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# Evaluation of Effects of Methods Used for Forage Analysis and Dietary Inclusion of Buffer on Fiber Digestibility and Rumen Fermentation in Diets High in Distillers Grains

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# EVALUATION OF EFFECTS OF METHODS USED FOR FORAGE ANALYSIS AND DIETARY INCLUSION OF BUFFER ON FIBER DIGESTIBILITY AND RUMEN FERMENTATION IN DIETS HIGH IN DISTILLERS GRAINS

BY

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This thesis is approved as a creditable and independent investigation by a candidate for the Master of Science in Biological Sciences with a specialization in Dairy Science degree and is acceptable for meeting the thesis requirements for this degree. Acceptance of this thesis does not imply that the conclusions reached by the candidate are necessarily the conclusions of the major department.

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# LIST OF ABBREVIATIONS

| | |
|---|---|
| ADF | Acid detergent fiber |
| ADG | Average daily gain |
| ADIA | Acid detergent insoluble ash |
| BCS | Body condition score |
| BW | Body weight |
| CCS | Conventional Corn silage |
| CHL | Conventional Haylage |
| CP | Crude protein |
| CV | Coefficient of variation |
| DDG | Distillers dried grains |
| DDGS | Distillers dried grains with solubles |
| DM | Dry matter |
| DMI | Dry matter intake |
| DWGS | Distillers wet grains with solubles |
| EE | Ether extract |
| FA | Fatty acids |
| HCS | Hybrid Corn Silage |
| HHL | Hybrid Haylage |
| $K_2$EDTA | potassium ethylene diamine tetra-acetic acid |
| Mcal | Mega calories |
| ME | Metabolizable energy |
| Mg | Magnesium |
| NaFl | Sodium fluoride |
| NDF | Neutral detergent fiber |
| NFC | Non-fibrous carbohydrate |
| $NE_g$ | Net energy gain |
| $NH_3$ | Ammonia |

| | |
|---|---|
| OM | Organic matter |
| PSI | Pressure per square inch |
| PUN | Plasma urea nitrogen |
| RDP | Rumen degradable protein |
| RUP | Rumen Undegradable protein |
| SEM | Standard error of the mean |
| TMR | Total mixed ration |
| VFA | Volatile fatty acids |
| WCCS | Wet Conventional Corn Silage |
| WCHL | Wet Conventional Haylage |
| WHCS | Wet Hybrid Corn Silage |
| WHHL | Wet Hybrid Haylage |
| wk | week |

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# ABSTRACT

## EVALUATION OF EFFECTS OF METHODS USED FOR FORAGE ANALYSIS AND DIETARY INCLUSION OF BUFFER SUPPLEMENTS ON FIBER DIGESTIBILITY AND RUMEN FERMENTATION

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Forage quality is an important factor affecting intake and utilization of forage, thus making it imperative to evaluate the methods used to determine the nutritive values of forage in order to predict animal performance. Additionally, fibrous feeds for ruminants are less subject to competitive demand. The plant cell wall is the largest hindrance to complete digestion of feeds, particularly forages and by-products and to the utilization of the nutrients and energy they contain, necessitating effective strategies for increasing the rate and efficiency of utilization of forage fiber and the energy therein. It is critically important to increase fiber digestion for productivity and environmental reasons.

Two studies were conducted in an attempt to make recommendations based on the methods used for forage analysis and rumen fermentation of dietary inclusion of buffer in diets high in distillers grains. The first study compared five forages: Hay (**Hay**), Conventional Corn Silage (**CCS**), Conventional Haylage (**CHL**), Hybrid Corn Silage (**HCS**), and Hybrid Haylage (**HHL**) in an *in situ* (dry/wet) and *in vitro* trials for differences in dry matter and fiber degradability. Results showed different methods vary in digestibility values, but difference among forage followed similar patterns among method. Further research will be warranted to standardize procedures to be used for methods to evaluate forage quality.

The second study evaluated the effects of High buffer (**HiBuffer**) and Low buffer (**LoBuffer**) inclusion on nutrient digestibility, rumen parameters, and blood metabolites in steers limit-fed diets high in distillers grains. Five dairy cannulated steers (Brown Swiss and Holstein) 303.4± 45 d of age were used in a cross over design experiment within a 2-week period. Two treatment diets containing 40% DDGS with High (**HiBuffer**) or Low (**LoBuffer**) buffer inclusion concentrations were fed. Results show differences in DMI and G:F, while BW and ADG were similar among treatments. The rumen total VFA, acetate: propionate, and pH were similar among treatments. For blood metabolites there were treatment effects for glucose and cholesterol, while plasma urea nitrogen concentrations were similar among treatments. Total tract digestion of nutrients was similar among treatments. Result demonstrates that buffer inclusion had limited impact on utilization of DDGS. However, future research is warranted to determine the precise amount of buffer inclusion and DDGS feeding rate.

# INTRODUCTION

Nutrient consumption and digestibility are estimated to be the closest approximation to the real nutritional value in feed (Romero et al., 2014). The knowledge of the nutritional value of feed and its balance in ration formulation is a fundamental tool to meet animal needs, so as to express their genetic potential for production. Currently, there are varieties of forages that may be used in ruminant nutrition and most especially in dairy production. However, their nutritional value and quality are determined by the complex interaction between nutrients and the microorganisms in the digestive tract, digestive process, absorption, transport, and metabolite use, apart from the animal's own physiological condition.

Historically, forages have been the major and most economical source of energy in ruminant diets. Fiber is unusual in that the nutritional concept of fiber is based on biological attributes. Therefore, the amount of available energy in forages has been the most important nutritional information expected from forage evaluation (Mertens, 2000). The true nutritive value of forage is related to the performance of the animal that consumes it. Thus, forage quality is determined not only by the amount of nutrients it contains, but also by feed-animal interactions associated with intake, digestibility, and metabolic efficiency (Mertens, 2000). Chemical and physical analyses can accurately measure nutrient and energy contents, but there are no laboratory methods to directly measure the potential intake, digestibility, and metabolic efficiency of the nutrients. Unfortunately, intake, digestion and utilization by the animal cannot be measured consistently. The search for tools for determining nutritional value of forages in formulating efficient rations has led to ongoing investigations on the dynamics of fermentation and digestion of forages in processes related to production in animal nutrition.

In addition, it has been estimated that about 50-75% of the differences in productive response of forages is related to intake, 25-50% is related to digestibility, and 5-15% is related to metabolic efficiency (Mertens, 2000). The digestibility coefficient of forage is important to indicate the real available nutrients in ration for the animal, apart from describing the nutritional value, because this will show how much of the nutrients the microorganisms in the rumen actually use, which helps to express the capacity of the animal to use each nutrient in a higher or lower scale (Romero et al., 2014).

Co-products of the food and biofuels industry are also sources of fiber and there is limited research on how it is utilized by dairy cattle. Some of the energy derived from distillers dried grains with solubles (DDGS) is digestible fiber which complements the fiber in forages. Availability of distillers dried grains with soluble has increased substantially, thereby an interest in using these feeds in cattle diets has also increased. Feeding DDGS is a viable option for growing dairy heifers and steers to provide supplemental rumen undegradable protein and energy, with equal performance with competitive feeding grain alternatives (Manthey et al., 2016; Anderson et al., 2015). Typically, the primary carbohydrate fraction in DDGS is NDF and feeding large amounts of DDGS will increase the concentration of NDF in the diet. Due to particle size considerations it is likely that with a higher inclusion amount of DDGS, the ability of DDGS to replace the effective fiber of forages will be limited (Manthey et al., 2017), which can be problematic for normal rumen function. Additionally, DDGS has a low pH due to its sulfur content which can cause further issues with rumen fermentation. Therefore, interest and research into buffer supplements inclusion when diets high in distillers dried grains with soluble are fed to growing heifers and steers was conducted.

Our hypothesis for the first study was that different methods will vary in digestibility result but differences among feedstuffs will follow similar patterns among

methods. The hypothesis for the second study was that different amounts of buffer inclusion in limit-fed diets high in distillers dried grains will have different effects on rumen fermentation profile and nutrient utilization.

# CHAPTER 1:

# LITERATURE REVIEW

Forages typically account for 40-100% of the ration of dairy cows and are vital for maintaining animal productivity and health (Adesogan et al., 2019).Variation in the digestibility of forage fiber affects the concentration of energy in the diet, the energy available for microbial protein synthesis in the rumen, and dry matter intake (DMI) of ruminants. Therefore, it is an important factor affecting ruminant productivity. However, increasing fiber digestibility will increase the energy density of diets, which will provide more energy to ruminal microbes and result in higher DMI (Allen, 1997). But, current empirical feed evaluation systems are unable to address the complex interrelationships in the rumen following the ingestion of certain feeds. These systems aim to match nutrient requirements with nutrient intake, but are not suitable to predict the responses to dietary changes in terms of product composition, digestion of nutrients and excretion of waste end-products to the environment. Thus, change from a requirement to a response performance requires prediction of the profile of absorbed nutrients and its subsequent utilization.

## Importance of Forage Quality

Forages are necessary components of diets for ruminant animals and most especially in the dairy industry because they provide coarse fiber needed to optimize rumen function. Fiber has been defined as the slowly digesting fraction of forage that occupies space in the gastrointestinal tract (Allen, 2000). Fiber values reflect the energy content of forages, and dairy cows require tremendous amount of energy for growth and physiological activities. Most nutritionists consider the energy value of forages to be more important as forage is typically the highest expenditure in dairy production (Amaral-Phillips, 2001). However, the cheapest energy source is that provided through

the forage portion of the diet. So, forage is the foundation of most cattle diets (Blezinger, 2002), thereby considering and regarding forage is the backbone of dairy production.

Forage quality is a broader term that not only includes nutritive value but also digestibility and subsequent forage intake. The forage quality has a direct impact on the amount of each nutrient that the forages can supply in a balanced ration, which helps to best utilize available forages in dairy operation. But, because forage quality is highly variable, their quality must be assessed before diets are formulated due to their direct effect on diet formulation for consistent productivity in dairy cows (Oba and Allen 2003). Therefore, forage analyses are extremely helpful and valuable tools that can be used to assess management practices and provide a quantitative measure of nutritive value. Forage analysis provides many of the inputs needed to formulate rations, so that the forage is used effectively (Mertens, 2000).

Furthermore, the animal's productive response is not only a function of the amount of nutrients and energy in the forage, but also the animal's intake, digestibility and metabolic efficiency. However, the ultimate response of forage quality is the performance of the animal that consumes it. The difficulty of separating forage intake potential from the actual intake that is obtained by animals with specific energy demand is one of the reasons that intake can be neglected as a factor in forage quality, even though it is the most important factor affecting animal performance.

## Biochemical and Physiological Challenge of Forage Digestibility

It appears that the major biochemical and physiological factors limiting digestion are associated with the plant cell wall or NDF in the forage. Since, the factors that limit digestion reside in fiber, it is logical that fiber fractions are most highly correlated with digestibility measurements, thus predicting DM digestibility is essentially estimating NDF digestibility. Also, most fiber digestion (70-100%) occurs in the rumen (Mertens,

2000), and for consistency among forage testing, acid detergent fiber (ADF) and neutral detergent fiber (NDF) content can be use to evaluate the quality of forage. Consequently, it should be evident that ruminal digestion can measure digestibilities with greater accuracy.

The ADF content reflects the digestibility and amount of energy that can be obtained from the forage, and the NDF content reflects the potential intake of this forage. As the fiber content increases (both ADF and NDF), the digestibility, energy content and potential forage intake decreases, therefore the changes in this fiber content ultimately affects production and performance (Allen, 2000).

## Fiber in Forage and Feeds

Forage is extensively used in dairy production because they provide coarse fiber needed to optimize rumen function. Forages are made up, predominantly of different types of fiber with certain types of fiber being more digestible than others. The high fiber content of forages is the main nutritional factor that differentiates them from concentrate (Adesogan et al., 2019). Fiber plays a fundamentally important role in ruminant livestock production, health and welfare. In addition to being an important energy source, it stimulates chewing and salivation, rumination, gut motility, regulates feed intake and is the structural basis of the scaffolding of the ruminal mat, which is vital for digestion of solid feed particles in the rumen. Fiber was defined as the undigested bulk in feed or fraction of plants that cannot be digested by mammalian enzymes (Allen et al., 2000). These fibers are found primarily in the cell walls of plants which together create a physical matrix that provides structure to the plant. Within the fiber matrix and within the plant cells are other nutrients such as proteins, fat, minerals and vitamins. In order to access these nutrients, the fiber component has to be broken down. The more digestible or degradable the fiber component is, the more accessible the nutrients will be to the

animal for digestion. Forage fiber is composed of complex carbohydrates including cellulose, hemicellulose, and pectins, as well as lignin.

*Cellulose*

Cellulose is associated with hemicellulose by hydrogen bonds (Morrison, 1979) and is often the most abundant component of the plant cell wall, comprising 10-45% dry matter (DM) (Giger-Reverdin, 1995; Saha, 2003). It is made up of repeated linear chains of glucose monomers. Glucose molecules are linked together in a $\beta$ 1,4 linkages. Only microbial enzymes can digest the $\beta$ 1,4 linked glucose in cellulose. In the plant, cellulose chains are highly structured within the secondary cell wall where they densely packed into microfibrils, but in the primary cell wall cellulose chains run in random directions (O'Sullivan, 1997).

*Hemicellulose*

Hemicellulose is closely associated with lignin that has a strong negative influence on fiber digestion. They are comprised of 10-25% dry matter (DM) of forages and up to 50% of the lignocellulosic biomass (Saha, 2003) in their free form. They are the most accessible and easily digested components of fiber because of their amorphous organization. Hemicellulose is also dependent on microbial enzymes for digestion because it has a complex structure made primarily of xylose that also has $\beta$ 1,4 links.

*Lignin*

Lignin is not a carbohydrate but a polyphenolic substance that is resistant to fermentative degradation and provides no nutritive value to the animal. It is the third most abundant component of fiber with many different molecules and bonds, thus making it very difficult to enzymatically degrade. Lignin can be classified as core lignin and noncore lignin. Core lignin is composed of highly condensed cinammyl alcohol

polymers (Grisebach, 1981) and is the component extracted in acid detergent lignin. Noncore lignins are comprised of p-coumaric acids, which are largely associated with core lignin and ferulic acids, which act as a bridge between lignin and hemicellulose (Jung, 1989).

Lignin is covalently bonded to hemicellulose, but it is not bonded to cellulose, rather it has a branched structure that fills the space between hemicellulose and cellulose, acting as a barrier to fiber digestion (Jung, 1989). The structure of lignin impacts digestibility by physically and biochemically inhibiting microbial enzymes due to the presence of hydrophobic phenylpropanoids (Besle et al., 1994). Lignin content in plant stems increases as they mature due to more lignified sclerenchyma tissues and vascular bundles which contribute to the structural integrity of the plant (Akin, 1989; Kong et al., 2013). This makes stems more recalcitrant to digestion.

