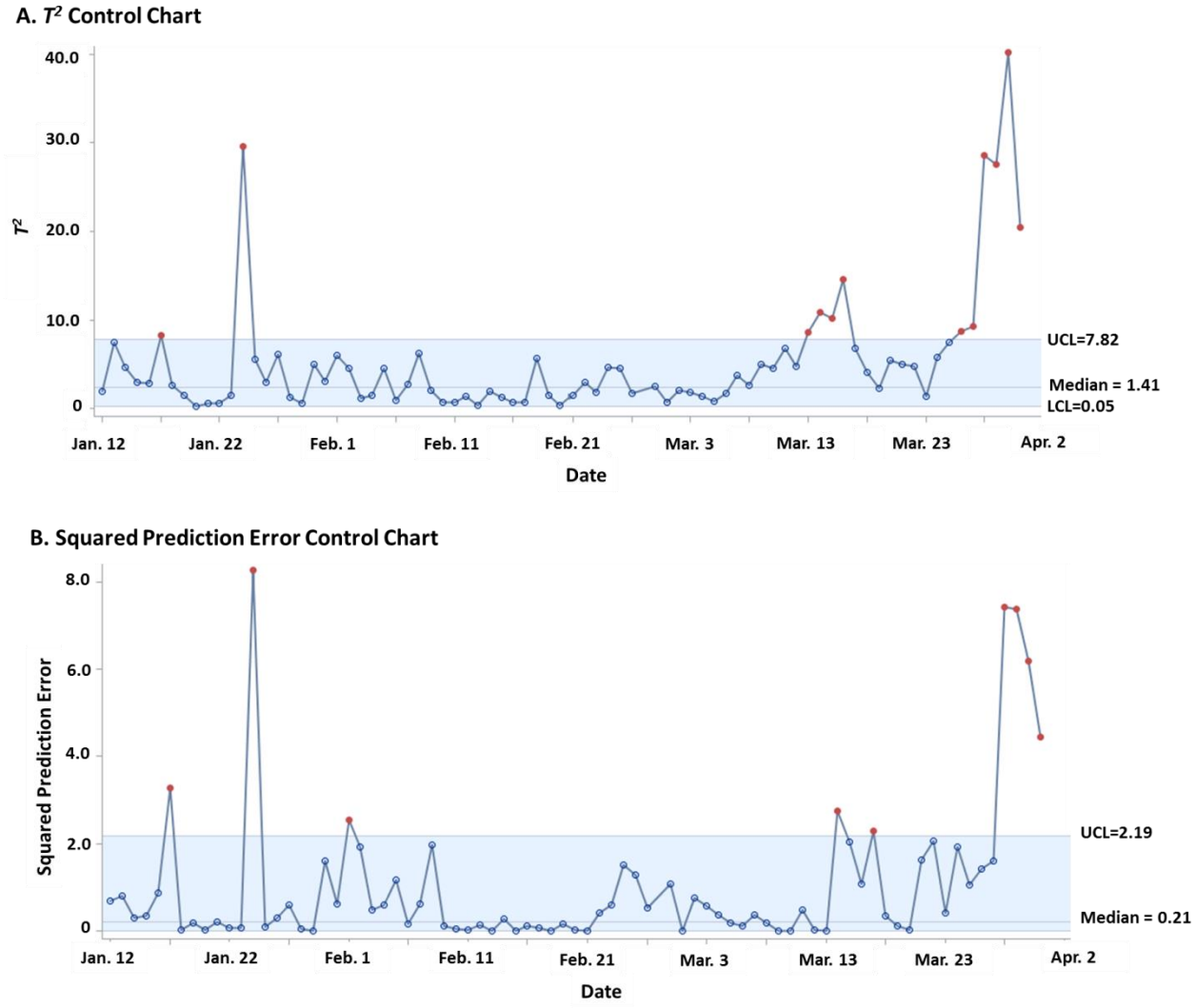

Figure 5-3. Phase II analysis of MVPMONITOR procedure using the last 80 data points of collected data with 2 principal components. Panel A is a Hotelling's T^2 control chart and Panel B represents a squared prediction error control chart. $\alpha = 0.05$ for control limits (upper, UCL; lower; LCL). Data points outside the shaded area indicate an out-of-control data point.

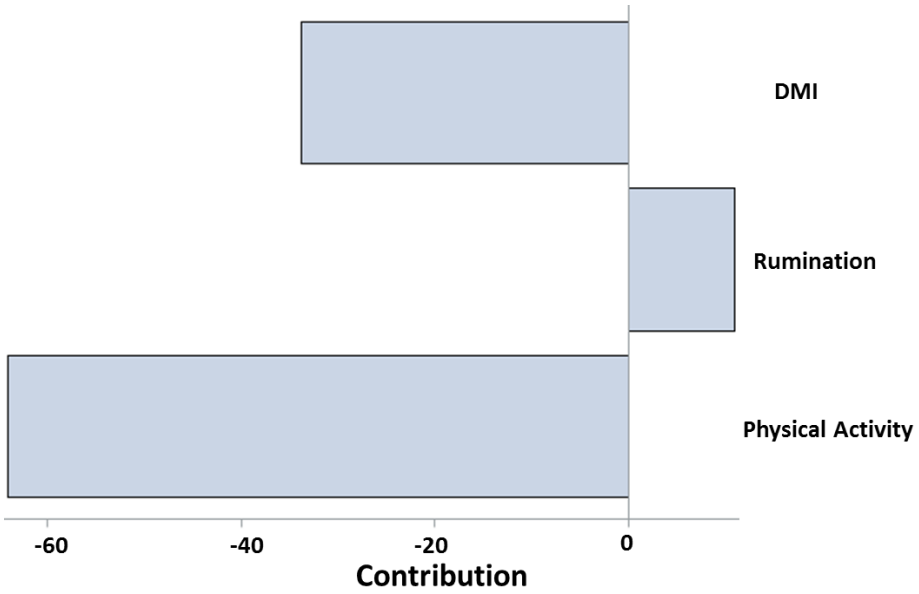

Figure 5-4. Example of a squared prediction error contribution plot derived from the MVPDIAGNOSE procedure. The contribution plot displays how each variable from the original measurement variables contribute to the variation of a particular data point. The contribution plot presented is from the out-of-control data point on Jan. 24th.

Chapter 6. Thesis Conclusions and Further Research.

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The objectives of this thesis were to characterize variance structure in data streams on a individual cow and pen level (Chapters 1 – 5), evaluate animal performance in a controlled research environment (Chapters 2 and 3) as well as on commercial dairies (Chapters 4 and 5), and begin to integrate data streams on a dairy farm (Chapters 4 and 5) using statistical process control techniques.

In Chapters 2 and 3, we conducted a feeding trial evaluating the effects of tall fescue hay inclusion on lactating cow performance (Chapter 3) and cow feeding behavior and meal patterns (Chapter 2). Our objectives were two-fold. First, our objective was to evaluate nutritional effects of inclusion of tall fescue hay on lactating cow performance and behavior. Second, our objective was to characterize variability in data streams such as DMI, milk production, rumination, and physical activity collected at the individual cow-level, and summarized on a group basis. Although the nutrition and fiber-related objective was outside the scope of the central theme of this thesis, conducting a feeding trial in the facilities as indicated in Chapters 2 and 3 allowed us to determine DMI on individual cows, which is not typically available on commercial dairy farms. In additional, these studies offered a controlled research environment and thus analyzing the variability in the data streams in this environment could be much different than that of a commercial dairy farm.

Chapters 4 and 5 focused on data collected from two commercial dairy farms that differed in management strategies. Our first objective was to analyze variability in data collected at the cow level and analyzed at the pen level. Our second objective was to monitor variability of data streams using statistical process control (SPC). Although SPC has been used for decades in other industries to reduce variability, adaptations to agriculture industries have been minimal. Univariate SPC techniques offered the ability to determine

frequency of out-of-control data points to begin to pinpoint when a process has too much variability.

