THE PENNSYLVANIA STATE UNIVERSITY
SCHREYER HONORS COLLEGE

DEPARTMENT OF VETERINARY AND BIOMEDICAL SCIENCE

# Characterization of the daily rhythm of intake, rumination, and rumen pH in cows fed diets differing in starch and fiber concentration and fatty acid profile

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SPRING 2015

A thesis
submitted in partial fulfillment
of the requirements
for a baccalaureate degree
in Veterinary and Biomedical Sciences
with honors in Veterinary and Biomedical Sciences

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# ABSTRACT

The cow has a daily pattern of intake and rumination that causes a daily rhythm in rumen pH. It is expected that the daily pattern of intake and rumination is regulated by the physiology of the cow, the nutrient composition of the diet fed, and the environment and herd management. However, the effect of diet on these rhythms is not well characterized, as intake, rumination, and rumen pH are commonly reported as sums or averages over the day. Rumination, feed intake, and rumen pH data observed every 5 seconds over 4 days in two experiments using Holstein cows were analyzed to determine the effect of diet on the daily rhythms. Two experiments used a duplicated 4 x 4 Latin square design with a 2 x 2 factorial arrangement of treatments. Diets for the first experiment contained either brown midrib (BMR) or its isogenic normal control corn silage at two different concentrations of dietary neutral detergent fiber (NDF; 29% and 38%). The second experiment included diets of ground high-moisture (HM) corn or dry ground (DG) corn at two dietary starch concentrations (32% and 21%). A third experiment used a replicated 4 x4 Latin square design that examined the effect of saturated and unsaturated fatty acid supplements (FS). Treatments included a control diet with no added fat and a 2.5% added fatty acid supplement from a saturated FA, an unsaturated FA, or an intermediate mixture of the saturated and unsaturated FS. For the first two experiments, intake followed a similar daily rhythm regardless of corn type or starch and NDF level. Rumen pH was affected by NDF level for both experiments, with diets with low NDF resulting in lower rumen pH values than high NDF diets, but the daily rhythm was similar between treatments. The DG and HM corn diets did not differ in daily rumination, but BMR decreased rumen pH compared to the normal diets. Type of corn silage also did not significantly affect rumination behavior, but low NDF levels did result in less rumination throughout the day. The third diet showed no significant effect of supplementation of fat or supplement fatty acid profile on intake, rumination, and rumen pH behavior. Overall, intake, rumination, and rumen pH follow a daily pattern that is minimally modified by dietary fiber and starch type and level or fat level and fatty acid profile.

# TABLE OF CONTENTS

## ACKNOWLEDGEMENTS

Thank you to all those who provided support during my time at the Pennsylvania State University, especially those who helped make the completion of my thesis possible. I am extremely grateful for the help and opportunities Dr. Kevin Harvatine has given me over the three years I have had the privilege to work in his lab. Without him this thesis would not have been completed and I am very much indebted to him for his time and patience. I would also like to sincerely thank Jackie Ying, whose assistance and teaching has been invaluable and who can always be relied upon to solve any problem that is encountered. I also owe a sincere thank you to Dr. Lester Griel, who has helped guide my academic career and enabled me to succeed in my dream of attending veterinary school. Finally, I would like to thank my parents for their love and support throughout not only my time as an undergraduate, but my entire life. I am beyond grateful for their unwavering support, without which I would not have achieved many of my goals. Thank you for always being there for me.

# LIST OF FIGURES

# LIST OF TABLES

## ABBREVIATIONS

| | |
|---|---|
| BMR | Brown Midrib |
| DG | Dry Ground |
| DMI | Dry Matter Intake |
| FA | Fatty Acid |
| FS | Fatty Acid Supplement |
| HM | High Moisture |
| MFD | Milk Fat Depression |
| NDF | Neutral Detergent Fiber |
| pdNDF | potentially digestible Neutral Detergent Fiber |
| peNDF | physically effective Neutral Detergent Fiber |
| PSPS | Penn State Particle Separator |
| SARA | Subacute Ruminal Acidosis |
| TMR | Total Mixed Ration |
| VFA | Volatile Fatty Acid |

# Chapter 1

## INTRODUCTION

The daily rhythm of feed intake and rumination produces a daily pattern in rumen pH in dairy cows. These processes are closely related to health, wellbeing, and production in dairy cows, with the value of rumination a potential tool that can be utilized to gauge reproductive status, nutrition, and health. The regurgitation, remastication, and resalivation that characterizes rumination is an essential cyclical process that facilitates the digestion of the animal's ration through the reduction of forage particle size, buffering of rumen pH, and expediting of digesta passage. Factors that impede normal rumination are therefore detrimental to animal health and production. Environmental stress, disruptions to feed intake, and disease may all hinder the rumination process, with the potential to create severe problems (Gregorini et al., 2013).

By understanding the normal pattern of rumination and feed intake behavior in dairy cows and their consequent effects on rumen pH, more precise management may be possible. Deviations from normal behavior may be used as a method of early detection of illness, heat onset, and parturition (Calamari et al., 2014). In order to determine when an animal is diverging from normal conditions, a baseline of comparison needs to be established. While all cows have similar dietary requirements, they do not necessarily consume similar diets due to varying management practices. As a result, the impact dietary differences, such as starch and fiber level, in a ration have on rumination is of importance.

The objective of the thesis was to determine the daily rhythm of feed intake, rumination, and rumen pH in dairy cows through the analysis of feeding behavior data from three separate experiments that investigated the effect of diet starch, fiber, and fatty acid concentration and type. The hypothesis of the project was that the daily pattern of rumination depends on the type of diet a cow is fed. As commercial rumination monitoring systems become more readily available to producers, information on what is considered to be a normal daily pattern for rumination and what factors alter this normal pattern are expected to be of interest to nutritionists and dairy managers.