Cellulose and hemicellulose are incompletely fermented to volatile fatty acids (VFA) and the extent of their fermentation is limited by the degree of lignification. Pectin is generally rapidly and completely fermented to VFA. As forages mature, cellulose, hemicellulose and lignin contents increase and pectin content decreases (Allen et al., 2000). Highly digestible fiber improves rumen health and production primarily by increasing dry matter intake. Currently, fiber is measured routinely as either ADF or NDF. Although ADF can indicate relative differences in fiber within a feed or forage type, it does not discriminate in fiber value among feed types and is not the measure of fiber in a feed (Mertens, 2000). NDF is the best for measuring the total fiber in a feed. Not all NDF is potentially fermentable due to lignification. The indigestible fraction of forage NDF is a major factor affecting the utilization of fiber carbohydrates as it varies greatly and may exceed one half of the total NDF (Allen et al, 2000). It is related to forage intake potential and separates feeds into a soluble fraction that is essentially

completely digestible (NDS) and a NDF fraction that contains all of the indigestible and slowly digestion components in feeds (Mertens, 2000). Therefore, as forage quality decreases the total percentage of fiber increases and forage digestibility including intake decreases. Research reported by Mertens (1985) indicated that as the NDF content of forage increases, the ability of the animal to consume adequate amounts decreases. Neutral Detergent Fiber (NDF) includes hemicellulose as well as cellulose and lignin. Lignin is indigestible while cellulose and hemicellulose vary in digestibility. Cellulose is typically more slowly digestible than hemicellulose. As fiber content increases, the NDF digestibility decreases. High fiber forages pass out of rumen slowly and makes the animal feel fuller for longer thereby reducing intake (Ward, 2008). Research by Mertens (1987) indicated that cows consume about 1.2% of their body weight per day as NDF when rumen fill limits intake. Other researchers have proven that rumen fill and intake are also affected by the digestibility of the forage NDF (Oba and Allen, 1999), and reported that one percent increase in NDF digestibility boosted dry matter intake by 0.37 pound which pushed up 4% fat-corrected milk production by 0.55 pound. An estimate of NDF digestibility can be obtained by *in vivo, in situ and in vitro* procedures.

## Rumen Fermentation of Fiber

Rumen fermentation is a process that converts ingested feed into energy sources for the host. Fiber scratches the rumen wall to start a series of contractions. These contractions lead to rumination, which is the process that physically breaks down the fiber source. Rumen fermentation plays a major role in forage (feed) digestion and microbial production in ruminants. The rate and extent of forage (feed) digestion in the rumen, rumen fermentation pattern and amount of microbial protein production ultimately determine the feed and forage value, nutrient provision and animal productivity. It has been well documented that bacterial populations in the rumen largely

determine the extent and rate of fiber degradation (Akin and Barton, 1983; Miron et al., 2001; Pan et al., 2003; Khampa et al., 2006). Also, the attachment of fibrolytic bacteria is an obligatory step in fiber degradation (Sung et al., 2007). However, carbohydrates are the major source of energy for rumen micro-organisms and they represent the major component of net energy for growth and maintenance. Therefore, rumen microbial population is the central component of the rumen ecosystem and rumen is essentially a fermentation chamber, where pH is the central issue to a healthy flow in microbial population, stable for fiber and feed intake to be digested at a maximal rate with a pH range from 6.2 to 6.8 (Sung et al., 2007). When the rumen pH falls below 6, fiber digestion diminishes and dry matter declines from a considerable loss in endogenous enzyme functionality and a drop in microbial yield and effectiveness. Low digestibility feed take longer to digest, consequently the rumen remains full for longer, dry matter intakes are lower and energy intakes are reduced.

Thus, rations improperly balanced or managed for carbohydrates can have a profound effect on rumen health and animal performance because under these conditions, cattle may not produce sufficient quantities of saliva, which naturally buffers the rumen. Rumen microbial activity increases following feed and forage ingestion. Therefore, determination of feed and forage digestibility in the rumen is necessary to predict animal performance and optimum ration formulation. However, the use of animal to measure feed and forage digestibility is a reliable approach but disadvantages are numerous and unsuitable for large-scale feed and forage evaluation. As a result, many biological methods which simulate the rumen fermentation process have been developed.

The rumen is the main site of microbial digestion which contains a complex variety of different microorganisms which act synergistically to break down the feed. The most important end products of carbohydrate breakdown in the rumen are volatile

fatty acids (VFA). Volatile fatty acids (VFA) are the main end products of rumen fermentation and they are the major source (70%) of energy for the ruminant (Wasielewska and Zygmunt, 2015). Virtually all of the VFA formed in the rumen are absorbed across the ruminal epithelium and then transported in the blood to the liver where they are converted to other sources of energy. The energy produced is used to perform various functions, therefore reduction in VFA is nutritionally unfavorable. The rumen microbes produce three primary volatile acids: acetic, propionic and butyric. The primary VFA is acetic acid which is produced mainly by the digestion of fiber and represents 55 to 70 percent of the total VFA formed. Propionate is produced by starch and sugar digestion bacteria and the level varies from 15 to 30 percent of the total VFA production, while butyrate contributes to 5 to 15 percent of the VFA produced. When evaluating VFA patterns, the ratio of acetate to propionate or the A:P ratio (60 percent acetate:25 percent propionate or 2.4:1) reflects the rumen fermentation pattern. High levels of acetate can indicate high fiber-low fermentable carbohydrate content. High levels of propionic acid can indicate reduced fiber digestion. VFA analysis in the field has not been performed, but would be a useful tool to evaluate rumen fermentation and digestion (Wasielewska and Zygmunt, 2015).

*Forage Particle Size*

In addition, fermentation rate and digestion can be limited by forage accessibility to rumen microbes, thus forage particle size is critically important which must contain sufficient physically effective NDF (Hall and Mertens, 2017) to stimulate rumination, chewing and saliva production for normal rumen pH and normal fiber mat. The amount and size of fiber particles is important to maintaining optimal rumen. Long fiber in the rumen forms the rumen “mat”. The mat is where fibers are entangled because they are too long to pass to the lower gut. Fiber from the mat is regurgitated and chewed

producing large amounts of saliva that naturally buffers the rumen and ultimately elevating rumen pH (Beauchemin, 1991). The physical effectiveness of dietary particle can affect feed intake, digestive efficiency and health of the cow (Allen, 1997). Several researchers have demonstrated that attachment of ruminal microorganisms to their substrate is a prerequisite for the digestion of forage particles in the rumen (Varga and Kolver, 1997). And, it has been reported that there is interaction between forage particle size on dry matter digestibility because particle size reduction in the rumen leads to increased surface area for microbial attachment and digestion (Kung, 2014).

*Ruminal pH*

Ruminant animals and ruminal microorganisms have a symbiotic relationship that facilitates fiber digestion. The rumen is essentially a fermentation chamber, where pH is the central issue to healthy flow in a microbial population and feed intake to be digested at a maximal rate. When physiological mechanisms of homeostasis are disrupted, ruminal pH declines and microbial ecology is altered (Russell and Rychlik, 2001). The structural carbohydrate fermenting microbes are usually limited by a ruminal pH of less than 6 (Hoover, 1986). Therefore, ruminal pH is one of the most important factors affecting fiber digestion (Sung et al., 2007). The pH of the rumen has profound effects on the growth of rumen microbes for fermentation and digestion of fiber and fibrolytic bacteria in the rumen grow best when the pH of the rumen is between 6.2 and 6.8 (Kung, 2014). In addition, it is apparent that low ruminal pH changes the rumen microbial population (Tajima et al., 2001). It is therefore crucial to maintain a ruminal pH above 5.8 to prevent decline of fiber digestion. As the pH decreases, fibrolytic bacteria in the rumen become less active and fiber digestion begins to decrease (Kung, 2014). When ruminal pH falls below 5.8-5.9, the rumen is mildly acidic and fiber digestion in the rumen ceases completely (Blezinger, 2013) and dry matter intake declines from a

considerable loss in endogenous enzyme functionality and a drop in microbial yield and effectiveness. Moreover, Murino et al., 2001 reported that low adhesion of rumen microorganisms was considered a causative factor of reduced fiber digestion at low ruminal pH. But, buffers help to promote thriving rumen micro flora and fermentation, by resisting changes and maintaining optimal rumen pH.

## Methods to Evaluate Fiber Digestibility and Quality

The most accurate data for fiber digestibility comes from animal digestibility experiments in which fiber intake and excretion in the feces is measured over time. This requires complete collection of fecal output for determination of weight and fiber content and is very labor intensive. For many purposes, the use of animals to measure fiber digestibility is not practical (Allen et al., 2000). Therefore, several different methods have been used to evaluate large numbers of samples for fiber digestibility including fermentation by ruminal microbes. The *in situ* and *in vitro* techniques have been correlated with animal performance (Ørskov, 1989), food intake (Blummel and Ørskov, 1993; Kamalak et al., 2005a), microbial protein synthesis (Krishnamoorthy et al., 1991) and *in vivo* digestibility (Khazaal et al., 1993; Kamalak et al., 2005a).

Thus, *in situ* and *in vitro* methods are two major ruminal techniques that can be used to determine ruminal digestibility directly. Each method has advantages and disadvantages in estimating digestibility, and results are often variable (Mertens, 2000). However, both methods have several significant problems in common for prediction of digestibility (Allen et al., 2000), thereby initiating the need to address the question of which analysis should be requested. Typically forage do not enter the rumen as finely ground particles, which has led to the suggestion that digestion should be measured on whole or very coarsely chopped material. Although, it is reasonable to assume that using finely ground samples in *in situ* and *in vitro* systems may over- estimate digestion

kinetics, and it is just as likely that whole or coarsely chopped samples may under estimate them. So, one of the issues in measuring digestibility is how to prepare the sample for measurement. The best particle size for measuring digestion *in situ* or *in vitro* for an optimal way in adjusting data to reflect *in vivo* performance has not been defined (Mertens, 2000). Also, the correct measurement of moisture content is crucial for the determination of the nutritive value of forage because it is the dry matter in the forage that contains the nutrients, but significant volatiles that are created during silage fermentation are evaporated by oven drying and are analyzed as water, thus making sample preparation very critical since it is important to know the exact composition of forage as the first step in determining its value and its potential contribution to animal's diet.

*Sample Preparation*

Sample preparation for *in situ* and *in vitro* trials in evaluating the nutritive value of forage is becoming a challenge to ruminant nutritionists. The results obtained from those trials are often influenced in comparison to *in vivo* trial. Therefore, there is a need to have a consistent standard for digestibility measurements. Lowman et al., 2002 stated that with regards to particle size for fibrous and more slowly degraded feeds, fermentation rate increases as particle size decreases. This was attributed to increased surface area as a result of grinding, thereby allowing better microbial access.

It was stated that freeze-drying is preferred to oven drying for the drying of high moisture substrates. Rymer et al., 2005 stated that implementing a standardized protocol for preparing substrates to be incubated is critical and the most critical issue appears to be the methods used to dry fresh material. Moreover, it was stated that comparisons of freeze-drying with oven drying are often contradictory. Nevertheless, a prior freeze-drying is the method of choice for minimizing cell damage that potentially alters the

dynamics of microbial attachment, substrate degradation and altering bioactive compounds (Rymer et al., 2005). Given the increasing and wide-scale use of *in situ* and *in vitro* techniques, there is need to critically examine and review the recommendations on sample preparation from previous researchers. Thus Adoption of a standardized approach to sample preparation may enable comparisons between independently produced trial data (Yanez-Ruiz et al., 2016).

## Distillers Dried Grain with Soluble (DDGS)

Forage comprised of grass and maize silages is currently used in the diet of ruminants to ensure high energy and nutrient supply. But, research has proven that the effective fiber in DDGS is comparable to forage and replacing forage with DDGS at an increased inclusion rate of up to 50% in limit-fed rations for growing heifers can maintain growth performance (Manthey and Anderson, 2016). Increased amount of distillers dried grains with soluble (DDGS) has been considered a viable option in the dairy industry because of the widespread availability of DDGS and low cost as an alternative feedstuff (Schingoethe et al., 2009), making it a highly attractive feed to include in dairy replacement heifer diets. Dried distillers grain with solubles is co-product of ethanol production. It is relatively inexpensive feed, yet it provides an excellent nutrient profile. Distillers grains are high in crude protein (CP), fat, acid detergent fiber (ADF) and neutral detergent fiber (NDF) content, it is also high in digestible energy value (Spiehs et al., 2002). Thus, DDGS can be used to replace both concentrates and roughages because of its nutritional content (Spiehs et al., 2002). However, high concentrations and high variation of minerals affect the value and end use of DDGS as animal feed (Liu, 2011).

Studies by Manthey et al., 2016 and Anderson et al., 2015 evaluated the use DDGS in growing heifer diets and found it to be nutritionally suitable feed. However, the

high sulfur and phosphorus content of DDDGS can have detrimental effects on nutrient digestibility and excretion when fed at high inclusion rates with a negative impact on animals both in terms of health and performance (Uwituze et al., 2011) including the environment. Therefore, it is important to understand possible ways to improve the inclusion rate of DDGS in order to increase its use in dairy heifer production.

The increased concentrations of fermentable fiber and undegradable protein (RUP) found in DDGS compared with other feed sources such as corn and soybean meal are thought to be the factors of improvement in animal production (Schingoethe et al., 2009). They are high in digestible NDF, however, this fiber is ineffective due to its small particle size and their net energy value is high because of the higher concentration in oil. Although, nutrients in distillers grains make it a desirable feedstuff, using distillers grains in heifer diets can be challenging. The need to challenge animals with energy dense diets will require strategic method of feeding in order to maintain a recommended average daily gain, thus limit-feeding may be an option.

Diets typically use for limit-feeding are proportionately high in concentrates and nutrient dense (Manthey and Anderson, 2016), but are fed at a set rate or amount to meet, but not exceed requirements. Higher ration energy density allows cows to obtain necessary energy for greater fermentation. In addition, high concentrate diets will produce more acid and coupled with the fact that DDGS will have a high concentration of sulfur, appears to create an acidosis condition in cattle and reduces feed intake prompting cattle to occasionally go off-feed. Greater fermentation will lead to more acid production decreasing the rumen pH. Therefore is practical to serve a buffering agent with the diet to prevent acidic condition in the rumen to prevent weakened feed digestion.

Additionally, distillers grains are high in phosphorus, and the phosphorus requirement of dairy heifer is low. An irreconcilable nutritional issue with feeding distillers grains to dairy heifers up to 20% of dietary DM is that excessive levels of phosphorus will be fed and nutrient management programs may be compromised because excess phosphorus will be excreted in the feces (Zhang et al., 2016). Distiller grains with soluble may also be high in sulfur, whereas, microbial growth and yield is also affected by the concentrations of trace minerals and vitamins (Sniffen and Robinson, 1987). Dietary sulfur and phosphorus concentration has been found to affect microbial growth (Sniffen and Robinson, 1987).

*Sulfur*

Sulfur is an important mineral in ruminant diets for various reasons. It is essential in animal diets to synthesize sulfur-containing amino acids and growth of rumen microorganisms. It has been shown to increase cellulose, OM and ADF digestibility, especially in diets where sulfur is limited (Martin et al., 1964; Barton et al., 1971; Kennedy et al., 1971). Sulfur is necessary for cellulose digestion, and research has shown a threefold increase in cellulose digestion when sulfur was added to a diet with no sulfur (Patterson et al., 1988).