A final objective was to begin to integrate data streams using multivariate SPC techniques. We believe that by combining several data streams, out-of-control performance at the pen level could be detected earlier than a simple univariate analysis. Our research presented in this thesis offers the first step in setting up models to explore out-of-control data at the pen-level using multivariate SPC techniques.

Considerable further research must be conducted in order for these techniques to become widely implemented. First, further research must explore causal relationships between events occurring on-farm and detection of out-of-control data streams. The main questions to be answered in this area of research are "What data streams are most influenced by different events occurring on farm?" and "Does variability in different data streams serve as a better predictor of milk production over other data streams?".

Another area of research is to explore the sensitivity of SPC techniques. Chapter 1 explored several different SPC techniques that can be used to explore variability in a data stream, and several of these control charts were presented in Chapters 4 and 5. However, a sensitivity analysis of each chart in relation to out-of-control data was not conducted. In addition, research in this area should explore the effects of adjusting the parameters used to determine the control limits on control charts. For example, does decreasing the control limits to two standard deviations from the mean instead of three standard deviations from the mean (as in Chapters 4 and 5) improve the detection times to changes in management?

Further multivariate SPC applications are another area of research that should be explored. We presented a relatively basic multivariate SPC control chart in the Hotelling's

T^2 example in Chapter 5. However, multivariate SPC control charts that are much more sensitive to smaller shifts in the mean were explained in Chapter 1. These charts, such as the multivariate cumulative sum control chart and the multivariate exponentially weighted moving average control chart, could provide a more sensitive analysis and earlier detection of deviant data on commercial dairies.

Finally, we suggest that further research should also focus on integration of several different data streams on commercial dairy farms outside of the data presented in this thesis. For example, weather data (temperature, barometric pressure, humidity, etc.) has considerable impact on animal performance. Forage quality and nutrient content changes affect animal performance and are influenced in part by weather. Thus, attempting to integrate diverse data streams outside of simple animal performance indicators could improve decision-making on a commercial dairy as well.

We conclude that while this thesis provides the framework for utilizing SPC techniques to evaluate animal performance and aid in detection of out-of-control data, further research is warranted before a practical application to a commercial dairy exists.

Appendix 1. Comparison of *In Situ* Versus *In Vitro* Methods of Fiber Digestion at 120 and 288 Hours to Quantify the Indigestible NDF Fraction of Corn Silage Samples.

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Abstract

Ruminal digestion of neutral detergent fiber (NDF) is affected by the proportion of NDF that is indigestible (iNDF), and the rate at which the potentially digestible NDF (pdNDF) is digested. Indigestible NDF in forages is commonly calculated as a function of the lignin concentration using the ratio: (2.4 x acid detergent lignin)/NDF. Our objective was to compare estimates of iNDF based on lignin ratio, to *in vitro* (IV) and *in situ* (IS) measurements at two fermentation end points (120 h and 288 h). Further objectives were to compare the subsequent rate, lag, and estimated total tract NDF digestibility (TTNDFD); evaluate the effectiveness of utilizing multiple time points in the *in vitro* method to determine the NDFD; and evaluate the quality of fit between a one-pool and two-pool model of fiber digestion. Thirteen corn silage samples were dried and ground through a 1 mm screen in a Wiley mill. Lignin was determined by wet chemistry. A 2 x 2 factorial trial was conducted to determine the effect of time of incubation and method of iNDF analysis on iNDF concentration; the two factors were method of iNDF analysis (IS vs. IV) and incubation time (120 vs. 288 h). Four sample replicates were utilized, and approximately 0.5 g/sample was weighed into each Ankom F57 bag (Ankom Technology, Macedon, NY; pore size = 25 µm) for all techniques. The IV-120 (37.8%) had a higher proportion iNDF than all other methods; IS-120 (32.1%) and IV-288 (31.2%) techniques were similar, but both were higher than the IS-288 technique (25.7%). Utilizing the lignin values to calculate the ratio of iNDF to lignin yielded similar results: IV-120 had a higher ratio (6.9) than all other methods; IS-120 (5.7) and IV-288 (5.5) techniques were similar, but were both higher than the IS-288 technique (4.6). The resulting rate and lag calculations showed that the IV-120 treatment had the fastest digestion rate (-2.8% h^{-1}) but the longest lag (10.3 h), compared to the IS-120 and IV-

288 with an intermediate rate (-2.3% h^{-1} and -2.4% h^{-1}, respectively) and lag (9.7 h and 9.8 h, respectively). As a result, the TTNDFD estimates did not differ between treatments, and averaged 35.5% of NDF. Thus, digestibility techniques at different times yielded vastly different iNDF estimates. All techniques also yielded ratios that were higher than the commonly used ratio of 2.4 x lignin.

Key words: fiber digestion, indigestible NDF, lignin

Introduction

Neutral detergent fiber (NDF) concentration of forages varies from 30 to 80 % of dry matter and also can differ widely in digestibility. Forage NDF consists of two fractions: an indigestible fraction (iNDF) and a potentially digestible fraction (pdNDF). The potentially digestible fraction disappears from the rumen either by microbial digestion or passage, while the indigestible NDF fraction leaves the rumen only by passage. Therefore, in order to model the digestion kinetics of pdNDF, accurate prediction of the iNDF fraction is a critical first step in determining the digestibility of the forage.

The iNDF fraction is commonly estimated as 2.4 times the acid detergent lignin (ADL) content, as originally proposed by Mertens (1973) and Chandler et al. (1980) and discussed in Van Soest et al. (2005). However, others have shown that the iNDF determination by this method is highly variable and does not correlate well to long term *in vitro* or *in situ* incubations (Huhtanen et al., 2006; Kramer et al., 2010; Krizsan and Huhtanen, 2013). The iNDF fraction can be approximated using a measurement of undigested NDF (uNDF) remaining at a predetermined incubation time, such as 96, 120, or 216 h, in either an *in vitro* or *in situ* system (Van Soest et al., 2005; Schalla et al., 2012;

Krizsan et al., 2013). Others propose that longer incubation times are required to precisely estimate the iNDF concentration of a feed (Nousiainen et al., 2004). The Nordic Feed Evaluation System (NorFor) defines iNDF as the NDF residue remaining after 288 hours *in situ* incubation (NorFor, 2011). Throughout this manuscript, the authors use the term "iNDF" when referring to the intrinsic characteristic of the forage, and uNDF when referring to the amount of NDF undigested at a particular time point.

Ruminal fiber digestion is a competitive process of passage and digestion. The rate of ruminal fiber digestion of the pdNDF can be estimated by measuring the change in pdNDF from *in vitro* incubations at 24, 30 and 48 h (Goeser et al., 2009). However, a more common approach is to index the relative fiber digestibility from a single time point in vitro incubation, most often a 30 h or 48 h. This method fails to account for variations in retention time of NDF in the rumen, as well as the inherent variation in lag times of *in vitro* digestion assays. In addition, Lopes et al. (JDS – in press Jan. 30th, 2015) found no correlation between *in vivo* digestibility and the 30hr NDFD over 21 diets from seven research trials conducted at the University of Wisconsin - Madison. However, Lopes et al. (2015) reported that the total tract NDF digestibility (TTNDFD) can be predicted by *in vitro* incubation of forage samples at several time points using the predetermined endpoint of digestion (iNDF) and measuring the rate of fiber digestion (Kd) to optimize forage utilization.

The Nordic feed evaluation system determines NDF digestibility based on *in situ* degradation (NorFor, 2011), however in the United States, *in vitro* systems are largely used to index fiber digestibility.