# Chapter 2

## LITERATURE REVIEW

### *Importance of Rumination*

Ruminants, which include dairy cows, possess a unique digestive system with four stomach compartments that allow specialized microbial fermentation and utilization of fiber that is not well digested by non-ruminant animals. The rumination process assists in the breakdown of difficult to digest fibrous materials through regurgitation of partially digested feedstuffs. During rumination, particle size of digesta is decreased, additional surface area is created for attachment of microbes, and saliva with important buffers is mixed with the digesta. Rumination also allows for rapid consumption of fibrous feed materials during meals that can then be broken down later at the animal's leisure (Ambriz-Vilchis et al., 2015).

The first two sections of the stomach, the reticulum and the rumen, are usually considered together, as they are adjoining compartments. The reticulum is the first section of the stomach, with a honeycomb-like structured wall that assists in the sorting of digesta. During rumination, the digesta in the regurgitated bolus originates from the reticulum. The adjacent rumen compartment is a much larger fermentation compartment with walls covered in papillae that increase surface area for absorption. The rumen is very large, holding between 40 and 60 gallons of material and taking up much of the left hand side of the abdominal cavity. Most fermentation and microbial digestion occurs in the rumen. The omasal compartment, also called the manyplies, consists of many muscular layers that increase surface area to allow efficient removal of excess water before the digesta enters the final stomach compartment, the abomasum. The abomasum is also called the true stomach since it is the location of the secretion of enzymes and hydrochloric acid, similar to the non-ruminant stomach, and has an acidic pH ranging from 2 to 4. From the abomasum, digesta continues on its path through the small intestine and large intestine, with the undigested material passing into the feces (Ishler et al., 1996).

The ruminant digestive system allows animals to quickly consume feed and then finish chewing and digestion at a later time. Ingested feed undergoes the process of pregastric fermentation within the rumen. Rumination occurs when this partially digested feed is then regurgitated, allowing size reduction of larger particles which increases the ability of microbes within the rumen to break down the fibrous materials (Ambriz-Vilchis et al., 2015). The regurgitated material, also called a bolus or cud, is then swallowed again. The digesta is continually mixed within the muscular rumen compartment, with smaller, denser particles on the bottom. Recently consumed material may form a floating mat in the rumen, providing some of the segregation of particles that require additional rumination (Ishler et al., 1996).

Microbes, such as bacteria, protozoa, and fungi, are essential to the fermentation process that occurs within the rumen of dairy cows. They break down fibrous materials such as cellulose and hemicellulose, in addition to starch and protein, and produce volatile fatty acids (VFAs) and high quality microbial protein. The microbial population within the rumen is greatly affected by the content of the diet and rumen pH. The VFAs produced are absorbed through the papillae on the rumen wall and are an essential component of ruminant metabolism. Microbes also biohydrogenate polyunsaturated acids and convert poor quality dietary protein or non-protein nitrogen into high quality microbial cell protein which can then be utilized by the animal.

Rumination ensures microbes have access to particles so that this process can take place (Mohammed et al., 2014).

Proper rumination is essential for dairy cows to maintain health and wellbeing. Rumination has been found to be at its highest when a cow is in a state of low stress. Excitement, anxiety and disease may inhibit the rumination process, further compounding the strain on the health of the animal. Additionally, rumination is an indication of the amount of digesta in the rumen and, thus an indication of the level of intake. For example, the rumen of a cow that is not eating will empty over time and cause rumination to decrease because there are no long particles present in the rumen that require rumination. As a result of these connections, monitoring rumination may be one of the easiest ways to quickly identify health issues for a cow. It may also be used as a method of detecting estrus in animals, allowing efficient reproductive management with less use of exogenous hormone protocols,, and can also be used to indicate when parturition is about to occur (Braun et al., 2013). Since rumination is a recurring process that tends to follow a daily pattern, it can serve as a fairly accurate estimate of health. A study that utilized rumination collars by Calamari et al., for example, suggested that a decrease in rumination time after parturition indicates a higher risk of disease and severe inflammation. Cows at risk of disease and complications may be identified sooner and treated more effectively when rumination time is continuously observed and compared to a baseline for that cow (Calamari et al., 2014). The potential exists to utilize this information in a commercial setting to prevent disease, promote wellbeing, and increase profitability, as a number of automated rumination observation systems have been commercialized.

***Rumen pH***

Rumen pH can vary between 5.8 and 6.4 over the day and depends on the balance of forage and grain in the animal's diet, cow specific factors, and feeding patterns. Most individual cows will vary approximately 1.2 pH units over the day. It is at this pH range that the microbial species essential to the ruminant digestive process are able to grow and function. If rumen pH deviates from normal, a number of potential problems may develop that can decrease productivity and severely impact the health of an animal. Organic acids are produced within the cow's rumen through the anaerobic fermentation process practiced by the rumen microbes. To mitigate this, a cow's saliva contains high amounts of sodium, phosphate, bicarbonate and other mineral ions that act as a buffer within the rumen, and assists in maintaining a rumen environment appropriate for microbial growth. Cows produce large amounts of saliva, potentially generating over 47.5 gallons per day. Saliva production is directly related to a ruminant's diet and the amount of time it spends chewing and ruminating (Ishler et al., 1996). It has been estimated that 30 to 40% of the neutralization of acid byproducts from fermentation is performed by salivary buffers (Yang and Beauchemin, 2006).