Sulfur metabolism in ruminant animals occur ruminally and postruminally. Sulfur in the rumen is reduced to $H_2S$ and then converted into microbial protein, and any excess $H_2S$ is absorbed. Postruminal metabolism of sulfur includes digestion and absorption of sulfur-containing substances, such as protein, amino acids and sulfates. Inorganic sulfate can be reduced to $H_2S$ by bacteria in the rumen. Sulfur recycling can decrease the requirement of sulfur for the animal and sulphide is converted to sulfate where it is incorporated into extracellular fluid. Once in the rumen, recycled sulfate is reduced to sulphide and can then be use to synthesize protein. Sulfur is lost through the excretion of

urine and feces (McDonald and Wilbur, 1974) and it can also be expelled as $H_2S$ gas via eructation.

Toxic concentrations of sulfur can be achieved when DDGS is included at high inclusion levels because of its sulfur content. The ideal environment for successful formation of $H_2S$ requires a more acidic environment with a pH of 6.5 (Lewis et al., 1954) compared to the near neutral pH found in the rumen. However, research has shown using pH to decrease $H_2S$ production by lowering the pool of hydrogen ions available to interact with sulphur to create hydrogen sulphide gas. Therefore, suggesting that a method by which an increase in pH could lead to a greater efficiency in digestion of DDGS due to a more favourable environment for rumen microbes.

*Phosphorus*

Phosphorus is a required nutrient for all livestock because of its numerous essential physiological functions in the body that include buffering pH changes in the rumen (salivary phosphate), energy transfer (ATP), structure of bone, teeth, and membranes. Ruminants use a larger proportion of dietary phosphorus because rumen microbes produce phytase, the enzyme that hydrolyzes phosphorus from phytate. Large amounts of inorganic phosphorus are secreted into the gastrointestinal tract of ruminants via saliva because phosphate is a crucial buffer for ruminal fermentation. The majority of phosphorus in most concentrate (grains) is in phytate form (Satter et al., 2005), and phytate phosphorus is almost totally available to ruminants because of phytase production by rumen microbes. During fermentation when processing DDGS, the percent phytate phosphorus in total phosphorus decreased significantly, whereas percent inorganic phosphorus in total phosphorus increased (Liu, 2011).

The dairy industry uses large amounts of co-products feeds, many of which serve as important sources of protein in the dairy diet. Feedstuffs vary greatly in total

phosphorus content, and the amount of phosphorus content of ruminant diets has been increasing overtime as the amount of grain, protein supplement, and by-products fed to beef and dairy cattle has increased. Besides concentration, bioavailability of phosphorus in animal is another important factor that affects retention of phosphorus in ingested feeds by animals and the amount of phosphorus excreted in wastes (Liu, 2011). The phosphorus requirement is essentially the same for beef and dairy (Satter et al., 2005). Research by (Aguerre et al., 2002) indicates that phosphorus availability in concentrates may average 75% or higher. However, phosphorus availability is likely to be greater with the highly digestible feedstuffs (Satter et al., 2005). With highly concentrated diets, a significant amount of phosphorus will be excreted by ruminants, and normally greater than 95% of the phosphorus is excreted in feces. In addition, 50% of fecal phosphorus is in rumen microbial residues (Wu et al., 2000), and the microbial mass is directly related to fermentable energy intake or DM intake (Satter et al., 2005).

## Limit Feeding Strategies

The strategy of limit-feeding utilizes rations greater in concentrates and lesser in forages to allow for a more energy dense diet that provides vital nutrients and a decrease in nutrient wastes (Zanton and Heinrichs, 2007; Zanton and Heinrichs, 2008). It is a feeding management with restricted intake to achieve a similar or controlled ADG. This is a more economical alternative to feeding ad libitum forage to dairy heifers which is the common practice among producers, heifers that are limited exhibit greater diet digestibility, greater feed efficiency, and lesser amounts of nutrient excretion (Hoffman et al., 2007; Zanton and Heinrichs, 2009b). It is an effective method to improve energy efficiency and has been explored by several researchers (Shi et al., 2018). Limit feeding was implemented to avoid excessive ADG that could be caused by increased dietary proportion, for a targeted ADG (Zanton and Heinrichs, 2007).

*Forage:Concentrate Ratio*

The ratio of forage:concentrate also affects ruminal pH. Starch is generally fermented faster than NDF. As concentrates increase in the diet, total acid production in the rumen increases, causing a decrease in pH (Kung, 2014). Efficiency of microbial growth was reported to decrease when the supplementation of concentrate is high (Huber and Kung, 1981), resulting from an uncoupled fermentation (Poland, 1988). The use of nonforage fiber feeds with high fiber contents will have similar effects because since the particle size is small, they will not be well chewed resulting in low production of saliva to buffer the rumen.

Therefore, the use of buffer for high ruminal fermentation that is consistent over time will help to maintain ruminal pH above 6.0.

**Rumen Buffers**

Research has shown buffers minimizing wide fluctuations in rumen pH to improve fiber digestion, although, the buffering capacity of saliva helps to increase ruminal pH. Requirements for buffering supplements in dairy cow diets are a function of salivary buffer secretion, feedstuff buffering capacity, acid-producing potential of the diet and feed acidity. Rumen buffer should react and tie up available hydrogen ions to have an equivalence point of pKa near the desired pH of 6.2-6.8 in rumen. Dietary buffers are widely used in the dairy industry, still much more research is necessary (Americandairymen.com, 2010). Ideally buffers should either be released during the interval of most severe acid production in the rumen, or should provide a continuous release to prevent fermentation-related increases in free proton, which might be detrimental to fiber digestion.

Research studies in formulating rations for carbohydrates have suggested that buffer inclusion amount is required to be considered in meeting NDF or NFC value, since proper buffering of the total digestive tract will increase dry matter intake. Buffers help to promote thriving rumen micro flora and fermentation, by resisting changes and maintaining optimal rumen pH. Moreover, research has proven that buffers' minimizing wide fluctuations in rumen pH improves fiber digestion, VFA-acetate:propionate ratio for enhanced dry matter intake. Hence, modifications on buffer inclusion amount when feeding DDGS may provide opportunities for better nutrient utilization in heifer and steer diets. Research has also proven that a combination of buffers is more effective than single ingredient buffers because buffers have different sites, durations and modes of action.

Therefore, maximizing rumen fermentation increases VFA production, providing more energy and microbial protein.

## Conclusions

Accurate evaluation of feed and forage value is a key economic issue due to its impact on animal health and production. The rate and extent of ruminal degradation of forage can vary strongly with stage of maturity (Jojnson et al., 2002; Jensen et al., 2005) and hybrid (Ngonyamo-Majee et al., 2009), but these variations cannot be properly understood until there is a standardized method to evaluate rate and extent of ruminal degradation of forage fiber (Peyrat et al., 2014).

Therefore, our study was on evaluation of methods used for forage analysis to determine effects on fiber digestibility. Our objective was to increase forage utilization and dairy productivity through an improved standardization of procedure for forage analysis. We hypothesized differences in fiber digestibility values among methods.

The energy concentration of DDGS is equal to or greater than the concentration of corn (Brirkelo et al., 2004). However, rather than coming from starch, the energy is provided by fat, digestible fiber, and CP. The primary carbohydrate fraction in DDGS is NDF. Feeding high amounts will increase the concentration of NDF in the diet. Several experiments have shown that DDGS is comparable to forage as a source of effective fiber, but due to particle size considerations it is likely that with higher levels of DDGS inclusion, the ability of DDGS to replace the effective fiber of forages will be limited. Although, DDGS particle size has the potential to provide a greater surface area for attack by enzymes, utilization of structural carbohydrate is not increased; rather, improvements in animal performance arise primarily from an increased digestible energy intake (Bourquin et al., 1990).

DDGS has high fat content and the variability in fat content can be problematic which must be controlled for normal rumen function. In addition, the high levels of minerals in DDGS might be a challenge to nutrient digestibility and excretion. Thus, limiting the utilization of DDGS and all these concerns must be accounted for when using this feed source at levels above 10% to 15% to obtain optimum rumen function, cow productivity and nutrient excretion (Penn State Extension, 2017). Unfortunately, there is limited research on improving the utilization of DDGS for growing dairy cattle.

Therefore the objective of this research is to determine how the dietary inclusion of buffer affects nutrient digestibility and excretion when feeding dairy heifer diets high in distillers grains. The study is imperative to help us understand how buffer inclusion rates impact fermentation profile and nutrient utilization. We also hypothesized that total tract digestibility and excretion of nutrients will be different at different buffer inclusion amounts.

# CHAPTER 2:

# EVALUATION OF METHODS USED FOR FORAGE QUALITY ANALYSIS.

## Abstract

Forages are important component of dairy diet which is less subject to competitive demands. However, plant cell wall is the largest hindrance to complete digestion and utilization of their nutrients, necessitating effective strategies to determine the nutritive values of forage in order to predict a commensurate animal performance. Research in this area is significantly increasing in demand due to increased grain prices coupled with decreased grain availability. Our study was on evaluation of methods used for forage analysis to determine effects on digestibility and rumen parameters. Objective was to compare use of *in vitro* and *in situ* methods in prediction of animal performance as results are often variable. It was hypothesized that differences in digestibility values among methods but a similar pattern among methods. Consequently, five forages including Conventional Corn Silage (CCS), Conventional Haylage (CHL), Hybrid Corn Silage (HCS), Hybrid Haylage (HHL) and Hay were processed (dry/wet) for *in situ* and dry for *in vitro* trials. *In situ* and *In vitro* measurements were conducted using three multiparous mid-late lactation ruminally cannulated Holstein cows to study DM and NDF degradation for the *in situ* and DM degradation, gas production, and fermentation parameters were from the *in vitro* trial. During the *in situ* trial, cows averaged 719.9 ± 59.9 kg of BW, 120 ± 91.1 days in milk (DIM) and 31.01 ± 3.7 kg/d of milk yield. Cows were milked twice per day at 0600 and 1800 h. Duplicate 5g samples were weighed into 10 x 20 cm Dacron bags and ruminally incubated for 0, 2, 4, and 8h incubation time periods for both dry and wet set trial. For the longer incubation time periods, 16, 24, 48, and 72h, bags were prepared in triplicate for both dry and wet set of sample because of expected degradability and amount of residual material required for post-incubation

analyses. The rate of passage out of the rumen was calculated to average 6.55 % for the three cows. The forage quality of five forage samples was evaluated on rumen fermentation and kinetics in an *in vitro* batch fermentation system. Rumen fluid was collected from 3 lactating multiparous Holstein cows at mid lactation via the rumen cannula at approximately 4 h after feeding. The *in vitro* batch fermentation experiment was repeated three times for 24 h on different days using the same 3 lactating multiparous Holstein cows. Data were analyzed using SAS 9.4 and means compared with Tukey's test. Degradation curves were calculated using Non-Linear procedure for *in situ* study. Rumen degradable dry matter was greater for dry *in situ* samples ($P$ <0.05) compared to wet samples and haylage had greater values of digestibility ($P$ < 0.05) compared to silage. The DM and NDF degradation values varied among the five forages with the highest values being observed in CHL and HHL, while lowest values were observed in CCS and HCS. The ammonia and total volatile fatty acids concentrations from the *in vitro* study followed a similar pattern of digestibility with *in situ*, but digestibility values were different. Result showed significant effects ($P$ < 0.05) in digestibility values resulting from procedural effect in sample preparation and methods used for forage analysis. Thus, accurate prediction of animal performance from forage will require an improved standardization of procedure.

**Keywords:** Forage, digestibility, method

## Introduction

Forages are a necessary component of diets for ruminant dairy cows because they provide coarse fiber needed to optimize rumen function. Forage quality can have a very significant effect on the overall nutritional plane of the animal as it affects the digestibility and intake; hence will have profound effect on performance. But forage quality is highly variable and forages have been traditionally analyzed for fiber concentrations because of their direct effect on diet formulation, thus their quality must be assessed before diets are formulated. Analysis of forage is an important troubleshooting tool to identifying forage with greater quality and it is crucial to be able to draw relevant conclusion on the performance of an animal from the forage quality.

Forage analysis provides useful data in nutritional management including forage management strategies. Consequently, it is important that forage quality be routinely analyzed because many parameters of forage quality affect diet formulation (Oba et al., 2005). The dry matter digestibility is a key characteristic describing the intake and production potential of forages in ruminant feeding. Meanwhile, there are technical difficulties in evaluating forage analysis because *in vivo* measurements are laborious, expensive and require a large amount of feed. Therefore, several other techniques have been developed to predict digestibility of feeds. The commonly used procedures to evaluate forage quality are *in situ and in vitro methods.* The rumen fluid-based *in vitro* technique (Tilley and Terry, 1963) and its modifications have been widely used for predicting digestibility and as a selection tool for improving the nutritional quality of forages (Stern et al., 1997). The advantages of using *in vitro* techniques as compared to other methods include low cost, simplicity, small feedstuff requirements and particularly, the ability to screen large numbers of samples under similar experimental conditions. However, a number of factors including inoculum source, recording system of gas

production, sample size and method of substrate preparation could alter fermentation results (Yang, 2017). Dry matter content of forage is important because it reveals the actual amounts of various nutrients available to the animal consuming it. But the method of drying forage for in vitro digestion experiments was studied and there was a significant difference in favour of freeze-drying (Clark et al., 1959). And, it was also reported by Clark et al., 1959 that oven drying the samples tend to decrease differences between stages of growth. It is important to note that forage including the moisture content is an accurate representation of forage offered to the animal and it has been recommended that samples for digestion studies be processed uniformly and research has reported a faster rate of digestion with dried ground samples. Unfortunately, there has been a dearth of knowledge on evaluations of the effect of sample preparation on forage analysis, So it is imperative to investigate the effects of sample preparation on the accuracy of forage analysis results for proper estimation of forage quality. This will be a milestone research into this particular area of forage analysis as a way to improve on animal performance.

### *In situ* Digestibility

*In situ* technique has been used for many years to provide estimate of both rate and extent of dry matter degradation of forages (Mehrez and Ørskov, 1977). The digestibility is measured with ground forage samples placed in small porous bags and inserted into the rumen through a rumen cannula. The pore size of the bag is usually ~ 50 $\mu$m, which allows entry of microbes but retains feed particles. However, the intrinsic problem that limits *in vitro* digestibility is not resolved (Oba et al., 2005). It has been argued that the *in situ* system is more similar to actual digestion determined in the animal because the ruminal contents are not placed in an artificial environment. But the *in situ* system is somewhat artificial in that the diet of the cannulated cow does not represent the

forage suspended in the bags, and the bags are an artificial environment themselves. However, the ruminal microorganisms remain in their natural environment and natural salivary buffers are secreted continuously by the cow, as the end products of digestion are continuously removed. Microorganisms from the rumen of the cow can pass into the bag and be measured as undigested forage, thus washing of the bags to remove ruminal contamination and microorganisms is a crucial step in *in situ* procedure. Finally, the ruminal environment is variable throughout the day depending on the feed and feeding management provided to the cannulated cow, which may not happen when the test forage in the bag is to be actually fed. The *in situ* technique mimics *in vivo* conditions and are widely adopted as the standard of method as providing reference values against which *in vitro* techniques are correlated. However, the procedure is prone to various sources of error (Edmunds et al. 2012), and therefore, there is need to have a consistent standard for digestibility measurements.