The objectives of the present study were to compare estimates of iNDF determined by two methods (*in situ* and *in vitro*) at two different time points (120 and 288 hours); compare

the subsequent rate, lag, iNDF/lignin ratio, and total tract NDF digestibility (TTNDFD); evaluate the effectiveness of utilizing multiple time points in the *in vitro* method to determine the ruminal NDFD; and evaluate the quality of fit between a one-pool and two-pool model of fiber digestion.

Materials and Methods

The *in situ* (IS) and *in vitro* (IV) studies were conducted at the University of Wisconsin – Madison using dairy cows housed at the Arlington Blaine Dairy. Animal protocols were approved by the Institutional Animal Care and Use Committee in the College of Agricultural and Life Sciences. The IS uNDF data was generated using four ruminally-cannulated late lactation dairy cows fed a high forage TMR diet (44.5% alfalfa silage, 26.8% corn silage, 10.7% alfalfa hay, 6.5% straw, 11.5% concentrate mix; DM basis) with a measured nutrient composition of 14.7% CP, 46.1% NDF, 10.6% starch, and 3.4% ether extract.

Thirteen corn silage samples were obtained from a commercial forage testing laboratory (Rock River Laboratory, Inc., Watertown, WI), where they were analyzed for CP, starch, and ether extract by NIR analysis for general characterization of the corn silage. Samples were dried at 60ºC for 48 h in a forced-air oven to determine DM content. Dried samples were ground to pass a 1-mm Wiley mill (Arthur H. Thomas, Philadelphia, PA) screen, and 0.5 grams/sample were weighed into each Ankom F57 bag (Ankom Technology, Macedon, NY) with a pore size of 25 µm for all *in vitro* and *in situ* samples for both the uNDF and NDFD assays. Acid detergent lignin was determined via method 973.18 (AOAC, 2006), modified to use 1.0 grams per sample in each Ankom F57 bag (Ankom Technology,

Macedon, NY). All Ankom bags were washed in acetone, dried in a fume hood, and further dried in a forced-air oven at 60°C for 1 h prior to weighing the sample.

A 2 x 2 factorial trial was conducted to determine the uNDF residue, with method of analysis (*in situ* vs. *in vitro*) and incubation time (120 vs. 288 h) as factors, creating the four treatment groups (IS-120, IS-288, IV-120, IV-288).

For each of the 13 samples and two incubation times, the aforementioned bags filled with each sample were inserted into a nylon laundry bag (30 x 40 cm) in duplicate and then positioned in the ventral rumen of each cow. Bags were pre-soaked in warm water for approximately 30 minutes prior to incubation. Each laundry bag contained eight blank Ankom bags containing 0 grams of sample to correct for any infiltration of NDF into the sample bags. Samples were removed after 120 or 288 h. After removal, samples were soaked in cold water before washing twice in a commercial washing machine with cold water during 12 min cycles. This procedure was then duplicated in a second run.

The IV uNDF data was determined by using the Combs-Goeser standardized *in vitro* incubation technique (Goeser and Combs, 2009; Goeser et al., 2009), modified to remove bags at two time points (120 and 288 h), instead of the original three (24, 30, and 48 h). Each of the 13 samples and two time point combinations were duplicated within a run and replicated in two runs. Each replicate contained eight blank Ankom bags containing 0 grams of sample to correct for infiltration of NDF into the bag. After removal, 0 h time point samples were included and rinsed in cold water.

The NDF digestibility was determined by the *in vitro* incubation technique described by Goeser and Combs (2009) and Goeser et al. (2009). The procedure was modified to remove bags at seven time points (12, 18, 24, 30, 36, 42, and 48 h). All 13 samples at each

time point in the *in vitro* incubation technique were replicated in four separate runs. Each replicate contained four blank Ankom bags containing 0 grams of sample to correct for any infiltration of NDF into the sample bags, as well as 0 h time point samples to correct for initial NDF content.

The NDF content of the intact sample and digested residue samples was determined by refluxing in neutral detergent solution containing α-amylase (Ankom Technology, Macedon, NY) and sodium sulfite (Sigma Aldrich, St. Louis, MO) prepared using the procedure described by Goering and Van Soest (1970) and adapted for an Ankom^{200} Fiber Analyzer (Ankom Technology, Macedon, NY). Neutral detergent fiber percentages of all samples were determined using the following equation:

1) NDF (g / g DM) = [(bag wt. + residue) – (bag wt. x bag correction factor)] / [(bag wt. + sample) – (bag wt.)].

The bag correction factor represents the average fractional weight change of 20 Ankom bags following the NDF wash procedure.

The *in vitro* NDF digestibility at each time point was determined using the following equations:

2) ivNDFD (g / g NDF) = ($NDF_{0\ h}$ – $NDF_{residue}$) / ($NDF_{0\ h}$).

3) ivNDFD (g / g pdNDF) = ($NDF_{0\ h}$ – $NDF_{residue}$) / (% pdNDF x $NDF_{0\ h}$).

For data sets using the linear model fitting techniques, potentially digestible NDF (pdNDF) as a fraction of NDF was calculated using equation (2), where the NDF residue is the residue at the 120 or 288 h time point. For data sets using the non-linear model fitting techniques, iNDF was estimated using a best fit model. The measured uNDF value will be close to the actual iNDF of the sample, therefore we bound the iNDF to +/- 10% of the measured uNDF

to ensure the calculated iNDF is not biased too severely by earlier time points. In addition, this allows for an estimation of the true iNDF from measured values. Therefore the pdNDF in the non-linear model fitting was estimated in the model and was the difference between NDF and iNDF.

To assess rate of digestion, the NDFD assay runs were fit to the following linear first-order differential equation:

4) $f(t) = 1$; for t < L

5) $f(t) = e^{k\,(t-L)}$; for t >= L

6) $R(t) = (1 - iNDF) * (1 - e^{k\,(t-L)})$; for t >= L and where uNDF (120 or 240)*0.9 < iNDF < uNDF(120 or 240) * 1.1

Where f(t) = remaining pdNDF (g/g) at time t; R(t) = NDF degraded (g/g) at time t; k = constant fractional rate of digestion; t = time (hours); and L = lag time before digestion occurs.

The assay results were fit to a linear (equation 4 and 5) and a non-linear (equation 4 and 6) equation. In the first method, the natural logarithm of the fractional pdNDF residue in each bag was calculated. The slope of the natural logarithm-transformed fractions versus time function is linear, thus the fractional rate of disappearance of pdNDF and the intercept (lag) of digestion can be determined. The slope and intercept was calculated using R^2 as the measure of best fit, thus minimizing the transformed errors from the model. At long incubation times, a time point result for remaining pdNDF (g/g) can be negative. In this circumstance, the natural logarithm transformation cannot be completed and the timepoint cannot be used. In addition, when the residual NDF fraction is close to 0, the log transformed value is a large negative number that exaggerates the actual slope of digestion.

Thus, we removed all residue values that were less than an arbitrary threshold of 0.05. This 0.05 threshold was also applied at the opposite end; samples that had not achieved 5% of pdNDF degradation where not used, as they could not be distinguished from samples that had not yet passed the lag phase. Another consequence of this method is that methods of iNDF measurement yielding a larger iNDF fraction (such as *in vitro* and earlier timepoints), and therefore a smaller pdNDF fraction, yield more data points being discarded for being too close to the iNDF estimate. After the data was best fit, the entire run was discarded as flawed if the goodness of fit, here measured by R^2, was less than 0.5. Of 52 total runs, 2 were entirely discarded.