Decreased rumen pH can become a significant problem in dairy herds, often due to high rates of starch digestion. Starch, such as processed corn grain, may be fermented to VFAs very quickly in the rumen, surpassing the buffering capacity of the cow's saliva and resulting in a decrease in rumen pH. This decrease in pH may in turn suppress appetite, decrease fiber digestion, and reduce microbial activity (Mohammed et al., 2014). Ruminal acidosis occurs when rumen pH declines below the optimal range for fiber digestion by ruminal microbes. Subacute ruminal acidosis (SARA) is a widespread issue among dairy cows and one of the most important metabolic disorders to the industry due to its impact on cow health and productivity.

SARA is associated with feeding diets that contain high amounts of fermentable concentrate and forage with low physically effective fiber (peNDF). These types of diets are intended as a means of meeting a high yielding cow's metabolic demands and maximizing milk production, but they may also result in adverse effects (Yang and Beauchemin, 2006). The animal's buffering system is unable to compensate for the amount of acidic fermentation product produced, causing the drop in rumen pH associated with SARA (Palmonari et al., 2010). These changes to pH can in turn have detrimental effects on the microbial population within the rumen, inhibiting the growth of cellulolytic bacteria and increasing problems such as laminitis, milk fat depression (MFD), inconsistent feed intake, and other health problems (Yang and Beauchemin, 2006).

Rumen pH appears to follow a daily pattern over the day, with some variation between cows. Factors such as diet composition, including concentration of starch and NDF, the digestion rates of the starch and NDF, and management factors including the number of times the cows are fed per day, are all variables that may influence the pH of the rumen. In cows fed once daily, it has been observed that ruminal pH experiences a decrease for several hours when the cow spends more time eating and less time ruminating, and then increases as the day progresses and fermentation byproducts are absorbed or buffered by increased saliva flow as the cow spends more time ruminating (Palmonari et al., 2010).

### *Daily Pattern of Feed Intake*

Feed intake is closely related to production in dairy cows, as increased daily dry matter intake (DMI) is associated with higher milk production and milk component levels. Dairy cows eat to meet energy requirements, but while lactating their metabolic requirements may surpass the limitation of physical fill. In this case, cows may enter negative energy balance in order to compensate for the gap in available nutrients to meet lactation requirements. Rations that have a digestibility that is too low may limit feed intake and cause suboptimal production. Rations are often formulated to minimize negative energy balance by adding concentrates. If too much concentrate and not enough forage is in the diet, however, rumen pH may become lowered, production may decrease, and health may be adversely affected. A proper ration balance that encourages feed consumption over the course of the day is therefore very important for production. Total mixed ration (TMR) feeding has been adopted on dairies as it can increase the consumption of unpalatable feedstuffs and supplements in the ration and also provides a constant concentration of nutrients across the day (Ishler et al., 1996).

There are a variety of stimuli that encourage feeding activity, which may be used to create a more even consumption of feed over the course of the day. The delivery of fresh feed has been found to be a strong impetus to feeding activity, as well as, to a lesser extent, is returning from milking. Cows that are fed more frequently over the day tend to have a more even intake after each feeding and consume feed more evenly over the day. This more even intake has been suggested to lend to a more consistent rumen pH and a supply of nutrients to the microbes and to the cow that can optimize milk synthesis (Hart et al., 2014). Research studies utilizing automated feed-observation systems have noted an expected high feeding frequency during the daytime (between sunrise and sunset) and a less-active feeding period during the nighttime hours, especially from midnight to the next morning. Cows consume a series of meals throughout the day, with the period between meals used for resting and rumination (DeVries et al., 2003b). In an experiment by Hart et al. (2014), cows fed three times daily did not have a significant difference in milk yield, composition, or milk production efficiency when compared to cows fed once or twice daily, but an increase in DMI was seen (Hart et al., 2014). In contrast, recent work from Penn State showed that feeding four times per day in equal meals increased milk fat of cows with lower milk fat (3.0%) and decreased the amplitude of the daily rhythm of milk fat synthesis (Rottman, 2014). It does appear that feed intake is a daily rhythm that is entrained by environmental cues, such as the frequency or timing of feeding, and has impacts on the metabolism of the cow, but further work is needed to untangle the complex interactions involved.

Certain conditions may limit feed intake, in addition to simple physical fill. Climate may be a significant determinate for feed intake, with temperatures exceeding 65˚F and humidity surpassing 65% causing a drop in DMI, milk production, and milk fat levels. In contrast, cold weather will often result in an increase in feed consumption due to the increased energy required to maintain body temperature. Feed intake is directly related to the health and production of dairy cows, making a balanced ration that stimulates good DMI over the course of the day important (Ishler et al., 1996).

### ***What Changes Rumination Time over the Day?***

Feed intake and composition influences rumination in a number of ways. A cow cannot ruminate while it is eating; therefore, rumination only occurs between meals. This is also the time rumination is necessary to ensure adequate digestion. Cows require forage in their diets in order to stimulate rumination and the associated salivary secretions that contain buffers that stabilize rumen pH (Allen, 1997). Forage fiber particles that are being actively digested have low density due to fermentation gasses produced by the microbes attached to the particle and these particles make up the fiber mat in the rumen. The physical properties of the NDF of the cell wall dictate the rate of digestion and, thus, the buoyancy of the particles within the rumen and the rate of breakdown and passage of the particles. Chewing is stimulated by higher NDF , poorer quality fiber, and longer particle size, with rumination time increasing and greater time spent chewing (Schulze et al., 2014).