### *In vitro* Digestibility

Menke et al (1979) and Menke and Steingass (1988) developed the *in vitro* gas production technique to evaluate the nutritive of forages and estimate the rate and extent of dry matter degradation indirectly using the gas production ($CO_2$) produced during fermentation. The *in vitro* digestibility of forages is determined by incubating dried ground forages in bottles with rumen microbes for a given period of time. Forages are dried and ground (usually to pass through a 1-mm screen), so that a representative sample can be taken. The bottles will also contain buffers, macro-minerals, trace-minerals, nitrogen sources, and reducing agents to maintain pH and provide nutrients required for growth of rumen bacteria. Because oxygen is toxic to rumen bacteria, bottles are gassed with carbon dioxide to maintain anaerobic conditions, and the temperature is held at 104°F (body temperature) during the incubation.

Every effort is made to provide the optimum environment for survival and growth of fiber-digesting bacteria in the incubation media. This is very important because digestion is a function of both enzyme activity and structural characteristics of substrates. If enzyme activity is limiting because of inadequate buffering or lack of essential nutrients, *in vitro* digestibility will be reduced, and more importantly, differences in *in vitro* digestibility among forages will be compressed and not reflective of the true differences among forages. Therefore, it is important to use an *in vitro* system that measures the maximum *in vitro* digestibility of forages, not one that limits *in vitro* digestibility because of the lack of buffering or essential nutrients.

It is crucial to note that *in vitro* digestibility is a biological evaluation of forage quality, therefore the microbial activity in rumen fluid can vary with diet and over time relative to feeding which can affect the results (Oba et al., 2005). Thus, measurements of *in vitro* digestibility are associated with greater intrinsic variation. This variation can be reduced by feeding the donor animals a high forage diet, sampling rumen fluid at the same time relative to feeding, and blending rumen fluid from several cows for each of the incubations.

*In vitro* digestibility is not necessarily the same as in vivo digestibility because the environment in the rumen is often less than optimum for fiber-digesting bacteria and forage fiber particles in the rumen are longer than those of ground forages use in *in vitro* measurements of digestibility. Longer particle size limits the surface area for microbial degradation per unit of fiber mass (Oba et al., 2005). Thereby, suggesting that *in vitro* digestibility of forages should be greater than in vivo digestibility as long as an optimum fermentation environment, such as pH, temperature, and anaerobic conditions, is carefully maintained in the incubation media. In addition, *in vitro* digestibility of forages

is greater than in vivo because the same retention time is used across samples, although actual retention time of forages likely varies with rate of digestion (Allen, 2000).

The objective of the study is to determine whether sample preparation and method will improve the accuracy of forage analysis and which alternative preparation will estimate more accurately. Therefore, a comparison was made between wet forage preparation and dried forage preparation for forage analysis. We hypothesized that different methods will vary in digestibility result but difference among treatment will follow similar patterns among methods.

## Materials and Methods

### *Animal Care and Feeding*

All animal procedures and uses were approved by the South Dakota State University Institutional Animal Care and Use Committee. This study was conducted at the South Dakota State University Dairy Research and Training Facility (**SDSU DRTF**; Brookings, SD). The study was completed from March 5, 2018 through March 7, 2018. Cows were observed daily for health problems and treated according to routine management practices at the DRFT.

*In situ* measurements were conducted using three multiparous mid-late lactation ruminally cannulated Holstein cows during Spring 2018. During the trial, cows averaged 705.9 ± 78.1 kg of BW, 120 ± 91.1 days in milk (DIM) and 31 ± 15.5 kg/d of milk yield. Cows were milked twice per day at 0600 and 1800 h. During the study, cows were housed in individual box stalls bedded with straw with ad libitum access to water. Cows were fed the same TMR as fed to the main lactating herd at the Dairy Research and Training Facility at the time of study (Table 1). Feed was offered daily at 1630 h and fed using individual feed boxes placed inside the stall. Individual feed intakes were measured

daily, and intakes were used to calculate passage rate. Average DMI for the three cows during the trial was 47.75± 39.64kg/d. Rations were fed on an ad libitum basis as a TMR with a forage-to-concentrate ratio of 53:47%. Feed samples were taken of the TMR offered each day and frozen (-20℃) until later analysis.

*Forage Collection and Processing*

Two corn silage and two grass silage samples with hay as control were obtained in 2016 from commercial farms in the USA. The corn and grass samples were harvested by a trained nutritionist from a feed company and ensiled. The corn silage and grass haylage were sampled and transported to the laboratory. After collection, the samples were stored at -20℃ until processing. The frozen samples were divided into three parts; one part was subjected to chemical analyses after oven drying and grinding, another part was stored at -20℃ for later in situ and in vitro incubations.

*Forage Hybrids and Sample preparation*

Hybrid and Conventional forage were harvested and conserved as Conventional Haylage (CHL) and Hybrid Haylage (HHL) and the corn was conserved as Conventional Corn Silage (CCS) and Hybrid Corn Silage (HCS). The samples were prepared in two ways before nylon-bagging for *in situ* (i) dried sample for 48h at 55℃ and then ground through the 4mm screen for the dry set *in situ* with Wiley Mill (model 3; Arthur H. Thomas Co. Philadelphia, PA). A wet set of samples were milled with a food processor (Intertek OST24160/CSKC0612) and separated with a Penn State separator to get uniform particles and directly bagged without drying for the wet set *in situ* rumen incubation to simulate forage particle size from chewed forages in the rumen. The third part was stored (-20℃) as a reserve for possible future analysis.

*In situ Procedures*

Five forages or feedstuffs were analyzed: Hybrid Haylage (HHL), Conventional Haylage (CHL), Hybrid corn silage (HCS), Conventional corn silage (CCS) and Hay (Hay) was the control. Five grams of each forage sample were weighed into 10 x 20 cm Dacron bags with a pore size of 50 um (Ankom Technology, Macedon, NY) and heat sealed using an impulse sealer. Duplicate sample bags were prepared for each forage and cow for the 0, 2, 4, and 8h incubation time periods for both dry and wet set trial. For the longer incubation time periods, 16, 24, 48, and 72h, bags were prepared in triplicate for both dry and wet set samples because of expected degradability and amount of residual material required for post-incubation analyses, but Hay was not prepared for the wet set. Prior to incubation in the rumen bags were soaked in warm water, approximately 39℃ for 20 min before insertion; during this time rumen fluid was also collected from cranial, ventral, and caudal locations in the rumen. Rumen fluid was collected to measure pH (Waterproof pH Tester 30, Oakton Instruments, Vernon Hills, IL) at each time point over the course of the 3-day study. The Dacron bags were inserted into the rumen before feeding on the first day of trial. The 0 h samples were subjected to the same soaking and rinsing procedures but were not placed in the rumen. Within each cow, sample bags were placed in a large nylon mesh bag (36 x 42 cm) with a weight to submerge samples beneath the particulate mat layer of the rumen. Bags were inserted into the large mesh bag in reverse order so they could be removed from the rumen simultaneously and rinsed at the same time. Duplicate and triplicate blank Dacron bags were also incubated for each time point in order to correct for microbial attachment and any accumulated material that might result in any changes in bag weights. After removal from the rumen, bags were submerged in a 15-L bucket, gently agitated, and rinsed manually in cold water until runoff was clear. Rinsing time was a minimum of 20 min for all bags. Bags were then frozen until analysis. Thawing and suspension in 0.1% methylcellulose solution along

with incubation in a shaking water bath at 37℃ for 30 min before rerinsing and drying is to help dislodge microbes attached to the feed particles and reduce the microbial protein contamination (Gargallo et al., 2006). Bags were thawed, suspended in methylcellulose, rinsed again individually for 10 min, and dried for 48h at 55℃ in a forced-air oven (Model V-23: Despatch Oven Co., Minneapolis, MN). Dry matter disappearance was calculated by the weight difference between the original sample and the residue of the post-ruminal incubation. Residues for each time point were pooled and composited for each forage sample by cow, ground through a Black and Decker Coffee Bean Grinder (100W) for NDF analysis.

*In vitro Procedures*

The forage quality of five forage samples was evaluated on rumen fermentation and kinetics in an *in vitro* batch fermentation system (Ankom Technology Corp., Macedon, NY). Rumen fluid was collected from 3 lactating multiparous Holstein cows at mid lactation via the rumen cannula at approximately 4 h after feeding. The *in vitro* batch fermentation experiment was repeated three times for 24 h on different days using the same 3 lactating multiparous Holstein cows. The rumen fluid (RF) from the 3 individual cows were mixed and strained through 4 layers of cheesecloth. And, the pH of the pooled rumen fluid (RF) sample was measured immediately to ensure the purity of the sample. Aliquots of 50 mL of strained RF were added to three 500 mL bottles containing a previously $CO_2$ gassed 200 mL of Mc Dougalls buffer (McDougall, 1947) and pre-warmed at 39℃. Each of the *in vitro* batch fermentation was performed in a shaking water bath (Cat#TSSWB27, Waltham, Massachusetts) at 39℃ for 24 h. In order to determine NDF digestibility, sufficient forage samples were ground with Wiley Mill 4 and 1 micron (model 3; Arthur H. Thomas Co. Philadelphis, PA). (Dacron (57 $\mu$m pore size, ANKOM, Macedon, NY) bags containing a total of 1g of ground forage samples

were weighed and sealed using a heat impulse sealer (Cat# MP-8 Intertek), the 2 bags containing the forage sample were placed in single bottle along with the blank according to experimental design for later determination of NDF.

Gas production was measured as pressure per square inch (PSI) in each bottle every 20 minutes using the ANKOM gas production system (ANKOM, Macedon, NY). This system is equipped with gas pressure sensor modules that transmit data via radio frequency, to be recorded by the computer. Gas produced was converted into mL using the equation $V_x = V_j P_{psi}$ x 0.068004084 where $V_x$ is the gas volume at 39℃ in mL, $V_j$ is the headspace of the bottle in mL (500 ML) and $P_{psi}$ is the pressure recorded by the gas monitor system software, moles of gas produced by n= Vp/RT, where n = quantity gas in moles, P = pressure in kPa, V = volume gas occupied in L, T = temperature in kelvin (K) and R = gas constant (8.314472 L/kPa·K/mol) and gas produced per 100 mg of substrate by the equation ml per 100mg = ml gas/(mg of substrate/ 100). Once the 24 h were completed, the filter bags containing the forage sample were washed with cold water and dried for 24 h at 105℃, after which drying bags were placed in a desiccator for 20 minutes and then weighed for dry matter digestibility analysis.

*Laboratory Analysis*

The DM of the forages and incubation residues was determined by oven-drying of a subsample at 55℃ and 105℃ respectively. The analysis of NDF was conducted using the filter bag technique method (ANKOM, Macedon, NY). Neutral detergent fiber (**NDF;** Van Soest et al., 1991) and acid detergent fiber for the forages (**ADF**; Robertson and Van Soest, 1981) were analyzed sequentially using the Ankom 200 fiber analysis system (Ankom Technology Corp., Fairport, NY). For NDF, heat-stable alpha-amylase and sodium sulfite were used.

The analysis of NDF was conducted using the filter bag technique (ANKOM, Macedon, NY). Ten milliliter of rumen fluid (RF) from each bottle was pipetted into a vial containing 2 mL of 25% meta-phosphoric acid and frozen at -20℃ to be analysed later for volatile fatty acid (VFA) concentration. Ten milliliter of rumen fluid (RF) was pipette into a vial containing 200 $\mu$l of 50% sulfuric acid ($H_2SO_4$) and frozen at -20℃ until analysis for ammonia N. For the analysis of the rumen fluid, the samples were thawed and centrifuged at 30,000 x g for 10 min at 10℃ ((Centrifuge: Eppendorf 5403, Eppendorf North America, Hauppauge, NY) and analysed for ammonia N using a colorimetric assay performed on a micro-plate spectrophotometer (Cary 50, Varian Inc., Walnut Creek, CA) according to Chaney and Marbach (1962). Rumen fluid samples that were preserved with metaphosphoric acid were thawed and centrifuged at 30,000 x g for 20 minutes at 4℃ and analysed for acetate, propionate, butyrate, isobutyrate, isovalerate, and valerate concentrations using an automated GC (model 6890; Hewlett-Packard Co., Palo Alto, CA) using a flame-ionization detector. Volatile fatty acids were separated on a capillary column (15 m x 0.25 mm i.d.; Nukol, 17926-01C; Supelco Inc., Bellefonte, PA) using 2-ethylbutyrate as an internal standard. The split ratio of 100:1 in the injector port was at a temperature of 250℃ with flow rate of 1.3 mL/min of helium. The column and detector temperature were maintained at 140℃ and 250℃ respectively.

*Statistical Analysis*

All data were analysed using SAS version 9.4 (SAS Insitute Inc., Cary, NC). Ruminal degradation constatnts of fiber were analysed using nonlinear regression modelling (Ørskov and McDonald, 1979; SAS 9.4). The following equation describes the model used to determine the ruminal degradation of dry matter and fiber as a percentage at time t (Y).

$$Y = A + B\,[1 - e^{-Kd\,(t)}]$$

The rapidly degradable fraction that disappears at 0 h after rinsing is represented by A; B represents the potentially degradable fraction; and $K_d$ is the rate of degradation of the B fraction and t represents time of incubation (h). The C fraction represents the indigestible fraction as 100- (A + B). The particulate passage rate (%/h) is the variable $K_p$ and is calculated according to the NRC (2001) equation for forages.

$$K_p = 2.904 + 1.375 \text{ x } X1 - 0.020 \text{ x } X2$$

In this equation X1 represents the DMI, as a % of BW; X2 is the forage amount, as a % of the diet DM. The passage rate for this particular study was found to average 6.0%/h among the three cows. Fraction A, B, C, and total digestible fiber were analysed using MIXED procedure of SAS 9.4. Mean comparisons were performed using Tukey's test with $P < 0.05$ designated as significant.

## Results and Discussion

*Feed Composition of the test forages*

The chemical composition of the tested forages and hay is presented in Table 3. As fibrous feed, the tested forages contained high variable CP (7% - 21%) and less variable fiber 27% - 40% NDF and 25% - 33% ADF as compared to hay. The CP content was numerically least for CCS and HCS, intermediate for HHL and the highest for CHL. In contrast, the NDF contents were apparently high in CCS and HCS and were close in values when compared with hay. But, the ADF values for CCS and HCS were higher when compared with hay. Variation in the other fiber fractions was considerable but the relative differences among the forages were not as great in magnitude as those in CP and NDF when compared with hay. The forages had similar fiber contents but varied widely in their CP contents.

*Ruminal Degradation Kinetics of Dry Matter and Fiber*

*In situ*

The mean DM degradation variables across the forages are presented in Table 4. The mean for the filterable and soluble DM A fraction ranged from 32.65 to 53.45 and HCS was the greatest with 53.45, intermediate with WCCS and least with WHHL ($P < 0.001$), whereas the mean of the degradable B fraction of DM ranged from 28.54 to 44.85 and highest was WHHL with 44.85. The intermediate were CHL and WCCS with 31.38-35.24, and the lowest were CCS and HCS with 28.54-29.89 ($P < 0.001$). The mean values of the C fraction, or ruminally undegradable DM was highest for WCCS and WHS, followed by CCS and WHHL with the least in CHL and HHL ($P < 0.001$). In the present study, all forages showed dry matter degradation rates above 2%, but needs longer time in the rumen to reach its maximum degradation. The parameters of the fractions A and rate of degradation are the most important in this classification. Table 4 shows the effect of sample preparation on the disappearance caused by drying and grinding. Fraction A indicates better degradable forage. The readily soluble fraction represents the feed part which immediately disappears when feed enters the rumen, this value proved to have the most variable values. Dried ground samples contained significantly ($P < 0.05$) more soluble DM than the wet samples. Drying and grinding the samples significantly ($P < 0.05$) increase the solubility of DM which agrees with a previous study by Kempton (1980). While higher value of rate of degradation reflects a shorter time for disappearance of readily degradable fraction, the values varied considerably in the present study when both the dry and wet samples were compared. The sample preparation method used to determine ruminal dry matter degradation displayed a wide range for the corn silage samples, which could have been caused by dry and wet sample preparation. Also, differences in forage were observed for the haylage

and corn silage. The effect of forage type was significant for the *in situ* degradability values.