The second method of model fitting was done using the NLIN procedure in SAS (version 9.4, SAS Institue, Inc., Cary, NC), which calculated the lag, digestion rate, and the iNDF. The NLIN procedure iteratively solves for the constants in the model, minimizing the SSE for the real errors, rather than the transformed errors of the linear method of model fitting. Using this modeling technique, the iNDF was also estimated as part of the model best fit, but was bounded to be within 10% of the measured uNDF120 or uNDF240 (equation 6). As with the previous linear model fitting approach, NDFD timepoints with pdNDFD residues of less than 0.05 or greater than 0.95 were rejected.

Digestion rate, lag, quality of fit (R^2) and an estimate of total tract fiber digestibility (TTNDFD) was calculated using the different pdNDF values yielded by each uNDF assay from each combination of method of analysis and incubation time as well as the NDFD values from the *in vitro* incubation technique. TTNDFD is calculated by the following equation (Lopes et al., 2015):

7) $$\mathrm{TTNDFD} = \mathrm{pdNDF} * \frac{k}{k-2.67} * \frac{1}{0.9}$$

Where k = digestion rate (% / hour); 2.67 represents the fiber passage rate (% / hr); and 0.9 represents the fraction of fiber digestion that occurs in the reticulorumen.

All data were analyzed via the MIXED procedure of SAS (version 9.3, SAS Institute Inc., Cary, NC), with the final model including the effects of method of analysis (IS vs IV), incubation time, and interaction between method of analysis and incubation time. Fixed effects for sample number and the interaction of sample number by method of analysis by incubation time were removed from the model ($P > 0.25$). Significance was declared when $P < 0.05$ and a tendency at $0.05 < P < 0.10$. In addition, a contrast was conducted to compare ruminal digestion parameters for the four treatments estimating iNDF to the 2.4 x Lignin iNDF estimation.

Results

Descriptive information of the nutrient composition and ruminal digestion parameters of the corn silage samples included in the analyses are provided in Table A1-1. Nutrient composition is typical of commercially produced corn silages utilized on dairy farms in the Midwest United States.

The effect of method of digestion and incubation length on ruminal digestion parameters in both a linear and non-linear model fitting approach is displayed in Table A1-2. For the linear model fit, the uNDF fraction as a proportion of total NDF was greater ($P < 0.001$) for the IV compared to the IS treatment (34.5 vs. 28.9%, respectively). In addition, the longer incubation time resulted in a lower ($P < 0.001$) uNDF fraction (28.4 vs. 35.0%, 288 and 120 h, respectively). As a result, the uNDF fraction estimated from the IV-120 treatment was greater, and the IS-288 estimated uNDF was lower, compared to the remaining

treatments. The calculated ratio of uNDF divided by lignin content had both a method ($P < 0.001$; 5.4 vs. 4.5, IV and IS, respectively) and time ($P < 0.001$; 4.4 vs. 5.5, 288 and 120 h, respectively) effect. Similarly, the IV-120 treatment had a significantly greater, and the IS-288 treatment a significantly lower, uNDF divided by lignin ratio when compared to the remaining treatments.

Data displaying the rate (% digestion / h), lag (h), quality of fit (R^2), and TTNDFD estimate (% of total NDF) are displayed in Table A1-2 and were determined using the IV NDFD digestibilities (12, 18, 24, 30, 36, 42, 48 h) and the endpoints of uNDF from each treatment explained above. For the linear model, the rate was significantly greater for the IV compared to the IS treatment (-2.4 vs. -2.1% / h, respectively). In addition, the shorter incubation time for uNDF resulted in a faster ($P < 0.001$) digestion rate (-2.6 vs. -2.3% / h, 120 and 288 h, respectively). As a result, the IV-120 treatment had a significantly greater, and the IS-288 treatment a significantly lower, digestion rate when compared to the remaining treatments. There was no main effect of method or time on lag, but the IV-120 treatment was greater ($P < 0.05$) compared to the IS-288 treatment. The quality of fit, as measured by R^2, did not differ among treatments and averaged 0.87 across all treatments. Similarly, the TTNDFD estimate as a proportion of total NDF did not differ among treatments and averaged 35.5%. No interactions between method and time were observed for any ruminal digestion parameters in the linear approach.

For the non-linear approach, the data followed the same general pattern as the linear model for both the rate and iNDF fraction as a proportion of total NDF. The uNDF fraction as a proportion of total NDF was greater ($P < 0.001$) for the IV compared to the IS treatment (32.0 vs. 26.6%, respectively). In addition, the longer incubation time resulted in a lower (*P*

< 0.001) uNDF fraction (27.1 vs. 31.5%, 288 and 120 h, respectively). As a result, the IV-120 treatment had a greater, and the IS-288 treatment a lower, uNDF fraction when compared to the remaining treatments. The rate was greater for the IV compared to the IS treatment (-2.3 vs. -2.0% h^{-1}, respectively). In addition, the shorter incubation time resulted in a lower (P = 0.002) digestion rate (-2.2 vs. -2.0% h^{-1}, 120 and 288 h, respectively). As a result, the IV-120 treatment had a greater, and the IS-288 treatment a lower, digestion rate when compared to the remaining treatments. There was no main effect of method or time on lag in the non-linear approach. Similar to the linear approach, the TTNDFD estimate as a proportion of total NDF did not differ among treatments and averaged 34.2%. No interactions between method and time were observed for any ruminal digestion parameters in the non-linear approach.

Table A1-3 provides a comparison of the ruminal digestion parameters using the laboratory determined estimation of iNDF compared to an estimated iNDF pool (2.4 x Lignin). Digestion rate of pdNDF, lag time, and iNDF fraction determined by the 2.4 x lignin method were significantly lower than when estimated by the other four methods. The calculated TTNDFD values however, were similar.

Discussion

Wide variations exist in methodologies in commercial feed testing laboratories for testing NDF digestion rate (Van Amburgh et al., 2005; Tylutki et al., 2008; Goeser et al., 2009) and iNDF (Nousiainen et al., 2004; Van Soest et al., 2005; Kramer et al., 2010; Krizsan and Huhtanen, 2013). This study evaluated methods of estimating iNDF, including *in vitro* and *in situ* at two incubation time points (120 h and 240 h), and its subsequent effects

on estimating the ruminal fiber digestibility using both a linear and a non-linear modeling approach. The study included thirteen corn silage selected to be diverse in composition in order to measure effects across a wide range of corn silage quality.

Contrary to previous studies and common industry practice of indexing ruminal digestion of NDF by using either one (30 h or 48 h) time point (Lopes et al., 2015 – submitted to JDS) or three time points (Goeser et al., 2009), we used seven time points (12, 18, 24, 30, 36, 42, and 48 h) in the present study. These time points were chosen because they represented points between the expected assay lag time and the last traditional time point (48 h) since we sought to determine the best possible fit of the data. We chose time points earlier than the traditional approaches because the physiologically relevant period of ruminal fiber digestion is earlier (Waldo et al., 1972), when a greater mass of fiber is present in the rumen. In addition, because inter-run variation was expected to be substantially greater than intra-sample variation (Mehrez and Orskov, 1977), in lieu of duplication of samples within run for each time point, duplication was completed across more runs. In a commercial lab, where turnaround time is of great importance, this may not be a viable strategy, but accounting for inter-run variation is important.

In addition to the traditional log-linear transformation to fit the data, our study also evaluated the use of a non-linear approach. In this approach, we removed from the analysis any time point value where pdNDF residue was <0.05 or >0.95 of the initial estimate of pdNDF, since it cannot be determined whether or not these samples have substantially started or finished digestion at this point. Another feature of the non-linear iterative solving approach is that the iNDF can be estimated without doing an uNDF assay, since adding a pdNDF term to the model will result in a solved value for iNDF. However, we recognize

that an iNDF value estimated from a model is still not as good as a measured value. To have a reasonable compromise between model fitting to solve for the degradation asymptote and not allow early time points (subject to variance) to poorly fit the asymptote, we put a limit on the modeled value of the asymptote; in this model the iNDF was bound to a range of +/- 10% of the uNDF measured from the assay. If an IV-120 is used as the iNDF estimate, it may be more appropriate to bound the value using a range of zero to some level of overestimation limit, as the IV-120 was found to be significantly lower than the "gold standard" IS-288. In this study all were treated equally with a +/- 10% range.