Ruminal fermentation of fiber is variable, depending on the digestibility of the fiber and environment in the rumen. Feeding a ration that is high in starch, potentially digestible neutral detergent fiber (pdNDF), or modifications of plant genetics or feed processing can greatly increase the rate of fermentation within the rumen (Allen, 1997). These highly fermentable feeds increase milk production but also result in an accumulation of VFA that exceeds the absorptive capability of the rumen, lowering ruminal pH and potentially resulting in health conditions such as SARA (Yang and Beauchemin, 2006). This process triggered by a low forage diet also reduces rumination time and salivary buffer secretion, further compounding the depression in ruminal pH and negatively impacting ruminal microbial communities (Mohammed et al., 2014).

Measures of fiber that are solely chemical in nature are not sufficient to balance diets of highly producing dairy cows, since fiber has a variable effect on chewing, in large part due to the diverse size of fibrous particles (Allen, 1997). Utilization of the Penn State Particle Separator (PSPS) is a simple and cost-effective way to estimate the particle size of TMR components like forage (Yang and Beauchemin, 2006). Reduction of forage particle size can increase DMI of high forage diets, especially when they are of low quality. Reduced particle size may also increase the rate of digesta passage, consequently reducing chewing time from rumination as a result. This reduction in chewing time as a result of forage particle size reduction may be decreased when forage quality is increased or concentrate is added to the diet. Since adequate chewing time is essential for maintaining rumen pH, forage particle size is a critical consideration in a ration due to its impact on rumination time (Woodford and Murphy, 1988). Secretion of saliva is increased when a cow chews during its eating and rumination time, so making adjustments to increase forage particle size could potentially improve low rumen pH levels and reduce the risk of ruminal acidosis (Yang and Beauchemin, 2006).

# Chapter 3

## MATERIALS AND METHODS

### Experiment 1: Effect of NDF level and digestibility

#### *Experimental Design and Treatments*

Feeding and ruminating behavior and rumen pH data from a previously published experiment by Oba and Allen (2000) were analyzed to determine the pattern of feed intake, rumination, and rumen pH over the course of the day. Briefly, eight multiparous Holstein cows (70 ± 7 DIM; mean ± SD) from the Michigan State University Dairy Cattle Teaching and Research Center were randomly assigned to duplicated 4 x 4 Latin squares balanced for carryover effects with a 2 x 2 factorial arrangement of treatments. Treatment factors evaluated were dietary NDF content at 29% (low) vs. 38% (high) and corn silage type of BMR vs. a normal isogenic control. All diets were fed once per day at 1200 h at 110% of expected intake. Cows were housed and milked in tie stalls for the duration of the experiment. Barn lights were turned off from approximately 2300 h until 0400 h each day and the experiment was conducted from late January until mid-April (Oba and Allen, 2000).

### *Data Collection and Statistical Analysis*

Cows were housed in a feed intake observation system described by Dado and Allen (1993). Briefly, hanging feed tubes suspended from an electronic load cell observed feed weight, a halter equipped with a pressure sensitive nose-piece observed chewing, and an indwelling pH probe observed rumen pH in the ventral sac. The sensors were wired to a computer data acquisition system and feed weight, chewing activity, and ruminal pH were recorded every 5 s from d 12 through 16 of each period. Raw feed weights were smoothed by averaging over 180 s and the rate of feed intake (percent of daily intake per hour) were calculated every 5 s over 10 min intervals and averaged over 2 h intervals. Rumination was defined as chewing with no feed disappearance. Rumination and rumen pH were averaged over 2 h intervals. Data was statistically analyzed as a 2 x 2 factorial design using the Proc Mixed procedure of SAS with repeated measures (version 9.3, SAS Institute Inc., Cary, NC). The model included the random effect of cow and period and the fixed effect of NDF level, corn silage type, and their interaction.

## Experiment 2: Effect of starch level and digestibility

### *Experimental Design and Treatments*

Feeding behavior and rumen pH data from a previously published experiment by Oba and Allen (2002) were analyzed to determine the pattern of feed intake, rumination, and rumen pH values over the course of the day. Eight multiparous Holstein cows (55 ± 15.9 DIM; mean ± SD) from the Michigan State University Dairy Cattle Teaching and Research Center were randomly assigned to duplicated 4 x 4 Latin squares balanced for carryover effects with a 2 x 2 factorial arrangement of treatments. Treatment factors evaluated were dietary starch concentration at 21% (high) vs. 32% (low) and corn grain type [high moisture (HM) vs. dry ground (DG) corn]. All diets were fed once per day at 1400 h at 110% expected intake. Cows were housed and milked in tie stalls for the duration of the experiment. Barn lights were turned off from approximately 2300 h until 0400 h each day and the experiment was conducted from mid-March until mid-June (Oba and Allen, 2002).

### *Data Collection and Statistical Analysis*

Cows were housed in a feed intake observation system similar to Dado and Allen (1993) as described above in Experiment 1. Feed intake, rumination, and rumen pH were similarly averaged over 2 h intervals. Data was statistically analyzed as a 2 x 2 factorial design using the Proc Mixed procedure of SAS with repeated measures (version 9.3, SAS Institute Inc., Cary, NC). The model included the random effect of cow and period and the fixed effect of starch level, corn grain type, and their interaction.

## Experiment 3: Effect of fat level and fatty acid profile

### *Experimental Design and Treatments*

Feeding behavior and rumen pH data from a previously published experiment by Harvatine and Allen (2006) were analyzed to determine the pattern of feed intake, rumination, and rumen pH values over the course of the day. Eight multiparous Holstein cows (77 ± 8.7

DIM; mean ± SD) from the Michigan State University Dairy Cattle Teaching and Research Center were randomly used in a replicated 4 x 4 Latin square designed experiment in which cows were randomly assigned to treatment sequence. Treatment included a low fat control and a 2.5% added fatty acid supplement as saturated (prilled FFA, Energy Booster-100, Milk Specialties Inc., Eden Prairie, MN), an intermediate mixture of saturated and unsaturated (50-50), and an unsaturated fat supplement (Calcium salts of FA; Megalac-R, Church and Dwight Inc.). All diets were fed as a TMR once per day at 0900 h at 115% expected intake. Cows were housed and milked in tie stalls for the duration of the experiment. Barn lights were turned off from approximately 2300 h until 0400 h each day and the experiment was conducted from mid-March until mid-June (Harvatine and Allen, 2006).