The mean value of ruminal fiber degradation for dry samples was presented in Table 5. The values varied considerably in the present study, which could have been caused by the high values of fiber in CCS and HCS.

Mean percentages for the degradation rate of the degradable B fraction for DM within 72 h of incubation, differed considerably among the five forages, ranging from 3.2 to 8.91 % $h^{-1}$, with the lowest rate being observed for HCS and WHCS and highest rate for CHL and WCHL as compared to Hay. The ruminal NDF degradation followed the same pattern.

*In vitro*

The *in vitro* dry matter degradability (DMd) showed a wide range of qualities in the five forages tested as presented in Table 6. The highest DMd value ranges from 49.34% - 53.24% found in HHL and CHL respectively corresponding to an intermediate decomposition rates, and the least values ranges from 34.21% - 36.59 corresponding to slow decomposition rates as found in CCS and HCS respectively. It is expected that the dry matter intake of the forages will increase with increased DMd because a higher value of the DMd is better. However, a poor relationship between NDF and DMd was observed due to the highly variable digestibility of NDF, which is in accordance with the report by Mertens, 2000.

The Neutral Detergent Fiber digestibilities (NDFd) were also found to be highest with a range value of 8.50% - 11.52% in CNHL and HHL respectively. The NDFd result for CCS and HCS are potential outliers with a negative impact on their digestibility and the result not presented, which might be because of the time to reach fermentation differs

between forages. Additionally, the *in vitro* lab result depicts that the CCS and HCS will require more time for digestibility than 24h.

Therefore, a faster disappearance of the NDF fraction from the rumen is expected for CHL and HHL as compared to CCS and HCS because of increased rate of digestion, which will reduce physical fill in the rumen over time and allows greater voluntary feed intake (Dineen et al. 2018). The rate of fiber degradation is positively related to DMI (Van Soest, 1982; Mertens, 1987). The uncommon wide range of NDFd is likely to have occurred because corn silage is harvested and stored within a relatively narrow range of maturity. Extremes in corn silage NDFd can occur when corn silage is harvested at an over mature stage (Hoffman et al. 2001).

*Gas Production*

Gas produced is directly from microbial degradation of forages, and indirectly from buffering of acids generated as a result of fermentation (Getachew et al., 2004). The amount of gas produced as an end-product from fermentation by rumen micro-organisms is not constant, but varies with the composition of the volatile fatty acids produced which in turn is substrate dependent as observed from Table 6. There is a very close relationship that exists between volatile fatty acid and gas accumulation. The high gas production values were observed in CHL and HHL. The least gas production levels were observed in CCS and HCS which shows that the degradability of CCS and HCS is low. The differences in gas production dynamics suggest that degradation of CHL and HHL was more rapid than that of CCS and HCS, most likely due to the high NDF and ADF fractions as influenced by the forage type.

*Ruminal Parameters*

The ruminal fermentation traits of CHL and HHL differ from those in CCS and HCS as shown in Table 7. The ammonia concentration varied considerably between HHL, CHL with a range value of 9.42 – 9.62 respectively and a range of 8.39 – 8.63 for CNCS and PMCS respectively, which might be from the variation in the CP contents of the forages. Ruminal ammonia is apt to be limiting with low dietary CP, since ammonia is a final product of CP breakdown in the rumen. It appears that CP content alone may not reflect potential ammonia availability (Yang, 2002), however, all the concentrations for the forages were above 4 to 6 mg/dL, which is considered adequate for ruminal fiber breakdown (Mackie and White, 1990). Van Soest (1982) indicated that forages containing larger amounts of CP generally have a greater digestibility. Van Kessell and Russell 1996 demonstrated that mixed ruminal bacteria only responded to amino nitrogen when carbohydrate fermentation was rapid. The results of two of the tested forages in the present study also showed a similar trend when comparing variations in CP contents and the *in vitro* NDF digestibilities, but a comparison including CCS and HCS negates the assumption. In fact the NDF digestibilities of these two forages were far below the $NH_3$-N concentration assumption. This implies that the effectiveness of ammonia concentration on fiber digestibility may vary, and may be dependent on some intrinsic factors from the forage.

Volatile fatty acids represent about 70 – 80% of energy absorbed by ruminants and both rates and proportions of individual VFA affect energy supply. The greatest total VFA concentrations over the 24 h were observed in CHL and HHL with range of 74.74 - 74.95 respectively, all of which contained low NDF concentration, whilst the least being produced by CCS and HCS from more fibrous forages with range value of 62.36 – 63.69. The acetate concentrations were high for CHL and HHL compared to the values

produced from CCS and HCS. But, propionate concentration was similar for the four forages. According to Grenet & Besle (1991) and Nagadi et al, (2000) degradability of cell wall carbohydrates is mainly limited by lignin content, accentuating its influence on feed utilization (Ahmad & Wilman, 2001). Bosch and Bruining (1995) confirmed that silages with high lignin content have a lower digestibility compared to silages that contained low levels of lignin. Differences among forages in the acetate-to-propionate ratio were high in CHL and HHL as compared to CCS and HCS. In terms of nutritional quality, CHL and HHL was superior to CCS and HCS due to higher values of VFA, Acetate, Propionate, A:P and $NH_3$-N, degradability and low content of NDF.

*Comparison of in situ and in vitro Result*

In this study an *in situ and in vitro* methods of forage DMd and NDFd were presented. Forage NDFd has not been satisfactorily predicted from feed chemical composition (Nousiainen et al., 2004; Huhtanen et al., 2006). Van Soest (1994) stated that chemical variables do not describe the differences between forage types in cell wall structure, which will have greatest impact on DMd and NDFd. For this reason, *in situ* and *in vitro* methods have been developed and related to *in vivo* study on DMd and NDFd. The true digestibility if NDF is nearly complete with small variation mostly caused by procedures (Huhtanen et al., 2006). Consequently, an *in situ* or *in vitro* method with accurate predictive ability should reveal and correlate to differences in forage NDF quality.

*In vitro* incubations indicate net yields from fermentation (ammonia and VFA), while *in situ* incubations measure actual losses through digestion in the rumen. Together, these results provide a comprehensive data-set defining digestion and fermentation of a range of conserved forages and enables ranking in terms of yield and glucogenic potential of the VFAs.

An important aspect of this study was the use of conserved forages prepared by mincing for the wet *in situ* rather than drying, grinding or chopping (Kolver et al., 1998; Barrell et al., 2000). The type of preparation has significant effects on degradation kinetics, which was in accordance with the findings by Barrell et al., 2000. Mincing the conserved forages was intended to replicate chewed material.

Means and standard deviations of soluble (a), insoluble but potentially degradable (b) and undegradable (u) fractions for the five forages for wet and dry set are in Table 4 and 5. It must not be forgotten that values generated from *in situ* techniques are known to have a high level of variation (Mihalet-Doreau and Ould-Bah, 1992; Schwab et al., 2005). And, it was also presented by Edmunds et al. 2012 that it seems likely that even higher differences will occur in different *in situ* procedure. Therefore, it implies that a higher level of agreement between methods cannot be expected.

The *in vitro* method was likely to be sensitive to forage type and prolong *in vitro* incubation time for silage, which was also observed by Krizsan et al., 2012 for some forage types when using an *in vitro* method to evaluate forage feeds. The study reflects that 24h *in vitro* forage analysis might not be appropriate for specific forage type with the dry samples. The wet samples for the *in situ* samples showed high values for potential degradable DM for all the forage samples and the rate of DM degradation was also very high for the wet samples as shown in Table 4. But, Ruminally degradable DM was observed to be low, which was not reflected by the dry samples. Therefore, despite efforts to standardize *in situ* and *in vitro* procedures, laboratory-specific sample preparation still seem to be needed to achieve accurate predictions of forage DMd and NDFd for animal performance.

## Conclusions

The rate and extent of fermentation in the rumen are very important determinants for the nutrients absorbed by ruminants, and goal of a feeding program is to achieve an appropriate balance in available forage nutrient to meet the nutritional needs of animal. But there is potential for significant variation in forage nutrient availability, which can be influenced by forage characteristics and intake potential, thereby suggesting the need for some information as to the nutrient availability in forage before feeding. The most practical approach is through *in situ* and *in vitro* techniques. However, research has proven that results obtained from *in situ* and *in vitro* techniques are often influenced by sample particle size and preparation. There is no standard protocol for sample preparation when using *in vitro* and *in situ* procedure to evaluate the nutritive value of forages. However, technical issues relating to sample preparation need to be considered to ensure that objectives of these trials are properly fulfilled. The result from the study showed that different methods vary in digestibility result, but differences among treatment follow similar patterns among methods, which was in accordance with our hypothesis. Thus, it is critically important to standardize procedures for methods used to evaluate forage quality. This will allow a more accurate ration formulation and animal response prediction.

**Table 1.** Ingredient composition of the total mixed ration fed to lactating cows during the *in situ* and *in vitro* experiment.

| Ingredient | % of DM |
|---|---|
| Corn Silage | 31.69 |
| Alfalfa Haylage | 10.93 |
| Alfalfa Hay | 8.53 |
| Ground corn | 2.02 |
| Whole cotton seed | 6.84 |
| Distillers dried grains | 2.40 |
| Dairy sugar[1] | 4.96 |
| Soybean Meal, 47% CP | 4.90 |
| Bypass SBM[2] | 4.86 |
| Limestone | 0.93 |
| Sodium Bicarbonate | 0.37 |
| Fat [3] | 0.65 |
| Salt | 0.65 |
| Urea | 0.20 |
| Calcium Phosphate 21% | 0.10 |
| Magnesium Oxide 54% | 0.10 |
| Vitamin Premix[4] | 0.16 |
| Yeast[5] | 0.01 |
| Vitamin E 20000 IU/lb | 0.04 |
| Omnigen[6] | 0.03 |
| Rumensin[7] | 0.007 |
| Biotin 1% 9979.2 mg/kg | 0.007 |

[1]Dairy Sugar (Quality Liquid Feeds, Dodgeville, WI). Liquid mixture of cane molasses, condensed whey, and tallow (assay DM basis: 5.7% protein, 27% fat).
[2]Soybest Pearl (Kemin, West Point, NE).
[3]Energy Booster bypass fat (Milk specialties, Eden Prairie, MN).
[4] Avail4 (Zinpro Corp., Eden Prairie, MN).
[5] Diamond V XP Yeast (Diamond V Mills Inc., Cedar Rapids, IA).
[6] Omnigen (Prince Agri Products, Teaneck, NJ).
[7] Rumensin, 198g/kg (Elanco Animal Health, Greenfield, IN).

**Table 2.** Nutrient composition of the total mixed ration fed to lactating cows during the *in situ* and *in vitro* experiment.

| Nutrient Composition[1] | % of DM |
|---|---|
| DM | 47.06 |
| CP | 18.08 |
| aNDF | 26.23 |
| aNDFom | 24.70 |
| ND-ICP | 2.89 |
| Starch | 27.15 |
| Fat (EE) | 5.31 |
| Ash | 7.71 |
| Calcium | 0.97 |
| Phosphorus | 0.36 |
| Magnesium | 0.37 |
| Potassium | 1.40 |
| Sulfur | 0.21 |
| Sodium | 0.47 |
| Chloride | 0.61 |
| DCAD mEq/100g | 25.59 |
| NFC | 47.09 |
| Vit. A (2200 IU/kg) | 6.23 |
| Vit. D (2200 IU/kg) | 1.56 |
| Vit. E (IU/kg) | 34.89 |
| $NE_L$ (Mcal/kg) | 1.72 |
| $NE_g$ (Mcal/kg) | 1.56 |

[1]Units expressed in % of DM unless otherwise noted.

**Table 3.** Chemical composition of test feeds used in the *in situ* rumen degradability and *in vitro* digestibility of Hay forage (Hay) in comparison to Conventional Corn Silage (CCS), Conventional Haylage (CHL), Hybrid Corn Silage (HCS) and Hybrid Haylage (HHL).

| | Treatments | | | | |
|---|---|---|---|---|---|
| Item[1] | Hay | CCS | CHL | HCS | HHL |
| DM, % | 88.44 | 35.13 | 47.38 | 30.69 | 39.70 |
| CP | 19.86 | 7.38 | 21.23 | 8.30 | 19.75 |
| ADF | 31.15 | 26.46 | 25.80 | 25.60 | 32.55 |
| NDF | 41.42 | 36.19 | 29.67 | 33.72 | 34.82 |
| Andf % | 37.48 | 41.12 | 29.45 | 39.67 | 34.87 |
| aNDFom | 35.73 | 40.13 | 27.32 | 38.07 | 31.87 |
| NDFD 30h, % | 46.89 | 50.28 | 53.40 | 56.09 | 49.60 |
| NDFD120h, % | 45.60 | 65.01 | 52.60 | 70.33 | 51.15 |
| NDFD 240h, % | 51.01 | 66.89 | 56.46 | 73.17 | 53.49 |
| uNDFom 30h, % | 18.97 | 19.95 | 12.73 | 16.72 | 16.07 |
| uNDFom 120h,% | 18.35 | 14.04 | 12.19 | 11.30 | 15.10 |
| uNDF 240h, % | 17.52 | 13.29 | 11.90 | 10.22 | 14.83 |
| ND-ICP, % | 3.27 | 1.26 | 3.47 | 1.38 | 3.20 |
| NFC % | 32.64 | 45.18 | 40.74 | 46.91 | 37.10 |
| Calcium | 1.69 | 0.25 | 1.51 | 0.44 | 1.29 |
| Chloride | 0.80 | 0.22 | 0.88 | 0.26 | 0.23 |
| DCAD mEq/100 | 26.32 | 3.11 | 26.45 | 8.39 | 44.01 |
| Magnesium | 0.39 | 0.16 | 0.35 | 0.20 | 0.34 |
| Phosphorus | 0.27 | 0.22 | 0.30 | 0.22 | 0.30 |
| Potassium | 2.25 | 0.61 | 2.43 | 0.88 | 2.43 |
| Sodium | 0.05 | 0.01 | 0.04 | 0.02 | 0.04 |
| Sulfur | 0.18 | 0.11 | 0.21 | 0.12 | 0.21 |

[1]Units expressed in % DM unless otherwise noted.
[2]% NFC= 100- (% Ash + % CP + %NDF + %EE) (NRC, 2001).