Accurate estimation of the pool size of pdNDF is important to determining NDF digestibility (Robinson et al., 1986). Different digestion methods (*in vitro* and *in situ*) yielded different estimates of the pdNDF pool at different time points. When the estimated value was close to the true pdNDF, overestimations of iNDF yielded overestimations of the digestion rate (Mertens, 1993) that effectively offset each other, resulting in the similar TTNDFD values (Table A1-2). When the estimations were not close, as in the scenarios depicted in Table A1-3, an estimation from lignin would result in approximately half the iNDF as measured by all four digestion assays. Thus, based on the present study, we recommend discontinuance of iNDF estimates from lignin for corn silage and recommend the use of *in situ* or *in vitro* digestion assays to make the iNDF estimation.

The non-linear model fit yielded similar but slightly lower results for TTNDFD. This may be due to selection criteria for individual runs being invalid, or due the inherent nature of the linear fit having transformed results that skew it toward higher digestion rates. However, the values were sufficiently close to that of the linear model, that no practical difference can be inferred. Most of the fiber-derived energy is in the early stages of digestion

(Waldo et al., 1972). Therefore, we recommend using the non-linear model fitting techniques to best fit a model with measured values from earlier stages of digestion. However, the linear fit method yielded similar results and we therefore feel it is an acceptable method, if outlier data points are removed that would otherwise skew the results.

Criteria for removing individual time points and runs needs to be employed as a quality assurance standard for any highly variable assay such as fiber digestion. The linear model choice of an R^2 of greater than 0.5 is quite conservative for inclusion. In a large dataset of random decreasing values, this same model yielded a mean R^2 value of 0.89, and only five runs were below this 0.50 threshold. Thus, the criteria described herein for selection of valid time points and runs is a good starting point. Run to run variation was a highly significant ($P < 0.001$) fixed effect in all models for both rate and lag. As such, we recommend duplicating across more runs, rather than duplicating within run.

The real value that producers and field nutritionists want to know is how much energy will a group of cattle derive from a particular fiber source. This can be determined via the TTNDFD. It accomplishes this by taking estimations of rate and the potentially digestible NDF fraction in the ruminant, and in combination with assumed passage rates and post-ruminal degradation rates, provides a value that is a reasonable estimation of fiber digestion (Lopes et al., 2015). It was also found to be a robust measurement across iNDF assay methods, yielding substantially similar results, regardless of assay method.

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Table A1-1. Descriptive information of the nutrient composition and ruminal digestion parameters of 13 corn silage samples used for analysis (% of DM unless otherwise indicated).

	Average	**Standard Deviation**	**Minimum**	**Maximum**
DM, % of as fed	39.2	7.3	26.6	55.6
CP	8.5	1.2	7.1	10.9
Starch	27.7	9.0	14.0	38.0
NFC	41.8	6.2	32.1	49.7
Ether Extract	2.1	0.3	1.4	2.5
NDF	36.8	4.8	29.7	45.2
Lignin	2.8	0.7	1.9	4.9
Ash-free Lignin	2.0	0.4	2.3	2.9
uNDF[1]	25.6	6.0	13.2	34.5
kd[2]	-2.1	0.5	-3.0	-0.9
TTNDFD[3], % of NDF	35.7	6.2	23.6	49.0

[1]Undigested NDF determined via *in situ* incubation for 288 h.

[2]Rate of degradation of pdNDF (kd) determined using iNDF (in situ 288 h) as an endpoint of fermentation.

[3]Total tract NDF digestibility (TTNDFD) determined as previously reported (Lopes et al, 2015).

Table A1-2. Effect of digestion method (*in situ* vs. *in vitro*) and incubation length (120 vs. 288 hours) on ruminal digestion parameters in both a linear and non-linear model fitting approach.

	Treatments					*P* - values		
	IS-120	IS-288	IV-120	IV-288	S.E.M.	Method	Time	M x T
Linear Model Fit								
Rate (%/hour)	-2.3[b]	-2.1[a]	-2.8[c]	-2.4[b]	0.2	<0.001	<0.001	0.22
Lag (hours)	9.7[ab]	9.4[b]	10.3[a]	9.8[ab]	0.6	0.10	0.18	0.75
R^2	0.87	0.87	0.86	0.87	0.02	0.51	0.58	0.84
uNDF (% of NDF)	32.1[b]	25.7[c]	37.8[a]	31.2[b]	1.8	<0.001	<0.001	0.82
uNDF/ash-free lignin	5.7[b]	4.6[c]	6.9[a]	5.5[b]	0.2	<0.001	<0.001	0.54
TTNDFD (% of NDF)[1]	35.5	35.7	35.3	35.5	1.5	0.73	0.76	0.95
Non-Linear Model Fitting								
Rate (%/hour)	-2.1[b]	-1.9[a]	-2.4[c]	-2.2[b]	0.1	<0.001	<0.001	0.69
Lag (hours)	8.7	8.5	9.0	8.8	0.6	0.43	0.59	0.99
uNDF (% of NDF)	28.7[b]	24.5[c]	34.3[a]	29.8[b]	1.7	<0.001	<0.001	0.78
TTNDFD (% of NDF)[1]	34.3	34.6	33.7	34.2	1.4	0.37	0.42	0.85

[a,b] Within response parameter, means with different superscripts differ ($P < 0.05$).

[1] Determined as previously reported (Lopes et al., 2014).

Table A1-3. Comparison of ruminal digestion parameters using uNDF[1] or with an estimated iNDF pool (2.4xLignin).

	4 Trts Averaged		**2.4xLignin**		
	Mean	S.E.M.	Mean	S.E.M.	*P*-value
Rate (%/hour)	-2.4	0.1	-1.6	0.1	<0.001
Lag (hours)	9.7	0.6	7.8	0.7	0.01
uNDF (% of NDF)	30.7	1.4	13.5	1.6	<0.001
TTNDFD (% of NDF)[2]	35.3	1.5	35.2	1.6	0.91

[1] The iNDF/lignin ratio averaged 5.7 across all treatments.

[2]Determined as previously reported (Lopes et al., 2015).

Appendix 2. Extension Publication: Measuring and Monitoring Rumination in the Dairy Cow

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What is Rumination?

Ruminants include a vast array of animals including domestic livestock species, such as dairy and beef cows, goats, and sheep, as well as wild animals, including deer, moose, antelope, giraffes, and llamas to name a few.

The main adaptation that differentiates ruminants from other species is the ability to acquire nutrients from fibrous plant materials. Ruminants have the unique ability to chew their cud, which breaks down fibrous feed particles to enhance fiber digestion and derive energy. This process, termed "rumination", is a repetitive, cyclical process that includes regurgitation, remastication, resalivation, and reswallowing of a feed bolus.

Cows have an inherent need to ruminate, and spend many hours throughout the day engaged in this process. Thus, decreased rumination can often be considered an indication of increased stress, anxiety, or disease. Rumination is also influenced by a multitude of nutritional factors, including fiber intake, diet composition, forage quality, and digestibility of feed. Saliva production, strongly correlated with the amount of time spent ruminating, contains buffers that moderate ruminal pH. Thus, accurate monitoring of rumination in dairy cattle may be an important way to track cow health on a dairy farm.