***Data Collection and Statistical Analysis***

Cows were housed in a feed intake observation system similar to Dado and Allen (1993) as described in Experiment 1. Feed intake, rumination, and rumen pH were similarly recorded every 5 s from d 16 through 19 of each period and were averaged over 2h intervals. Data was statistically analyzed using the Proc Mixed procedure of SAS with repeated measures (version 9.3, SAS Institute Inc., Cary, NC). The model included the random effect of cow and period and the fixed effect of treatment.

**Table 1: Summary of experimental treatments**

| | Treatment | | | |
|---|---|---|---|---|
| **Experiment 1** | NDF Level | | Corn Silage | |
| | Low (29%) | High (38%) | BMR | Isogenic Control |
| **Experiment 2** | Starch Level | | Corn Grain | |
| | Low (21%)<br>High NDF | High (32%)<br>Low NDF | Dry Ground | High Moisture |
| **Experiment 3** | Fatty Acid Supplementation | | | |
| | Control (NoFat) | Saturated | 50-50 | Unsaturated |

# Chapter 4

## RESULTS

### *Experiment 1*

There were no three-way interactions of NDF level, corn silage type, and time of day, but two way interactions of NDF level and time and corn silage type and time were observed. The analyzed data was able to evaluate the effect of fiber level and digestibility on feed intake, rumination, and rumen pH, the results of which are shown in Figure 4.1. A similar pattern over the day was observed, regardless of treatment. There was an effect of time of day on DMI intake over the day as a percent of daily intake per hour, but no diet by time of day interactions (Figure 4.1A and B). There was an interaction of dietary NDF concentration and time of day for rumination activity ($P$ = 0.02; Figure 4.1C). Decreasing dietary NDF decreased rumination across nearly the entire day, but there was a larger effect from approximately 1230 h until 2030 h (Figure 4.1C). Type of corn silage, however, had no effect on rumination and did not interact with time of day (Figure 4.1D). There also was an interaction of diet NDF concentration and time of day for rumen pH ($P$ = 0.01; Figure 4.1E). Similar to rumination, lower NDF levels reduced rumination across the entire day, but there was a larger difference after 1630 h. Lastly, BMR corn silage decreased rumen pH ($P$ = <0.01), but there was no treatment by time interaction (Figure 4.1F).

***Experiment 2***

There were no three-way interactions of starch level, corn grain type, and time of day, but two-way interactions of starch level and time of day were observed (Figure 4.2). An effect of time was observed for feed intake, rumination, and rumen pH. There was no effect of starch level or type of corn grain observed for DMI as a percentage of daily intake over the day, and no treatment by time interaction was observed (Figure 4.2A and B). There was a starch level by time of day interaction ($P$ = <0.01) for rumination activity over the day. High starch (low NDF) diets were observed to decrease rumination activity more between 0030 to 0830 h (Figure 4.2C). However, type of corn did not affect the pattern of rumination over the day (Figure 4.2D). NDF level impacted rumen pH ($P$ = 0.002), but no NDF level by time of day interaction was observed as diets low in NDF decreased rumen pH over the entire day (Figure 4.2E). Type of corn grain had no effect on rumen pH, although DG corn had a lower pH value at 1750 than HM corn, giving it a lower minimum pH value ($P$ = 0.02; Figure 4.2F).

***Experiment 3***

Supplementation of fat and the concentration of unsaturated fatty acid of fat supplements had no significant effect on the daily rhythm of intake, rumination, and rumen pH behavior (Figure 4.3). There were no two-way interactions of fat supplement treatment and time of day for DMI as a percent of daily intake, rumination over the day, or rumen pH over the day.

Additionally, there was no main effect of treatment observed, but an effect of time was observed for feed intake, rumination, and rumen pH.