**Table 4.** Ruminal DM degradation of Hay forage (Hay) in comparison to Conventional Corn Silage (CCS), Conventional Haylage (CHL), Hybrid Corn Silage (HCS), Hybrid Haylage (HHL) for *in situ* dry and wet trial.

| | Feeds | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| Item[1] | Hay | CCS | CHL | HCS | HHL | WCCS | WCHL | WHCS | WHHL | SEM |
| DM dis, % | | | | | | | | | | |
| A[2] | 40.49$^{de}$ | 48.96$^{abc}$ | 50.69$^{ab}$ | 53.45$^{a}$ | 45.75$^{bcd}$ | 30.02$^{e}$ | 42.18$^{de}$ | 44.52$^{cd}$ | 32.65$^{f}$ | 1.90 |
| B[3] | 39.56$^{ab}$ | 28.54$^{d}$ | 35.24$^{bcd}$ | 29.89$^{dc}$ | 38.10$^{abc}$ | 31.38$^{bcd}$ | 39.69$^{ab}$ | 31.41$^{bcd}$ | 44.85$^{a}$ | 2.06 |
| C[4] | 19.95$^{bc}$ | 22.50$^{abc}$ | 14.07$^{c}$ | 16.65$^{bc}$ | 16.15$^{bc}$ | 29.60$^{a}$ | 18.13$^{bc}$ | 24.08$^{ab}$ | 22.50$^{abc}$ | 2.21 |
| $K_d$[5], %/ h | 8.910$^{a}$ | 3.70$^{b}$ | 8.66$^{a}$ | 3.16$^{b}$ | 7.35$^{ab}$ | 7.18$^{ab}$ | 8.47$^{a}$ | 3.33$^{b}$ | 7.74$^{ab}$ | 1.89 |
| RDDM[6] | 63.12$^{bc}$ | 59.24$^{cd}$ | 70.57$^{a}$ | 63.01$^{bc}$ | 65.60$^{b}$ | 52.16$^{f}$ | 64.22$^{b}$ | 54.35$^{ef}$ | 56.58$^{de}$ | 1.09 |

[1] Units expressed in % DM unless otherwise noted.
[2] Soluble DM.
[3] Potentially degradable DM.
[4] Undegradable DM.
[5] Rate of DM degradation.
[6] Ruminally degradable DM.
W =wet samples
$^{abcdef}$ Values with unlike superscripts differ by $P < 0.05$ using Tukey's test.

**Table 5.** Ruminal NDF degradation of Hay forage (Hay) in comparison to Conventional Corn Silage (CCS), Conventional Haylage (CHL), Hybrid Corn Silage (HCS), Hybrid Haylage (HHL) for *in situ* dry samples.

| | Feeds | | | | | |
|---|---|---|---|---|---|---|
| Item[1] | Hay | CCS | CHL | HCS | HHL | SEM |
| DM dis. , % | | | | | | |
| A[2] | 21.76[a] | 3.85[c] | 15.16[b] | 3.42[c] | 16.57[b] | 1.429 |
| B[3] | 44.16[b] | 84.33[a] | 51.66[b] | 94.45[a] | 51.00[b] | 4.407 |
| C[4] | 30.09[a] | 11.83[bc] | 33.18[ab] | 2.13[c] | 32.44[ab] | 4.269 |
| $K_d$[5], %/ h | 4.74[a] | 1.10[b] | 5.43[a] | 1.11[b] | 4.78[a] | 0.843 |
| RDDM[6] | 39.96[a] | 15.91[b] | 38.12[a] | 17.11[b] | 37.21[a] | 1.759 |

[1] Units expressed in % NDF unless otherwise noted.
[2] Soluble NDF.
[3] Potentially degradable NDF.
[4] Undegradable NDF.
[5] Rate of DM degradation.
[6] Ruminally degradable NDF.
[abc] Values with unlike superscripts differ by $P < 0.05$ using Tukey's test.

**Table 6.** Ruminal fermentation and fiber digestion of Hay forage (Hay) in comparison to Conventional Corn Silage (CCS), Conventional Haylage (CHL), Hybrid Corn Silage (HCS), Hybrid Haylage (HHL) for *in vitro* trial.

| | Treatment[1] | | | | | | *P-values* |
|---|---|---|---|---|---|---|---|
| Item | Hay | CCS | CHL | HCS | HHL | SEM | |
| Acetate, mM | 59.38$^{a}$ | 49.38$^{b}$ | 61.72$^{a}$ | 49.91$^{b}$ | 62.49$^{a}$ | 1.355 | <0.01 |
| Propionate, mM | 8.05$^{b}$ | 8.74$^{ab}$ | 8.77$^{ab}$ | 9.19$^{a}$ | 8.55$^{ab}$ | 0.309 | 0.15 |
| Butyrate, mM | 2.61$^{d}$ | 3.24$^{ab}$ | 2.97$^{bc}$ | 3.45$^{a}$ | 2.76$^{dc}$ | 0.091 | <0.01 |
| Isovalerate, mM | 0.72$^{c}$ | 0.84$^{b}$ | 1.02$^{a}$ | 0.95$^{ab}$ | 0.91$^{ab}$ | 0.025 | <0.01 |
| Valerate, mM | 0.19$^{b}$ | 0.17$^{c}$ | 0.25$^{a}$ | 0.19$^{b}$ | 0.24$^{a}$ | 0.000 | <0.01 |
| A:P | 3.79$^{a}$ | 2.87$^{b}$ | 3.59$^{a}$ | 2.77$^{b}$ | 3.71$^{a}$ | 0.125 | <0.01 |
| Total VFA, mM | 70.95$^{a}$ | 62.36$^{b}$ | 74.74$^{a}$ | 63.69$^{b}$ | 74.95$^{a}$ | 1.526 | <0.01 |
| Acetate, % | 83.72$^{a}$ | 79.16$^{b}$ | 82.54$^{a}$ | 78.34$^{b}$ | 83.35$^{a}$ | 0.532 | <0.01 |
| Propionate, % | 11.34$^{b}$ | 14.02$^{a}$ | 11.78$^{b}$ | 14.44$^{a}$ | 11.42$^{b}$ | 0.404 | <0.01 |
| Butyrate, % | 3.67$^{b}$ | 5.20$^{a}$ | 3.98$^{b}$ | 5.43$^{a}$ | 3.68$^{b}$ | 0.112 | <0.01 |
| Isovalerate, % | 1.01$^{d}$ | 1.35$^{bc}$ | 1.36$^{ab}$ | 1.50$^{a}$ | 1.22$^{c}$ | 0.022 | <0.01 |
| Valerate, % | 0.27$^{c}$ | 0.27$^{c}$ | 0.34$^{a}$ | 0.30$^{b}$ | 0.33$^{a}$ | 0.007 | <0.01 |
| $NH_3$-N, mM | 9.11$^{ab}$ | 8.39$^{c}$ | 9.62$^{a}$ | 8.63$^{bc}$ | 9.42$^{a}$ | 0.116 | <0.01 |
| mL/100mg | 99.67$^{ab}$ | 74.42$^{c}$ | 104.44$^{a}$ | 79.60$^{bc}$ | 100.77$^{ab}$ | 7.014 | 0.01 |
| DMd | 42.85 | 34.21 | 53.24 | 36.59 | 49.34 | 0.994 | <0.01 |
| NDFd | 19.53 | -11.10 | 8.50 | -6.52 | 11.52 | 1.708 | <0.01 |

[1]Hay (**Hay**), Conventional Corn Silage (**CCS**), Conventional Haylage (**CHL**), Hybrid Corn Silage (**HCS**), Hybrid Haylage (**HHL**).

[abcd] Values with unlike superscripts differ by $P < 0.05$ using Tukey's test.

# CHAPTER 3:

## EVALUATION OF EFFECTS ON RUMEN FERMENTATION AND FEED DIGESTIBILITY OF BUFFER SUPPLEMENTS IN LIMIT-FED DIETS HIGH IN DISTILLERS DRIED GRAINS IN DAIRY STEERS

## Abstract

The objective of this study was to determine how dietary inclusion of buffer affects nutrient digestibility and excretion when feeding dairy heifers and steers diets high in distillers grains. The effects on growth, feed efficiency, rumen pH, rumen fermentation, nutrient digestibility, and metabolic profile were investigated. A feeding trial was conducted using 5 cannulated Holstein and Brown Swiss steers in a cross over design with 2-wk periods to evaluate effects of low and high buffer inclusion on limit-fed increased DDGS dietary treatment on dry matter intake (**DMI**), rumen fermentation, and nutrient digestibility. Experimental diets had a similar composition of ingredients but with different buffer inclusion amounts of low and high inclusion rates. Treatments were 1) 40% DDGS with 1.05% low buffer inclusion (**LOBUFFER**) and 2) 40% DDGS at 1.55% high buffer inclusion (**HIBUFFER**). The remainder of the diet consisted of grass hay. Diets were fed at 2.50% of BW. Steers were individually limit-fed using Calan gates. Steers were weighed every 2 wk and diet amount offered was adjusted accordingly. Frame measurements and body condition score (**BCS**) were recorded every 2 wk at the end of each period. Rumen fluid was collected at time points via rumen cannula during wk 0, 2 and 4 for pH, ammonia-N, and volatile fatty acids (**VFA**) analysis. Total tract digestibility of nutrients was evaluated at the end of each period using fecal grab sampling. There were no treatment by period interactions for any of the growth parameters measured and growth parameters did not differ among treatments. Steers had a tendency for lower DMI for **LoBuffer** treatment diet according to *p-values*

among treatments. There was no treatment effect for body weight among the treatments, but a numerical increase in ADG was observed in **LoBuffer** which resulted in significant treatment effect in gain to feed ratio (Gain:Feed) across treatments. As the buffer inclusion amount increased, rumen ammonia-N numerically increased. Acetate proportion and acetate:propionate did not increase with the buffer inclusion amount and the effect was similar among treatment according to *p-value*. There was treatment by time interactions for ammonia-N and total VFA production for the two treatment diets. Buffer inclusion did not increase the total tract digestibility of DM and organic matter (OM). Limit-feeding diets with buffer inclusion for increased amount of DDGS improved gain: feed and maintained frame growth without increasing BCS. Result was not in agreement with our hypothesis because the different amounts of buffer inclusion had similar effect on most parameters measured. However, treatment diets with two buffer concentration and increased amounts of DDGS in limit-fed rations was able to maintain steer growth performance. Although, there will be need for more research on the appropriate and precise inclusion rate of buffer.

**Keywords:** distillers grains, dairy steers, dairy heifer, buffer, growth performance.

## Introduction

Limited research regarding the feeding of dried distillers grains with solubles (DDGS) to growing dairy replacement heifers is available. Only few studies (Schroer et al., 2014; Anderson et al.,2015; Manthey et al., 2016) have been published. Remarkable increase in ethanol production has resulted in large amounts of distillers dried grains with solubles (DDGS) as a valuable feedstuff for dairy and livestock rations. However, the high fiber content in DDGS may reduce nutrient digestibility and feeding high-fiber feedstuffs to growing heifers may decrease diet digestibility (Zanton and Heinrichs, 2008), as the level of distillers dried grains with solubles increase compared to corn. Therefore, understanding of the digestible nutrient content is critical to achieve accurate diet formulation (Widyaratne et al., 2006), as DDGS has high concentrations of certain nutrients. Some understanding of the effects of feeding distillers grains to dairy heifers can be gotten from similar comprehensive research on steers (Klopfenstein et al., 2008; Schingoethe et al., 2009). Utilizing a limit-feeding strategy in which nutrient-dense diets are fed to meet but not exceed nutrient requirements to maintain growth performance, is an increasing area of interest in research for growing heifers and steers. Distillers Grains have become a global commodity for trade and DDGS has been proven to be a rich source of significant amounts of protein, amino acids, phosphorus, and other nutrients for dairy heifer feed. It is an excellent source of RUP (Powers et al., 1995). Although, inclusion of DDGS in diets of growing heifers has been found to support growth (Manthey et al, 2017), the effect of feeding on nutrient excretion needs to be further evaluated. Research has shown that cattle fed DDGS had higher concentrations of nitrogen, phosphorus and sulfur in the blood as the proportion of DDGS in the diet increased (Swanson, 2010). High concentrations of some minerals in DDGS affect the value and end use of DDGS as animal feed because of nutritional disorder and excessive minerals in waste.

Therefore, feeding a diet high in DDGS may cause environmental concern by increasing the excretion of those nutrients. However, studies on nutrient digestibility and excretion have been rarely considered when the inclusion rate of DDGS in a ration is determined for growing dairy heifers and steers.

Phosphorus and sulfur are of greatest interest and it has a significant implication in not only animal nutrition but also the environment. Although the concentration of phosphorus varies among papers, a range of 0.5-1.0% is generally agreeable (Liu, 2011), and sulfur may exceed 1%. Such a concentration range is much higher and exceeds the requirements for growing heifers and steers, thus the high phosphorus concentration of DDGS has become an emerging issue. In addition, nutrient excretion is a major concern for the dairy industry due to its potential impact on the environment. When growing heifers and steers consume diets containing high concentrations of phosphorus, such as diets with high DDGS inclusion, the amount of phosphorus and sulphur excreted in waste is increased.

Additives are commonly used in the dairy industry to increase N utilization efficiency and can alter ruminal S metabolism by altering rumen microbial community (Martineau et al., 2007). Increased apparent absorption and retention of certain minerals including P was observed (Greene et al., 1986). Some additives are fed as salt to function by creating a shift in ion transfer across the cell's membrane, thus shifting the rumen population to produce a different volatile fatty acid profile. Supplements that provide natural buffering agents secreted in cow's saliva have been reported to maintain healthy rumen environment and effective rumen buffer increases digestibility. Therefore, buffer supplements inclusion with high DDGS diet could potentially affect nutrient digestibility and excretion for dairy heifers and steers.

The objective of our study is to determine how dietary inclusion of buffers affects nutrient digestibility and excretion when feeding dairy heifers diets high in distillers grains. It was hypothesized that the different inclusion amounts of buffer would maintain steer growth performance over the short period length due to limit-feeding, but there would be changes in rumen fermentation for the different levels of buffer inclusion. It was hypothesized that total-tract digestibility and excretion of nutrients will be improved by increased buffer inclusion amount.

## Materials and Methods

### *Animal Care*

This study was conducted at the South Dakota State University Dairy Research and Training Facility (**SDSU DRFT**; Brookings, SD). The study was conducted from August 2018 through September 2018. Steers were observed daily for health problems and treated according to routine management practices at the DRTF.

### *Experimental Design*

Three Brown Swiss steers (336 $\pm$ 13d of age; 375 $\pm$ 28kg) and two Holstein steers (255 $\pm$ 1d of age; 285 $\pm$ 3kg) were used in a cross-over design with two treatment diets. Originally, there were six steers but a Holstein steer died just prior to starting the study. Steers were paired based on birth date, breed and BW. Treatments were randomly assigned to each animal in the pair. Steers were acclimated to the barns and feeding system followed with one week of covariate sampling and by 2 experimental feeding periods of 2 weeks. During the covariate week steers were fed the herd diet for ad libitum intake.

Treatment diets (Table 7) were: 1) 0.5% mineral mix, 0.3% Salt and 0.25% Calcium Carbonate (**LOW BUFFER)**, and 2) 0.5% mineral mix, 0.25% Salt and 0.8%

Calcium Carbonate (**HIGH BUFFER**) on DM basis. The remainder of the diets consisted of 58.97% of grass hay and 39.98% of DDGS for the Low Buffer diet, and 58.42% of grass hay and 40.03% of DDGS for the High Buffer diet. Both were fed at 2.5% of body weight (**BW**). Diets were formulated using the NRC (2001) to meet a target of 0.85 kg/d when fed to a 250 kg BW Holstein heifer and to provide similar energy intakes. The 250 kg BW was pre-estimated average BW for Brown Swiss steers during the study based on age and herd data. On the last two days of each two weeks interval, steers were weighed and then the amount of feed offered was determined for the next two weeks, except for the covariate week that it was just for one week. Amount of each ration offered was also adjusted using DM analysis of feeding ingredients. The diet for each animal was switched at the end of two weeks.