Technology

Technological advances have resulted in the ability to measure a wide array of information including health, reproduction, and production parameters on individual cows and on a herd level. On a commercial dairy, nutritionists have often relied upon visual observation of cud-chewing as a proxy for rumination time. In a university setting, methods to measure rumination have relied heavily upon visual observation or harnesses enclosing the

head that detect jaw movements – clearly not commercially-viable approaches. An indirect system to measure rumination activity, known commonly as rumination monitors, has become feasible on commercial farms in the last 5 years. Several companies utilize different approaches to detect rumination.

Activity Monitors Measure Physical Activity

Technologies to measure the physical activity of a dairy cow, such as those often used in detection of estrus, involve the use of an electronic activity monitoring system. These systems use a small sensor called an accelerometer, which is affixed to the cow to detect motion in three dimensional space: vertical/horizontal, front/back, and left/right. When the activity monitor is located on the neck or ear, the inclination of the head can be measured to determine the eating status of a cow. When a cow is eating, her head is typically tilted towards the ground. In addition, an activity monitor can also be used to determine how much motion is occurring. Therefore, by combining these signals, a cow that is eating often has a high degree of vertical/horizontal motion with a changing degree of head inclination as the cow takes a bite and lifts her head to chew.

Agis Automatisering BV: Physical Motion

Agis Automatisering BV, a company based out of the Netherlands, has recently developed a sensor to record cow feeding behavior and rumination, termed the CowManager SensOor system (Figure A2-1), which is also marketed by Select Sires. The technology is an electronic sensor that quantifies rumination, eating behavior, physical activity, and temperature. This sensor fits into an ear identification tag and contains an accelerometer to measure the cow's movement in three dimensions. Based on head position and motion, the

cows' behavior is classified into 4 different behavioral categories including "ruminating," "eating," "resting," or "active" (Bikker et al., 2014).

Specific details of the SensOor system are not available due to the proprietary nature of the device. However, we do have a general idea of how the data is collected, summarized, and presented to the user. Once data is recorded by the 3-dimensional accelerometer in the ear tag of the cow, it is expressed as a percentage of each behavior per hour and per day. Data are sent to a computer via a wireless signal, and can be stored for a maximum of 48 hours after the last communication with the computer. A web-based application allows the user easy access to the data.

The SensOor system is currently available through Selected Sires and Boumatic, who market the products as CowManager.

SCR Dairy System: Motion + Vocalization

SCR Dairy has developed and marketed the most widely available rumination system currently on the market. The Heatime System (SCR Dairy) is marketed under many different companies, including Micro Dairy Logic, Semex (called AI24), Select Sires, Lely, and Genex CRI. Although the exact mechanism of detecting rumination is proprietary, the technology integrates information from both motion and sounds recorded by a tuned microphone.

The rumination sensor is positioned by a neck collar on the left side of the cow's neck, as shown in Figure A2-2. The rumination sensor includes a microphone that records sounds made by the cow during regurgitation and rumination. The data is recorded and processed by the microphone, collected via a long distance antenna or infrared signal, and downloaded into the computer for analysis (Schirmann et al., 2009).

SCR Dairy's rumination sensors process the signal from the sensor unit in the collar, the data is then transmitted to a remote receiver, where the data is received and analyzed to determine whether the perceived action represents rumination or eating activities. The rhythm of the chewing actions is sampled periodically to determine the frequency and consistency of the chewing motion. During rumination, cows chew in a very steady, rhythmic manner. Each chewing motion takes approximately 0.5 – 1.5 seconds, with swallowing and bolus regurgitations taking place for 2 – 7 seconds at 32 – 81 second intervals. In contrast, when the cow is eating there are no bolus regurgitations and more sporadic chewing actions.

After determining whether the action is rumination versus eating, the data is summarized by two hour blocks. The data can then be viewed as the number of minutes ruminating per two hour block, total rumination minutes per day, or a calculated number to indicate change in rumination. The graph above (Figure A2-3) is a view of the rumination change over time from the SCR system. In this example, a seven day average baseline is calculated on an individual cow basis in two hour blocks. Each two hour block is compared to the similar segment from previous days. For example, if a cow ruminates 55 minutes on average over the past seven days in the 10am to noon time frame, and today the cow ruminates 45 minutes during the same time frame, the graph would plot -10 minutes as the change in rumination relative to that time frame. Further applications of this rumination graph will be discussed below.

What Does 'Typical' Rumination Look Like?

Key Features Over a 30 Day Period

'Typical' rumination can best be explained by viewing Figure A2-3, which is a graphic display from SCR Dairy's computer output of rumination data for one dairy cow over the course of a 30 day period from 30 to 60 days in milk. This graph shows several small, unexplained drops in rumination over the course of the time interval. On the X-axis is the time of day, with days in milk below. The left Y-axis corresponds to the gold line, or the "Weighted Rumination Change" from one 2 hour period to the corresponding 2 hour period averaged over the 7 days prior. The left Y-axis also corresponds to the bar graph, representing the raw rumination data, with each gray bar representing total minutes of rumination in a 2 hour period. The right Y-axis corresponds to the "Total Rumination Minutes In Last 24 hours" under the blue shaded area, essentially totaling the 12 prior 2 hour periods on a continual basis.

Key Features Over a 24-Hour Period

The total number of minutes ruminating is variable among farms, and even among cows within a farm. On average, cows will typically ruminate 7 to 10 hours per day (420 to 600 minutes per day), including ruminating while both standing and lying. Mature cows often ruminate more frequently than heifers. In addition, cows cannot ruminate while eating, thus, there is typically less rumination that occurs during the day when cows are fed than at night. At night, cows typically lie down for a greater duration of time, increasing rumination time. In addition, there is often a lag of when rumination begins after feeding.

How Can Rumination Be Used in Practice?

Rumination information can be used for many purposes. Currently, rumination data are primarily used to detect changes in chewing activity in individual cows. The most

common use is to detect a physiological change that is occurring in the cow, including detecting cows in estrus (heat), cows close to calving, and quick detection of sick cows.

Detecting Cows in Heat

A cow in estrus will dramatically reduce the amount of time spent ruminating. By tracking rumination change over time in conjunction with activity, a farmer can detect cows in estrus. To supplement the rumination data, many sensors also collect physical activity data that indicates the amount of physical movement a cow makes in a day. When a cow is in estrus, she will also dramatically increase her physical activity. Thus, an increase in physical activity and a decrease in rumination is a common signal to a producer that a cow is in estrus.

This pattern can be seen in the SCR graph (altered for ease of viewing) in Figure A2-4, where an activity increase and rumination decrease triggers the system to indicate the cow is in heat, as seen by the graphic near the bottom of the figure. After a period of 21 days, the pattern repeats itself, representing a typical reproductive cycle of a dairy cow.

Several studies support that cows have reduced rumination time during estrus (Reith and Hoy, 2012), but the benefits of breeding cows based on rumination changes is uncertain. Although the benefits of this program have been touted as a way to reduce the reliance on synthetic hormone injections, the impact on fertility of the dairy cow is unknown. Cows inseminated to advanced timed artificial insemination protocols generally have higher fertility than cows inseminated to estrus. Furthermore, 20% to 30% of cows on a dairy are anovular at the end of the voluntary waiting period and are by definition not cycling and will not be detected by the system. However, since most pregnancy diagnoses do not occur until

around 30-35 days after breeding, an optimal use of rumination collars may be to more quickly detect non-pregnant cows after an initial timed artificial insemination.

Monitoring Time of Calving

Before calving, cows will decrease rumination, thus, this data could be used as a predictor to determine when cows will calve. As seen in Figure A2-5, adapted from SCR Dairy, a sharp decrease in rumination and an increase in activity indicates calving, as depicted by the graphic at the bottom of the figure.