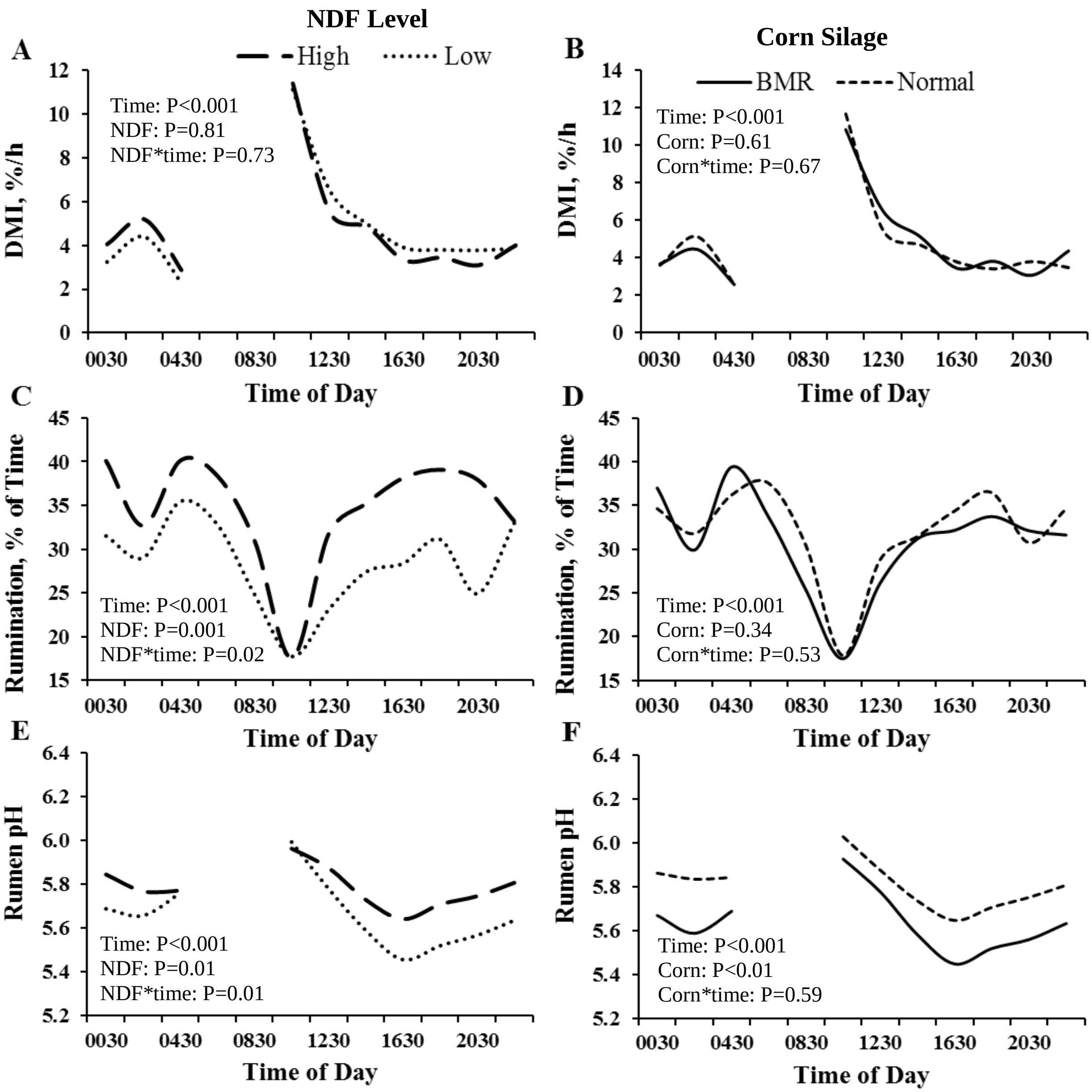


**Figure 4.1: Effect of fiber level and digestibility on feed intake, rumination, and rumen pH (Exp. 1).** Dietary NDF was 29% (low) and 38 % (high) and corn silage was either isogenic normal or brown mid-rib (BMR). Data was collected every 5 s from d 12 through 16 of each period.