*Animal Feeding*

All five steers were housed in a single pen of five steers. The pen had an inside roofed area (7m x 4m) and an outside dirt exercise lot (7m x 23.5). The inside areas of the pens were a bedded pack, and were bedded with wood shaving once a week. Because the consumption of bedding material can be a concern when limit-feeding, pens were bedded several days ahead of sampling. The pen was provided with water ad lilbitum. Steers were fed once daily at 0800 h using the calan gate feeding system (American Calan Inc., Northwood, NH) and individual intakes were measured. Bales of hay were coarsely pre-ground with a vertical tub grinder to ease hand mixing. Diet components were individually weighed and hand mixed for each steer. The mineral mix was hand mixed with the DDGS before mixing with the grass hay. Because steers were limit-fed and were expected to consume all feed, particle sorting was a minor concern. Any orts were weighed and recorded every morning before feeding. Samples of DDGS and grass

hay were taken each week and stored at -20℃ until analysis, but no ort sample was collected for analysis as there were no refusals for collection at the end of each period.

*Animal Measurements and Sampling*

Body growth measurements including BW, withers and hip heights, heart and paunch girth, body length, and hip width were measured on 2 consecutive days approximately 4 h post-feeding at the beginning of the study and then every 2 weeks at the end of each period. Body length was measured from the top point of the withers to the end of the ischium (Hoffman, 1997). Body condition scores (BCS) were assessed at the start of the experiment and then every 2 weeks thereafter for the remainder of the study by 3 independent observers based on the scale described by Wildman et al. (1982) with 1= emaciated and 5= obese.

Rumen fluid was sampled from each steer for one day during covariate, and at the end of each 2-wk period at 0.5 h before feeding and 2, 4, 6, 8, 12, 18 and 24 hours post-feeding, rumen fluid was collected via the rumen cannula. Approximately 50 mL of rumen fluid was collected from 3 to 4 different sites in the rumen. Samples were immediately measured for pH using a pH meter (Waterproof pH Testr 30, Oakton Instruments, Vernon Hills, IL) and 2 aliquots (10 mL) were collected with a syringe and acidified with either 200 $\mu$L of 50% (volume/volume) sulfuric acid or 2 mL of 25% (weight/volume) metaphosphoric acid and stored at -20℃ until later analyses of ammonia N (**$NH_3$-N**) and volatile fatty acid (**VFA**) analysis, respectively.

For analysis of total tract digestibility, fecal samples were collected during week 2 at the end of each feeding period. Acid detergent insoluble ash (**ADIA**) was used as an internal digestibility marker. Fecal grab samples were collected in a rotational schedule during 2.5 consecutive days at the end of each period, stored at -20℃ until processing

and analysis. Fecal sampling time points were scheduled so that the samples represented every 3 h in 24 h feeding cycle.

*Laboratory Analysis*

Total dietary nutrient concentrations were calculated based on analysis of grass hay and DDGS for each treatment. Feed samples were dried for 24 h at 105℃ for DM analysis in order to adjust dietary ingredient inclusion rates and determine DMI. Samples of DDGS and grass hay were collected once weekly and frozen at -20℃ until analysis. Samples of DDGS and grass hay were thawed and samples for each week were composited and sub-sampled on as-fed basis by weight for lab analysis. Composite samples were dried in duplicate for 48h at 55℃ in Despatch oven (Style V-23, Despatch Oven Co. Minneapolis, MN), ground to 4mm particle size with a Wiley Mill (model 3; Arthur H. Thomas Co. Philadelphis, PA), and then further ground to 1 mm particle size using an ultracentrifuge mill (Brinkman Instruments Co., Westbury, NY). In order to correct analysis to 100% DM, 1g aliquots of feed samples were dried for 4 h in a 105℃ oven. Ash content was determined by incinerating 1g sample for 8 h at 450℃ in a muffle furnace (AOAC 17$^{th}$ ed., method 942.05; 2002). Organic matter (**OM**) was calculated as OM= (100-%Ash). Samples were analysed for nitrogen content via combustion method (AOAC 16$^{th}$ ed., method 990.03), on a Rapid N Cube (Elementar Analysensysteme, GmbH, Hanau, Germany). Nitrogen content was then multiplied by 6.25 to calculate CP. Neutral detergent fiber (**NDF;** Van Soest et al., 1991) and acid detergent fiber (**ADF**; Robertson and Van Soest, 1981) were analyzed sequentially using the Ankom 200 fiber analysis system (Ankom Technology Corp., Fairport, NY). For NDF, heat-stable alpha-amylase and sodium sulphite were used. Petroleum ether was used to determine ether extract (**EE**; AOAC 2002, method 920.39) in an Ankom XT10 fat analysis system (Ankom Technology Corp., Fairport, NY). Petroleum ether has been recommended for

EE analysis (Thiex, 2009) because Diethyl ether tends to overestimate EE in DDGS. Non-fibrous carbohydrate was calculated as %NFC = 100- (% Ash + % CP + % NDF + % EE) according to the NRC (2001).

Dried and ground samples of grass hay and DDGS were composited and sub-sampled into weekly composites and sent to a commercial laboratory (Dairyland Laboratories, Inc. Arcadia, WI) for analysis of minerals (Ca, Cl, Mg, P, K, Na, and S) and starch. Mineral content, excluding chloride, was determined using inductively coupled plasma spectroscopy (AOAC International, 1995). Chloride content was determined using a direct reading chloride analyzer (Corning 926, Corning Inc., Corning, NY). Non- Fibrous Carbohydrate (NFC) was calculated as %NFC = 100- (% Ash + % CP + % NDF + % EE) according to the NRC (2001).

Rumen fluid samples preserved with sulfuric were thawed and centrifuged at 30,000 x g for 10 minutes at 4℃ (Centrifuge: Eppendorf 5403, Eppendorf North America, Hauppauge, NY) and analysed for ammonia N using a colorimetric assay performed on a micro-plate spectrophotometer (Cary 50, Varian Inc., Walnut Creek, CA) according to Chaney and Marbach (1962). Rumen fluid samples that were preserved with metaphosphoric acid were thawed and centrifuged at 30,000 x g for 20 minutes at 4℃ and analysed for acetate, propionate, butyrate, isobutyrate, isovalerate, and valerate concentrations using an automated GC (model 6890; Hewlett-Packard Co., Palo Alto, CA) using a flame-ionization detector. Volatile fatty acids were separated on a capillary column (15 m x 0.25 mm i.d.; Nukol, 17926-01C; Supelco Inc., Bellefonte, PA) using 2-ethyl-butyrate as an internal standard. The split ratio of 100:1 in the injector port was at a temperature of 250℃ with flow rate of 1.3 mL/min of helium. The column and detector temperature were maintained at 140℃ and 250℃ respectively.

Fecal samples for each steer were composited on as-is basis by volume. Aliquots of 100 ML of fecal samples were taken from each time point and composited. Samples were then dried and ground as previously described for feed samples. Fecal samples were analysed for DM, Ash, CP, NDF, and ADF as previously described for feed samples. Acid detergent insoluble ash analysis was conducted on all feed composites and fecal samples. The method for ADIA analysis consist of analysing the sample for ADF content (Robertson and Van Soest, 1981) and then determining the ash content using a modified procedure of the AOAC 17$^{th}$ ed., method 935.29 (2002). Digestibility calculations were determined according to Merchen (1988).

*Statistical Analysis*

All data were analysed using SAS version 9.4 (SAS Insitute Inc., Cary, NC). The MEANS procedure of SAS was used to estimate the means and standard errors of the nutrients of the weekly feed composites.

Steers intake, growth data, rumen fermentation parameters and total tract digestibility were analyzed using the PROC MIXED procedures of SAS (Littell et al., 2006). The model included treatment, period, and treatment x period interactions. Initial body size measurements and BW were included as covariates within the model. Least square means for each treatment are reported in the tables. Significant differences among treatments was declared at $P \leq 0.05$ and a tendency was declared at $0.05 < P \leq 0.10$.

Data from the analysis of steer plasma metabolites and rumen fermentation characteristics were analyzed with repeated measures by time points using MIXED procedures (Littell et al., 2008). The main time effects in the model were treatment, time and treatment by time interactions. Values for initial measurement were found to be different among treatments, so they were accounted for within the model as a covariate term. For the repeated measures, Akaike's criterions were used for each each parameter

to determine the most suitable covariance structure. Least square means for each treatment are reported in the tables.

## Results and Discussion

*Feed Analysis*

In the formulation of treatment diets, pre-trial samples and book values were used and the two diet compositions are presented in Table 7. The individual ingredients used in the experimental diet were also presented.

The ingredient nutrient compositions are presented in Table 8. The DDGS and hay percentages of the treatment diets differed slightly with high buffer diet having 40. 03 and 39.98 and low buffer diet having 58.42 and 58.97 for DDGS and grass hay respectively. Because the DDGS was supplied in one batch, nutrient composition of the DDGS did not vary much over the duration of the study; however, there was some variation in the nutrient composition of the grass hay during the experiment. Nutrient composition was based on individual ingredient analysis during the course of the study. The buffer concentration increased with increased buffer inclusion amount as expected due to experimental design. Non-Fibrous Carbohydrates concentrations were low across all diets. Therefore, the other nutrients including fat, fiber and protein rather than starch were the major energy sources in the diets and we speculate that fiber will be the major source of energy (Russell, 1998). Since, the concentration of DDGS in the diets were high, energy density of diets will also be high justifying the use of limit-feeding to avoid overconsumption and high ADG as seen by Anderson et al. (2009 and 2015d).

Sulfur toxicity which can occur when feeding large amounts of DDGS (Schingoethe et al., 2009), was not an issue in this study. Calcium carbonate was included in the experimental diets for buffering and to mitigate any risk of sulfur toxicity.

Steers did not have orts, except for days following straw bedding. Additionally, steers were weighed and then amount of feed offered was determined for the next two weeks, so as steers were always gaining weight daily the DMI as percentage of BW was constantly increasing during the 14 d intervals.

*Steer Growth Performance*

Body weight, DMI, and gain: feed results are presented in Table 11. The BW and ADG based on two week interval calculations were similar among treatments. And, no significant treatment effect was observed for BW at$P$ = 0.78 and ADG at $P$ = 0.93. The ADG in this trial was greater than the target recommendation of 0.8 kg/d (Zanton and Heinrichs, 2005). Because this research was intended to build upon the research conducted by Anderson et al. (2015a;b) and Manthey et al.(2016), the NRC (2001) model was used to formulate the diets. The results from this experiment and Anderson et al. (2015a, b); Manthey et al.(2016) suggest that the NRC (2001) model overestimates the energy requirements of growing dairy heifers or underestimates energy provided by DDGS. The current experiment and Anderson et al. (2015a;b); Manthey et al.(2016) demonstrate that heifers can be limit-fed diets with DDGS to control ADG, but the amount to be offered should be less than NRC (2001) recommendations to achieve this recommended ADG.

Dry matter intake had a tendency to be different among treatments with a *P-value* of 0.06 and gain: feed (Table 11) had a significant effect across treatments with a *P-value* of 0.003.

Frame size measurements and BCS are presented in Table 12. There was no treatment effects for most frame size parameters measured, Wither Height ($P$= 0.27), Paunch Girth ($P$= 0.29), Hip Width ($P$= 0.31) and BCS ($P$= 0.99) There were significant treatment effects for Hip Height ($P$< 0.01) and Hearth Girth ($P$= 0.03), although the

steers are still actively growing and frame size is expected to increase over time. There were also no differences in change per day for any of the frame growth measurements, suggesting that all treatment diets provided adequate ME and protein to maintain growth during the experimental period. There were no differences among treatment for BCS (Table 12). Throughout the experiment, steers maintained BCS above 3.0, indicating that the nutrient digestibility was high and buffer inclusion will require lesser amount of DDGS for increase production of heifers at a low cost. Also, the study was of short duration.

*Blood Metabolites*

Plasma concentrations of cholesterol, glucose and plasma urea nitrogen were presented in Table 14. There were treatment significances for mean concentrations of cholesterol and glucose. The treatment effect for cholesterol mean concentration was $P$=0.02 and tendency was observed for Treatment by time interactions ($P$= 0.08), but treatment effect was significance at 8 h time point ($P < 0.01$) as shown in Fig. 4. The cholesterol concentration for treatment diets were above 21 mg/dL which is close to the recommended value of 20 mg/dL (Stewart et al., 2017). There was time and treatment by time interaction effect for PUN as shown in Fig. 6. Blood urea nitrogen was also greater than 20 mg/dL, but blood urea nitrogen above 20 mg/dL has been associated with decreased conception and pregnancy rates in cattle (Elrod and Butler, 1993). In ruminants, blood urea nitrogen concentration is associated with dietary CP intake, which is ruminally degraded resulting in greater production of ruminal ammonia that is transported into the blood stream. When ammonia reaches the liver, it will be metabolized into urea and secreted into the blood (Broderick and Clayton, 1997). Then constant recycling of N to the rumen from the blood stream (Owens and Zinn, 1988) is occurring, excess ammonia is absorbed via the rumen wall and converted into urea in the

liver. The amount of N recycled to the rumen is reduced when ruminal $NH_3$-N concentrations are high (Owens and Zinn, 1988). This might be the driving factor for the high PUN concentration. This is indicating that buffer inclusion with DDGS will require lesser amounts of DDGS in ration formulation.

*Rumen Fermentation Characteristics*

The total ruminal volatile fatty acid, molar proportions of individual volatile fatty acid, pH and $NH_3$-N concentrations varied ($P < 0.01$) within each of the time points for each treatment as shown in Table 13. The pH result as shown from Table 13 was maintained within 6.4 throughout the entire time of rumen degradation by the microbes. There was a gradual release of buffer which helps to resist a change in pH as shown in Fig. 1 because there was treatment by time interactions across the treatment diets. All the rumen fermentation characteristics has significance time effect ($P < 0.01$). For the fermentation pH, effects of buffer amounts were evident within the first hours (up to two) hours of post feeding and which was consistent over time. Rumen pH can affect the relative proportions of rumen mirobes (fibrolytic vs. non-fibrolytic) and the quantity and ratio of end products produced (VFA). Feed efficiency will be impaired when rumen pH levels fluctuate widely throughout the day or when rumen pH is below optimum. By resisting changes in pH and maximizing fermentation, the cow obtained more VFA for energy and more microbial protein. The fibrolytic microorganisms activity was able to be sustained for a long time and which is in accordance with our speculation to improve the fiber digestibility

The rumen fermentation parameters were presented in Table 13. Rumen ammonia- nitrogen ($NH_3$-N) concentration was similar between the HiBuffer and LoBuffer treatments. The rumen ammonia concentration increased ($P < 0.01$) from 2h post feeding, then declined with the largest reduction noted at 12h as shown in Fig. 2.