Researchers at the University of Kassel in Germany evaluated rumination changes immediately before calving and discovered that during the final six hour period before birth, rumination time was decreased in dairy cows (Buchel and Sundrum, 2014). More research is needed to determine the practical applications of this information.

Early Detection of Sick Cows

When a cow is suffering from an illness, particularly post-calving, rumination time will sharply decrease. Rumination data may therefore be an early determinate of a sick cow and allow a quicker response. Figure A2-6 depicts a graph adapted from SCR Dairy and shows a severe, sustained drop in rumination outlined in a red box. This particular cow had a displaced abomasum that allowed the herd staff to identify the cow very early and intervene.

Italian researchers (Calamari et al., 2014) studied the relationship between rumination time and the incidence of disease during the transition period of dairy cows. They found that rumination time could serve as an early indication of disease onset.

Effect of Stress and Environment on Rumination

High levels of stress also negatively impact rumination. For example, a study from the Miner Institute in New York found that when cows are stocked at 130% of capacity in a

freestall setting, rumination time decreased by 25% compared to cows stocked at 100% (Krawczel et al., 2009). This study also found that overcrowding has a more pronounced effect on rumination in 1st lactation heifers compared to mature cows. In addition, other research has found that 1st lactation heifers housed separately increase rumination time compared to 1st lactation heifers housed in a commingled group with mature cows. Overstocking, prolonged time in headlocks, uncomfortable resting surfaces, social factors, and competition for feed and water all likely impact the stress level in a pen of cows, and therefore, the amount of time a cow spends ruminating.

Concluding Thoughts

Clearly the advent of commercially-viable rumination detection sensors holds great promise for the dairy industry. Although current utilization of rumination data focuses on the individual cow, for example, heat detection, calving time, and post-calving illnesses, there also exists a potential to utilize rumination data at the pen and/or herd level. Research indicates that rumination is greatly affected by management and nutritional changes on farm. Further research into utilizing rumination data at the pen and/or herd level holds promise to better understand and improve management and nutritional decision-making on farm.

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Figure A2-1. CowManager SensOor System from Agis Automatisering BV (Harmelen, Netherlands).

Figure A2-2. Heatime HR System from SCR Dairy (Netanya, Israel).

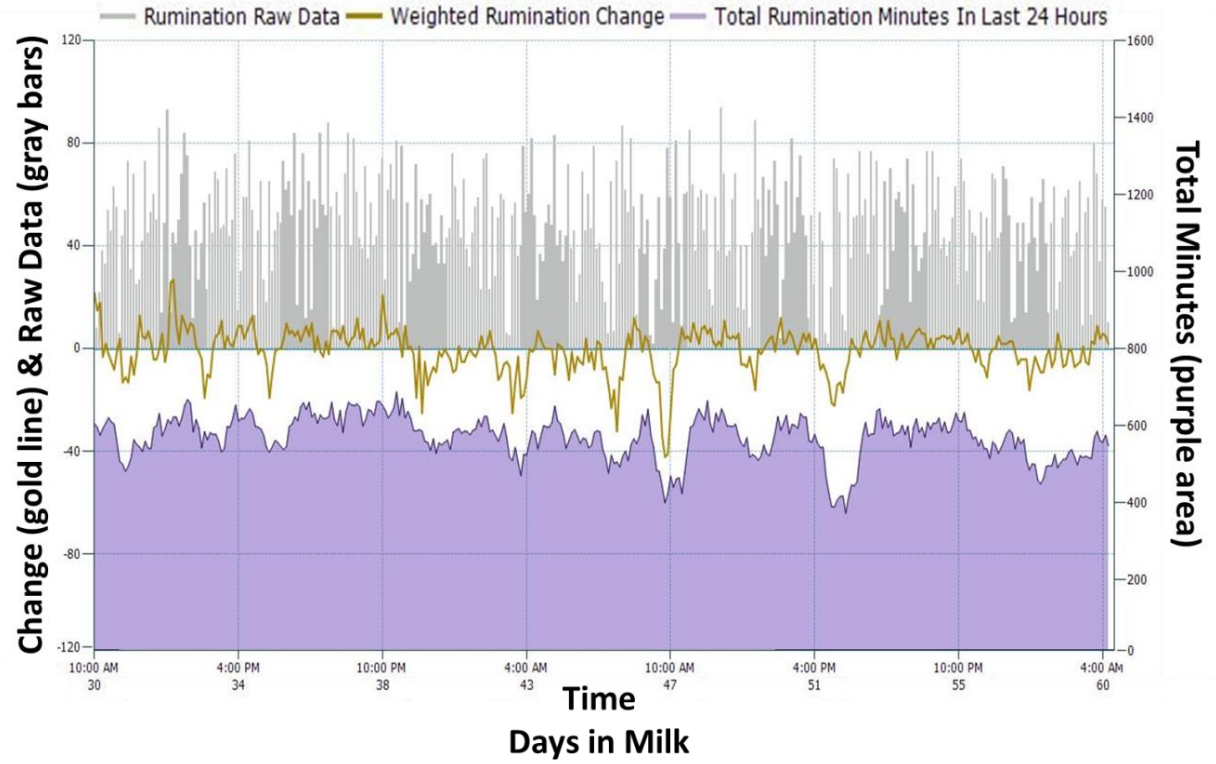

Figure A2-3. Example of typical rumination output, adapted from SCR Dairy.

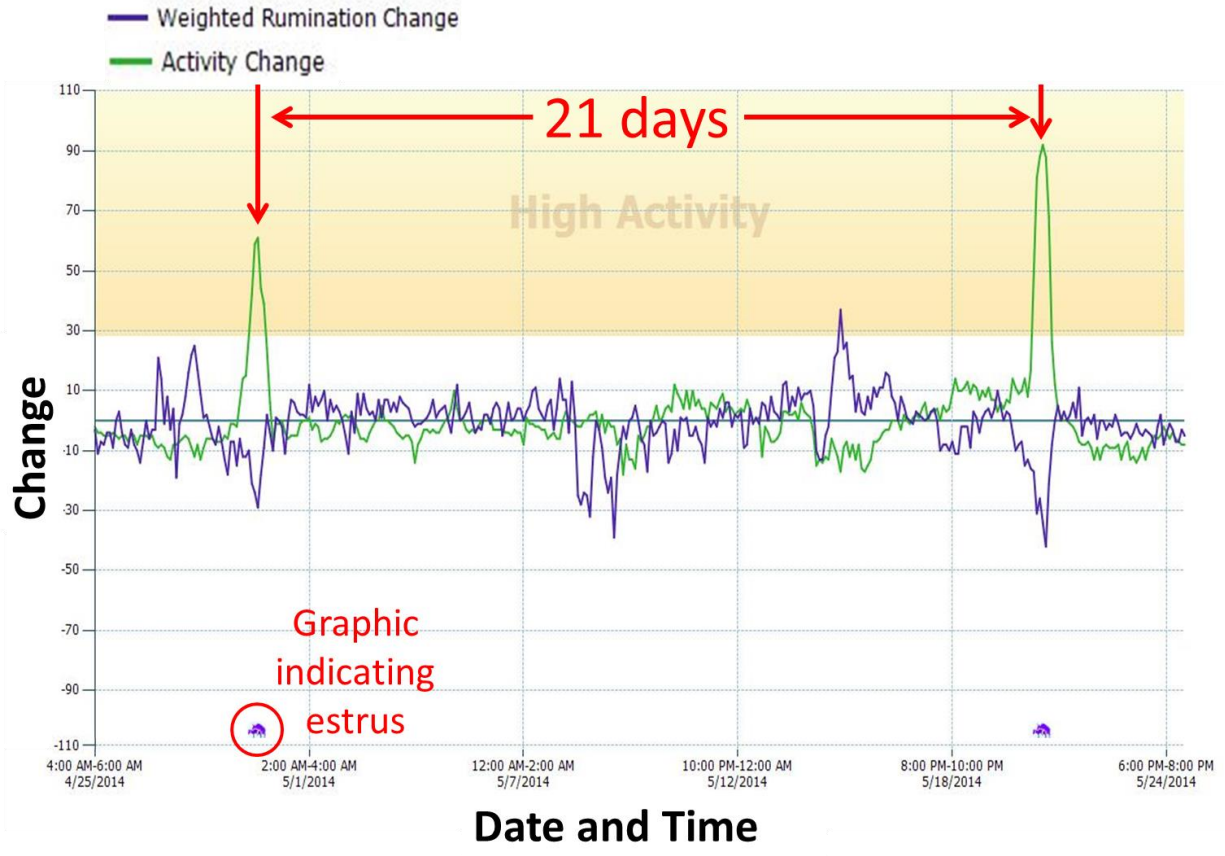

Figure A2-4. Rumination and physical activity data used for estrous detection, adapted from SCR Dairy.