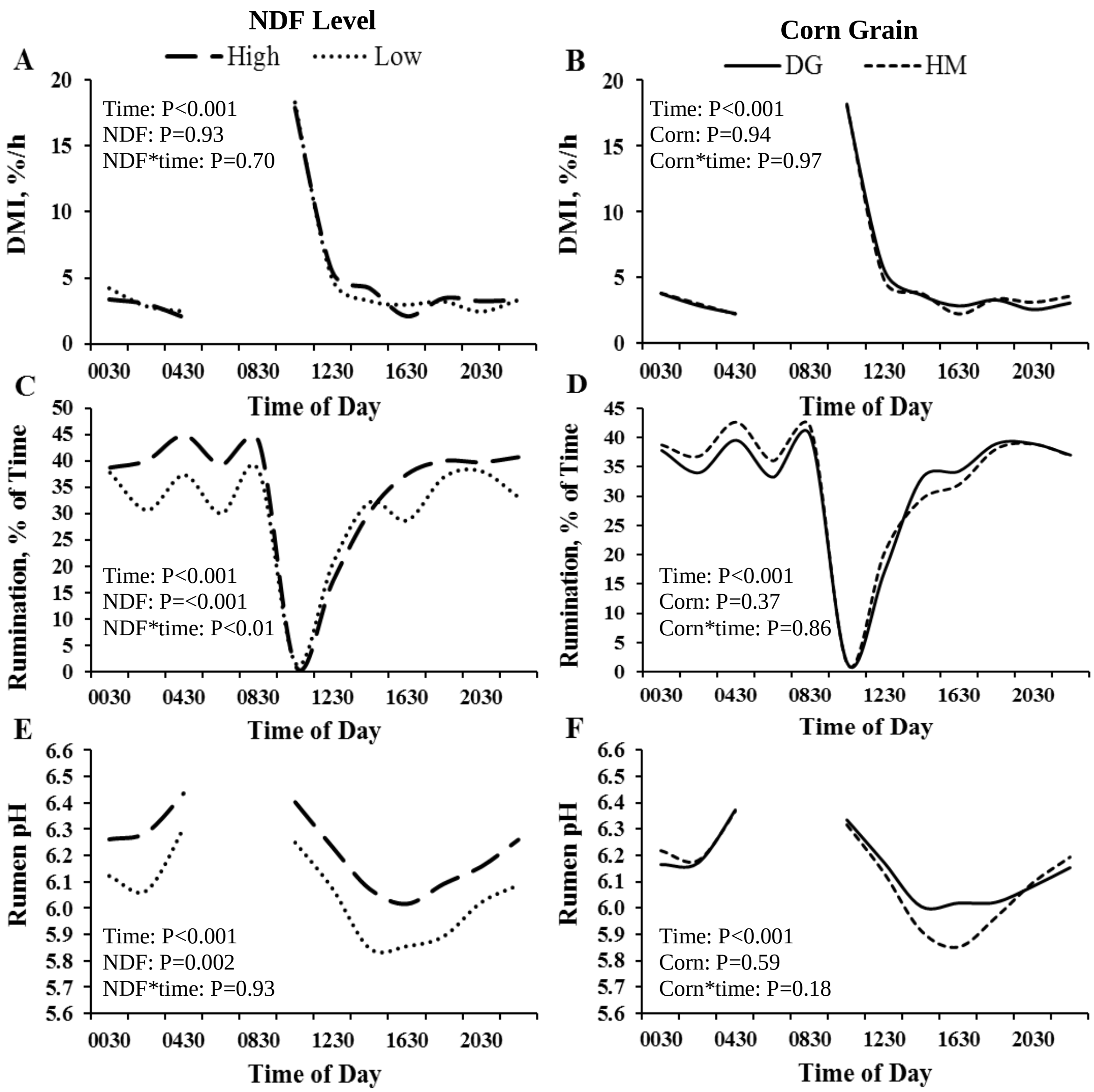


**Figure 4.2: Effect of starch source and level on feed intake, rumination, and rumen pH (Exp. 2).** Dietary starch was at 21% (low starch; high NDF) and 32% (high starch; low NDF) and corn grain was either high moisture (HM) or dry ground (DG). Data was collected every 5 s from d 12 through 16 of each period.

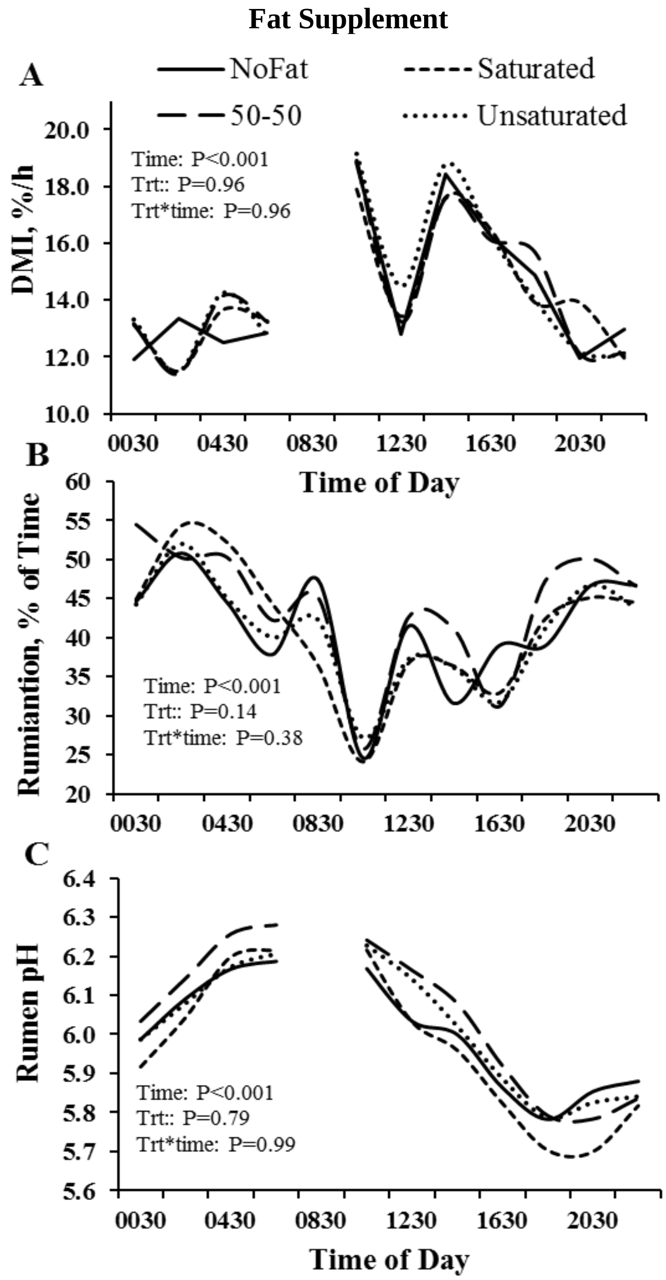


**Figure 4.3: Effect of fat level and fatty acid profile on feed intake, rumination and rumen pH (Exp. 3).** Treatments included a low fat control (No Fat), and 2.5% added fatty acid supplements that were saturated, unsaturated, or an intermediate mixture of saturated and unsaturated (50-50). Data was collected every 5 s from d 16 through 19 of each period.

# Chapter 5

## DISCUSSION

Feeding behavior data from three separate experiments was effectively used to ascertain if the daily pattern of feed intake, rumination, and rumen pH over the course of the day is significantly impacted by dietary starch, fiber, or fatty acid profile in dairy cows. A daily rhythm was observed for feed intake, rumination, and rumen pH in each experiment, as indicated by an effect of time apparent for each. Intake was not altered by dietary fiber and starch type or fatty acid profile. Rumination was impacted by NDF level, with lower NDF diets resulting in less rumination throughout the day. Corn silage, corn grain, and fatty acid profile did not impact rumination over the entire day. Low NDF level and BMR corn silage was observed to decrease rumen pH over the day, but type of corn grain and type of FA did not. It was found that decreasing dietary fiber and increasing starch decreased rumination across the entire day, but had a larger impact during the evening hours.