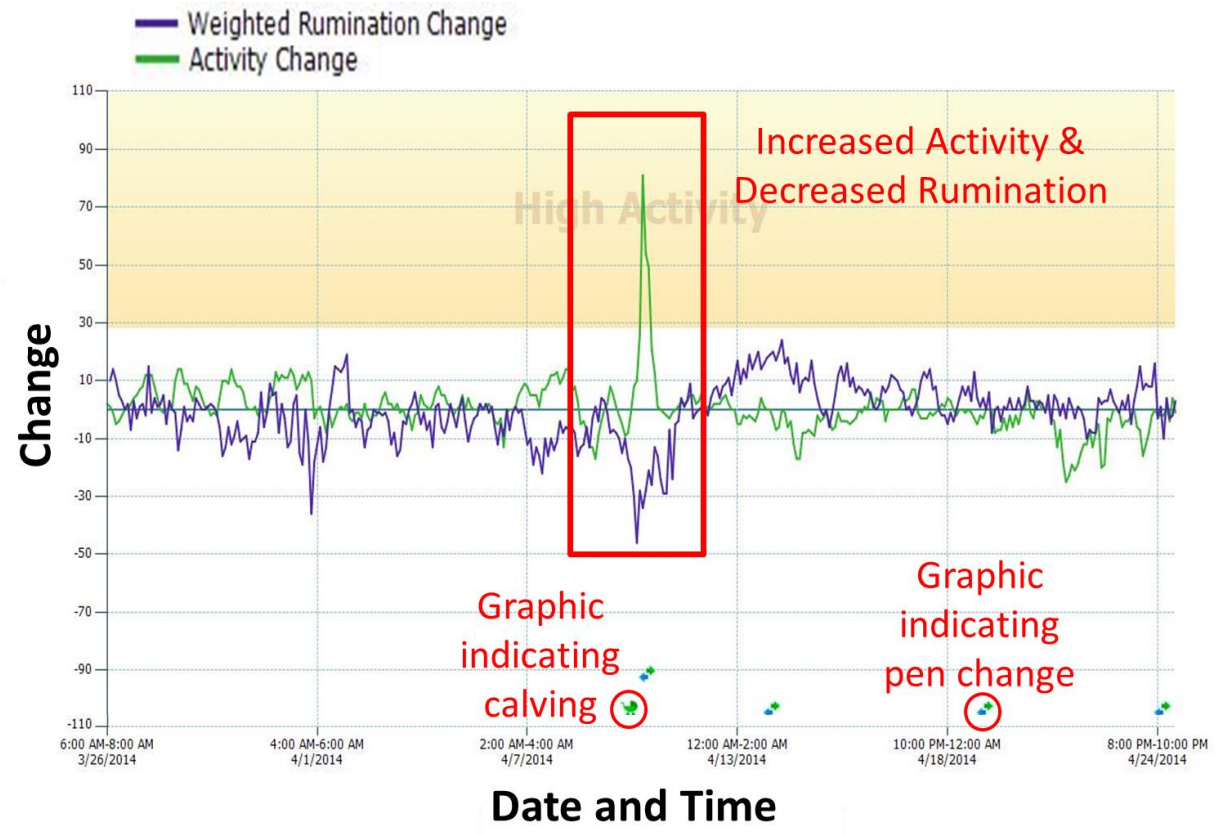

Figure A2-5. Rumination and physical activity data used for detection of calving, adapted from SCR Dairy.

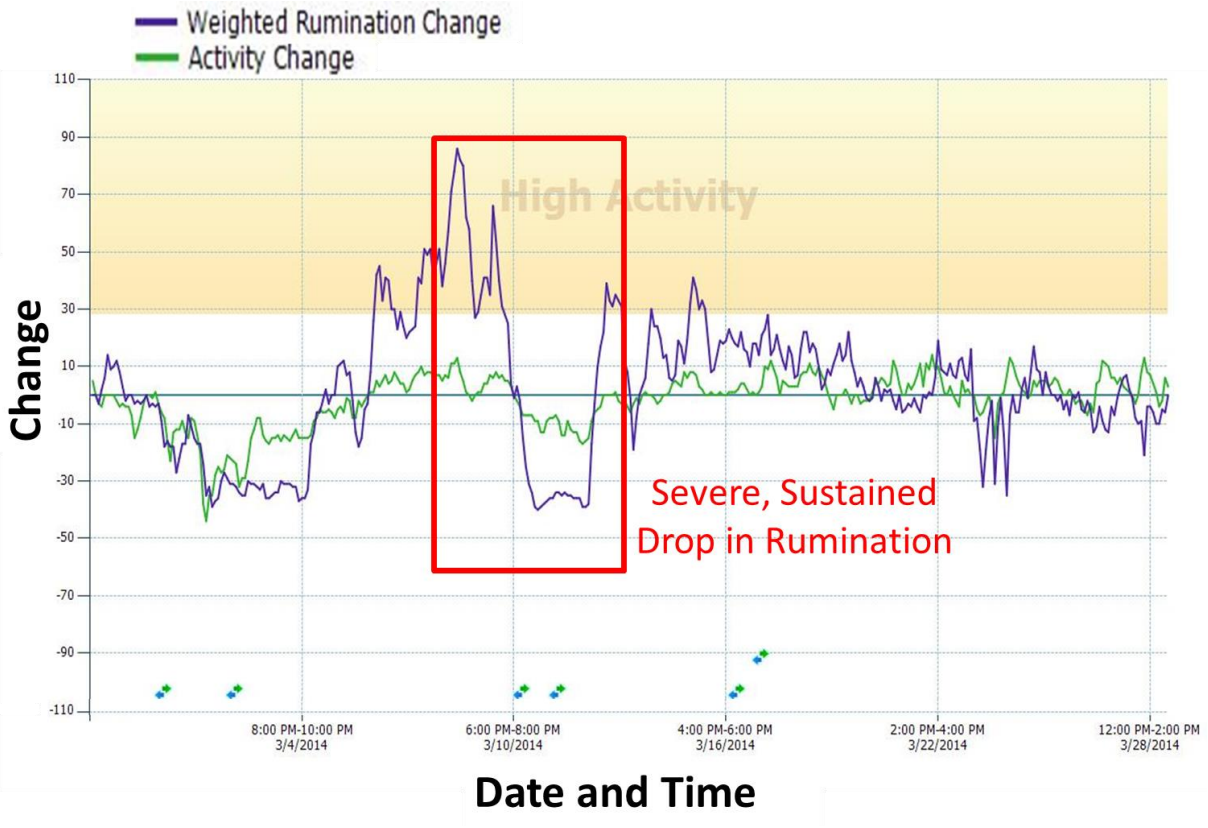

Figure A2-6. Rumination data used for early detection of sick cows, adapted from SCR Dairy.