Intake behavior for all three experiments followed a similar rhythm over the course of the day, as shown in Figure 4.1A and B, Figure 4.2A and B, and Figure 4.3A and B. DMI as a percentage of daily intake over the day is at its highest immediately after delivery of new feed, prompting a conditioned meal. Additionally, the majority of feed consumption occurred between dawn and dusk, in concordance with the findings of previous feeding behavior reports (DeVries et al., 2003a). Experiments 1 and 2 experienced a steady decline in the rate of intake after the conditioned feeding when spontaneous feeding occurred. Additionally, the experiment showed low rates of intake during the overnight hours, with only Exp. 1 showing an increase in intake activity around 0300 h. Exp. 3 has similar activity, but showed an increase in the rate of intake in the afternoon around 1500 h, which was not seen in Exp. 1 and 2. Regardless of corn type, starch level, NDF level, and fatty acid profile, intake followed a similar daily rhythm. The consistency of this rhythm regardless of diet implies that the daily pattern of intake is not highly driven by the temporal pattern of nutrient absorption and metabolism, but by an internal circadian timekeeping mechanism responsive to other environmental factors such as lighting schedule. Milking schedule could also have an impact as all cows were milked at similar times.

Rumination behavior over the day followed a daily pattern over the day for all three experiments (Figure 4.1C and D; Figure 4.2C and D; Figure 4.3C and D). Rumination was generally opposite to the feeding activity due to the fact that time spent eating is time that cannot also be occupied with rumination. Therefore, rumination is at its lowest when a new meal has just been fed (Schirmann et al., 2013). After the meal occurs, a steady increase in time spent ruminating is apparent. This time spent ruminating allows the cow time to further break down large particles recently consumed in the meal, facilitating the digestive process. Overnight rumination was also observed, with the highest amount of rumination occurring from 0030 to 0830 h. Amount of rumination was not just influenced by the time of day and meals, but also was affected by NDF level in the diet. Low NDF and high starch diets were found to lower the amount of rumination throughout the day, particularly during the overnight rumination period for both Exp. 1 and 2. Experiment 2 also had a significant reduction in rumination during the afternoon and evening hours between 1230 and 2030 h. Corn grain and silage type, nor fatty acid concentration and profile altered rumination, suggesting that these are not dietary aspects that alter chewing and rumination behavior.

Rumen pH was less variable over the course of the day than rumination and feed intake, since a relatively stable rumen pH is essential to maintaining a cow's digestive health (Figure 4.1E and F; Figure 4.2E and F; Figure 4.3E and F). However, it can be seen that shortly after active feeding periods rumen pH decreases, with the lowest pH at or shortly after 1630 h. Low NDF and high starch in the diet decreased the overall rumen pH throughout the entire day in both Exp.1 and 2, with low starch, high NDF diets having a higher rumen pH over the day. Since starch is rapidly fermented in the rumen, organic acid byproducts are quickly produced at a rate faster than the cow's buffering capacity can neutralize them, resulting in the lower rumen pH, especially shortly after meals. High NDF encourages chewing, increasing the availability of buffering saliva, while also having a lower degree of fermentability. BMR corn was also found to lower the pH of the rumen throughout the day.

The data supports that certain dietary factors impact DMI, rumination, and rumen pH over the day in dairy cows more than others. The smaller particle size of low NDF, high starch diets likely causes a faster passage rate of digesta and less of a need for regurgitation and rumination as a means of further particle breakdown. The rapid fermentation of these smaller particles in the rumen is likely therefore responsible for the decrease in rumen pH accompanied by the reduced time spent ruminating that occurred in Exp. 1 and 2. Likewise, BMR corn is more highly digestible than its isogenic normal control, and therefore is highly fermentable in the rumen causing the increase in pH, with an effect similar to a high starch or low NDF diet (Ferraretto and Shaver, 2015). The absence of a reduction in BMR diet rumination time may potentially be attributed to initial particle size of the BMR and control corn being about equal. Other dietary factors, such as FA profile and corn grain type did not appear to impact the intake, rumination and rumen pH behavior of dairy cows.

Feed intake was at its highest from dawn until dusk and rumination was generally at its highest during the overnight hours. The amount of light each day was the same for all cows in the three experiments, with the tie-stall housing having the lights turned off from approximately 2300 h at night until 0400 h in the morning when milking started. Cows have been known to experience a diurnal grazing pattern when kept on pasture, with their most active feeding behavior occurring at sunrise and sunset (Albright, 1993). Grazing bouts take place predominantly during daylight hours, but some grazing during darkness does still occur (Sheahan et al., 2011). However, the effect of day length has not been studied in cows housed in confinement, which are not as exposed to the patterns of light dictated by the sun as cows on pasture. More research would have to be conducted to determine to what extent day length influences intake and rumination on cows housed in confinement.

This research confirms that feed intake, rumination and rumen pH follow a daily pattern in dairy cows, which has many applications for research in commercial settings since feed intake, rumination, and rumen pH are often indicators of health. Rumination behavior, in particular, can be used to judge the health, nutrition, and reproductive status of a cow, making this data applicable in establishing a baseline for what is normal rumination behavior in consideration of dietary variations. These daily patterns should be incorporated into the interpretation of data collected by commercial rumination collars as a means of reducing error and increasing the precision of making observations and management decisions based off of these observations. Future directions could include determining the extent of the effect of cow specific factors other than diet to the baseline of rumination behavior.

# Chapter 6

## CONCLUSION

The objective of characterizing the daily rhythm of intake, rumination, and rumen pH over the course of the day and determining the effect of diets differing in starch and fiber concentration and fatty acid profile was completed. Intake, rumination, and rumen pH were found to follow a daily pattern that is minimally modified by dietary fiber and starch type and level. Feed intake over the day was not affected by the treatment diets. Low NDF, high starch diets were found to decrease both rumination time and rumen pH over the day, but a larger effect was observed in the evening hours. BMR corn did not affect rumination, but decreased rumen pH. DG and HM corn grain diets as well as fatty acid supplementation was not found to alter feed intake, rumination, or rumen pH.

# Chapter 7

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