The concentrations of ruminal $NH_3$-N were noted to be greater for HiBuffer treatment at various time points. The concentration was enough for efficient utilization of nitrogen (5mg/dL) (Satter and Roffler, 1974) for both treatments. Ammonia is used for protein synthesis within the rumen and accumulates when protein degradation exceeds microbial requirements (NRC, 2001). This suggests that protein digestion in the rumen was more efficient, which is reflected in the PUN result.

The total concentration of volatile fatty acids (VFA) did not differ among treatments. But, there was a time point effect as shown in Fig. 3. Acetate:propionate (A:P) was also similar among treatments and it is in accordance with the recommendation from Wasielewska et al., 2015 that under optimal rumen fermentation conditions, the A:P ratio should be greater than 2.2 to 1. The high concentrations of acetate with the increased amounts of DDGS indicate high fiber-low fermentable carbohydrate content, which is in agreement with our hypothesis for buffer inclusion to improve the fiber digestibility. Since, high ruminal fermentation that is consistent over time is the goal. In addition, the total VFA (81.5 vs 73.2) and A:P (2.2 vs 2.1) concentrations were greater compared to those reported by Manthey et al., 2016, which shows that acetate concentrations (43.8 vs 41.9) and propionate concentrations (25.5 vs 19.9) was greater at the same DDGS inclusion rate for the same 4 h time point.

*Apparent Tract Nutrient Digestion*

Apparent tract nutrient digestibility is presented in Table 15. Digestibility of NDF and ADF were similar among treatments. With feeding increased amounts of DDGS, greater amounts of fat consumed potentially could interfere with fermentation because of the effects of unsaturated lipids on microbial growth and negatively affect the digestibility of nonlipid energy sources (Jenkins, 1993; NRC, 2001). However, even with 40% inclusion rate of DDGS in the diet, total diet EE concentration was 3.9 as shown in

Table 9, which is less than 8% which is thought to be upper limit before fat concentration begins to have negative effects on rumen degradation of fiber and DM (Palmquist, 1994; NRC 2001). Anderson et al. (2015a) speculated that the fat from DDGS is bound within the feed particle and had less severe effects on digestion of nutrients because it is slowly introduced in the rumen.

The apparent tract digestibility of nutrients are within the normal digestibility values from previous research studied which is speculated to be as a result of the small particle size of DDGS resulting from a fast passage rate and low retention time. This is also in agreement with Van Soest, 1982, and Merchen, 1988 that suggested a reduction in total tract digestibility may be due to an increase in digestion rate and passage rate through the rumen. Although, physical processing of forages by grinding does provide a greater surface area for attack by enzymes, utilization of structural carbohydrate is not increased; rather, improvements in animal performance arise primarily from an increased digestible energy intake (Bourquin et al., 1990). In fact, fiber digestibility is reduced by 3.3% as a result of reduced residency time in the rumen (Varga and Kolver, 1997). However, the NDF (62 vs 57) and ADF (58 vs 52) digestibility from this study was higher than reported studies from Manthey et al., 2016 and Morris et al., 2018.

Nutrient digestibility of the treatment diets were similar despite differences in buffer inclusion amounts. Therefore, less buffer inclusion amount is likely to be needed to improve and increase fiber utilization of DDGS to achieve similar ADG.

## Conclusions

In disagreement with our hypothesis, limit feeding diets containing increased differ buffer inclusion in diets high in DDGS was maintained growth performance of dairy steers and likewise heifers based on BW, ADG, frame growth and rumen parameters. However, ADG was greater than NRC (2001) predictions for both

treatments. In addition, dietary buffer inclusion with increased amount of DDGS increased gain: feed. Rumen fermentation was maintained and not different between treatments. There were some shifts in blood metabolites between treatments. Overall, under conditions of this study. Results indicated that dietary buffer inclusion had limited impacts on DDGS utilization and rumen fermentation.

**Table 7.** Ingredient composition of buffer inclusion treatment diets for growing dairy steers limit-fed with increased amounts of distillers dried grains with solubles (DDGS).

| | Treatment[1] | |
|---|---|---|
| Item | HiBuffer | LoBuffer |
| Ingredient[2] % DM | | |
| DDGS | 40.03 | 39.98 |
| Grass hay | 58.42 | 58.97 |
| Mineral mix | 0.50 | 0.50 |
| Salt | 0.25 | 0.30 |
| Calcium Carbonate | 0.80 | 0.25 |

[1]High inclusion rate of Buffer (**HiBuffer)**; Low inclusion rate of Buffer (**LoBuffer)**.
[2] Formulated using NRC, 2001.
[3] Contained: 3.2 g/kg of lasalocid sodium, 18.9% Ca, 24.3% NaCl, 1.60% Mg, 0.50% K, 3,880 mg/kg Zn, 880 mg/kg Cu, 50 mg/kg I, 25 mg/kg Se, 550,000IU/lb Vitamin A, 110,000 IU/kg Vitamin $D_3$, and 4180 IU/kg Vitamin E (HeiferSmart No Phos B2909 Medicated, Purina Animal Nutrition, LLC.).

**Table 8.** Nutrient composition of the grass hay and distillers dried grains with solubles (DDGS) used in the treatment diets limit-fed to growing dairy steers.

| | Grass hay | | DDGS | |
|---|---|---|---|---|
| Item[1] | Mean | SE | Mean | SE |
| DM[2], % | 86.62 | 1.251 | 90.50 | 0.254 |
| Ash[2] | 11.29 | 0.549 | 5.18 | 0.068 |
| OM[2] | 52.19 | 0.322 | 37.94 | 0.058 |
| CP[2] | 10.03 | 0.444 | 30.88 | 0.037 |
| ADF[2] | 42.13 | 2.042 | 11.21 | 0.741 |
| NDF[2] | 75.45 | 1.087 | 35.12 | 1.417 |
| EE (Petroleum)[2] | 1.67 | 0.151 | 8.08 | 0.096 |
| NFC[2,3] | 2.25 | 1.239 | 20.75 | 1.550 |
| Ca[4] | 0.48 | 0.032 | 0.13 | 0.021 |
| Chloride[4] | 0.56 | 0.053 | 0.18 | 0.005 |
| Potassium[4] | 1.95 | 0.102 | 1.18 | 0.005 |
| Magnessium[4] | 0.20 | 0.011 | 0.38 | 0.005 |
| Sodium[4] | 0.03 | 0.011 | 0.24 | 0.011 |
| Phosphorus[4] | 0.20 | 0.005 | 0.91 | 0.005 |
| Sulfur[4] | 0.20 | 0.005 | 0.62 | 0.005 |
| DCAD[4]mEq/100g | 23.00 | 1.459 | -3.55 | 0.289 |

[1] % DM, unless otherwise indicated.
[2] Results from analysis of weekly composites (n=4).
[3] %NFC = 100 – (% Ash + % CP + % NDF + % EE) (NRC, 2001).
[4] Results from analysis of 4 weeks composites (n = 4).

**Table 9.** Nutrient composition of buffer inclusion treatment diets for dairy steers limit-fed with increased amounts of distillers dried grains with solubles (DDGS).

| | Treatment[1] | |
|---|---|---|
| Item | Hi Buffer | LoBuffer |
| Dry Matter | 86.69 | 87.40 |
| CP | 18.22 | 18.27 |
| ADF | 25.50 | 25.43 |
| NDF | 50.94 | 51.18 |
| EE | 3.91 | 3.90 |
| OM | 89.87 | 90.35 |
| NDF | 50.50 | 50.90 |
| NFC | 27.40 | 27.50 |
| DCAD, mEq/100g | 25.39 | 58.96 |
| Ca | 0.76 | 0.52 |
| Magnesium | 0.27 | 0.27 |
| Potassium | 1.61 | 1.62 |
| Sodium | 0.26 | 0.21 |
| Chloride | 0.61 | 0.67 |
| Ether extract | 3.60 | 3.60 |
| Phosphorus | 0.48 | 0.48 |
| Sulfur | 0.37 | 0.37 |
| Magnesium | 0.44 | 0.71 |
| ME, Mcal/kg | 2.36 | 2.37 |
| NEl, Mcal/kg DM | 1.48 | 1.49 |
| $NE_G$, Mcal/kg | 0.88 | 0.89 |

[1]Units expressed in % DM unless otherwise noted.
[2]% NFC= 100- (% Ash + % CP + %NDF + %EE) (NRC, 2001).

**Table 10.** Mean nutrient intakes for dairy steers limit-fed buffer inclusion treatment diets with increased amounts of distillers dried grains with solubles (DDGS).

| | Treatment[1] | | | *P-values*[3] |
|---|---|---|---|---|
| Intake, kg/d | HiBuffer | LoBuffer | SEM | Trt |
| DMI | 7.26 | 7.18 | 0.598 | 0.06 |
| ADF | 1.85 | 1.82 | 0.152 | 0.02 |
| NDF | 3.70 | 3.67 | 0.305 | 0.28 |
| CP | 1.32 | 1.31 | 0.109 | 0.16 |
| EE | 0.32 | 0.32 | 0.027 | 0.52 |
| OM | 6.52 | 6.48 | 0.539 | 0.33 |

[1]High buffer inclusion (**HiBuffer**), Low buffer inclusion (**LoBuffer).**
[2]Significance of effects for treatment (**Trt)**.

**Table 11.** Dry matter intake, body weights, and gain to feed ratios for dairy steers limit-fed buffer inclusion treatment diets with increased amounts of distillers dried grains with solubles (DDGS).

| | Treatments | | | *P-values* |
|---|---|---|---|---|
| Item | HiBuffer | LoBuffer | SEM | Trt |
| BW, kg | 164.70 | 164.50 | 1.430 | 0.78 |
| DMI, kg | 7.26 | 7.18 | 0.600 | 0.06 |
| ADG[2],kg/d | 1.09 | 1.12 | 0.185 | 0.93 |
| Gain:Feed | 0.15 | 0.16 | 0.013 | <0.01 |

[1]High buffer inclusion (**HiBuffer**), Low buffer inclusion (**LoBuffer).**
[2]Significance of effects for treatment (**Trt)**.

**Table 12.** Frame size measurements for dairy steers limit-fed buffer inclusion treatment diets with increased amounts of distillers dried grains with solubles (DDGS).

| | Treatment | | | *P - values* |
|---|---|---|---|---|
| Item | HiBuffer | LoBuffer | SEM | Trt |
| Withers Height, cm | 128.53 | 129.06 | 0.33 | 0.27 |
| Hip Height, cm | 131.83 | 133.10 | 0.333 | <0.01 |
| Heart Girth, cm | 158.54 | 159.58 | 0.457 | 0.03 |
| Paunch Girth, cm | 201.80 | 200.84 | 1.38 | 0.29 |
| Hip Width, cm | 41.43 | 41.64 | 0.163 | 0.31 |
| Body Length, cm | 123.59 | 122.59 | 0.603 | 0.09 |
| BCS[2] | 3.35 | 3.35 | 0.017 | 0.99 |

[1]High buffer inclusion treatment (**HiBuffer**), Low buffer inclusion treatment (**LoBuffer**).
[2]Body condition score with 1 = emaciated and 5 = obese (Wildman et al., 1982).

**Table 13.** Rumen fermentation parameters for dairy steers limit-fed buffer inclusion treatment diets with increased amounts of distillers dried grains with solubles (DDGS).

| | Treatment | | | *P – values* | | |
|---|---|---|---|---|---|---|
| Item | HiBuffer | LoBuffer | SEM | Trt | Time | Trt × Time |
| pH | 6.46 | 6.45 | 0.063 | 0.86 | 0.50 | 0.21 |
| $NH_3$-N, mg/dL | 9.66 | 8.87 | 0.608 | <0.01 | <0.01 | 0.39 |
| Total VFA,mM | 79.52 | 80.85 | 3.685 | 0.71 | <0.01 | 0.95 |
| Acetate, mM/100mM | 48.29 | 48.54 | 2.134 | 0.91 | <0.01 | 0.94 |
| Propionate, mM/100mM | 21.83 | 22.99 | 1.043 | 0.32 | <0.01 | 0.61 |
| Butyrate, mM/100mM | 7.13 | 7.21 | 0.592 | 0.86 | <0.01 | 0.90 |
| Isovalerate, mM/100mM | 1.32 | 1.21 | 0.055 | 0.04 | <0.01 | 0.44 |
| Valerate, mM/100mM | 0.98 | 0.91 | 0.433 | 0.19 | <0.01 | 0.74 |
| Acetate:Propionate | 2.25 | 2.21 | 0.043 | 0.60 | <0.01 | 0.50 |

[1]High buffer inclusion treatment (**HiBuffer**), Low buffer inclusion treatment (**LoBuffer**).
[2]Significance of effects for treatment (**Trt**), Time (**T**), and Treatment x Time (**Trt** x **T**).

**Table 14.** Plasma metabolites for dairy steers limit-fed buffer inclusion treatment diets with increased amounts of distillers dried grains with solubles (DDGS).

| | Treatment[1] | | | *P values*[2] | | |
|---|---|---|---|---|---|---|
| Item | HiBuffer | LoBuffer | SEM | Trt | Time | Trt × T |
| Cholesterol, mg/dL | 44.71 | 40.69 | 1.94 | 0.02 | 0.35 | 0.08 |
| 0 hr | 46.01 | 42.09 | 2.55 | 0.18 | | |
| 4 hr | 42.62 | 43.22 | 2.55 | 0.84 | | |
| 8 hr | 45.49 | 36.76 | 2.55 | <0.01 | | |
| Glucose[3], mg/dL | 80.97 | 83.43 | 2.06 | 0.01 | 0.11 | 0.13 |
| 0 hr | 83.33 | 83.12 | 2.27 | 0.90 | | |
| 4 hr | 79.17 | 82.44 | 2.27 | 0.05 | | |
| 8 hr | 80.40 | 84.73 | 2.27 | 0.01 | | |
| PUN, mg/dL | 21.98 | 21.21 | 0.97 | 0.23 | <0.01 | <0.01 |
| 0 hr | 22.42 | 20.73 | 1.15 | 0.13 | | |
| 4 hr | 24.61 | 21.82 | 1.15 | 0.02 | | |
| 8 hr | 18.91 | 21.07 | 1.15 | 0.06 | | |

[1]High buffer (**HiBuffer**), Low buffer (**LoBuffer)**.
[2]Significance of effects for **(Trt)**, Time (T), and Treatment x Time (**Trt x T**).
[3]Glucose was measured from serum samples instead of plasma.

**Table 15.** Total tract digestibility of nutrients for dairy steers limit-fed buffer inclusion treatment diets with increased amounts of distillers dried grains with solubles (DDGS).

| | Treatment[1] | | | *P-values*[2] |
|---|---|---|---|---|
| Item, % | HiBuffer | LoBuffer | SEM | Trt |
| DM | 62.06 | 61.96 | 1.961 | 0.92 |
| OM | 67.97 | 67.81 | 1.658 | 0.87 |
| CP | 74.00 | 73.13 | 1.558 | 0.24 |
| NDF | 62.25 | 62.75 | 1.778 | 0.70 |
| ADF | 58.30 | 58.86 | 1.930 | 0.69 |

[1]High buffer inclusion (**HiBuffer**), Low buffer inclusion (**LoBuffer).**
[2]Significance of effects for treatment (**Trt)**.

.

**Figure 1.** Rumen pH in dairy steers limit-fed buffer inclusion treatment diets with increased amounts of distillers dried grains with solubles (DDGS).

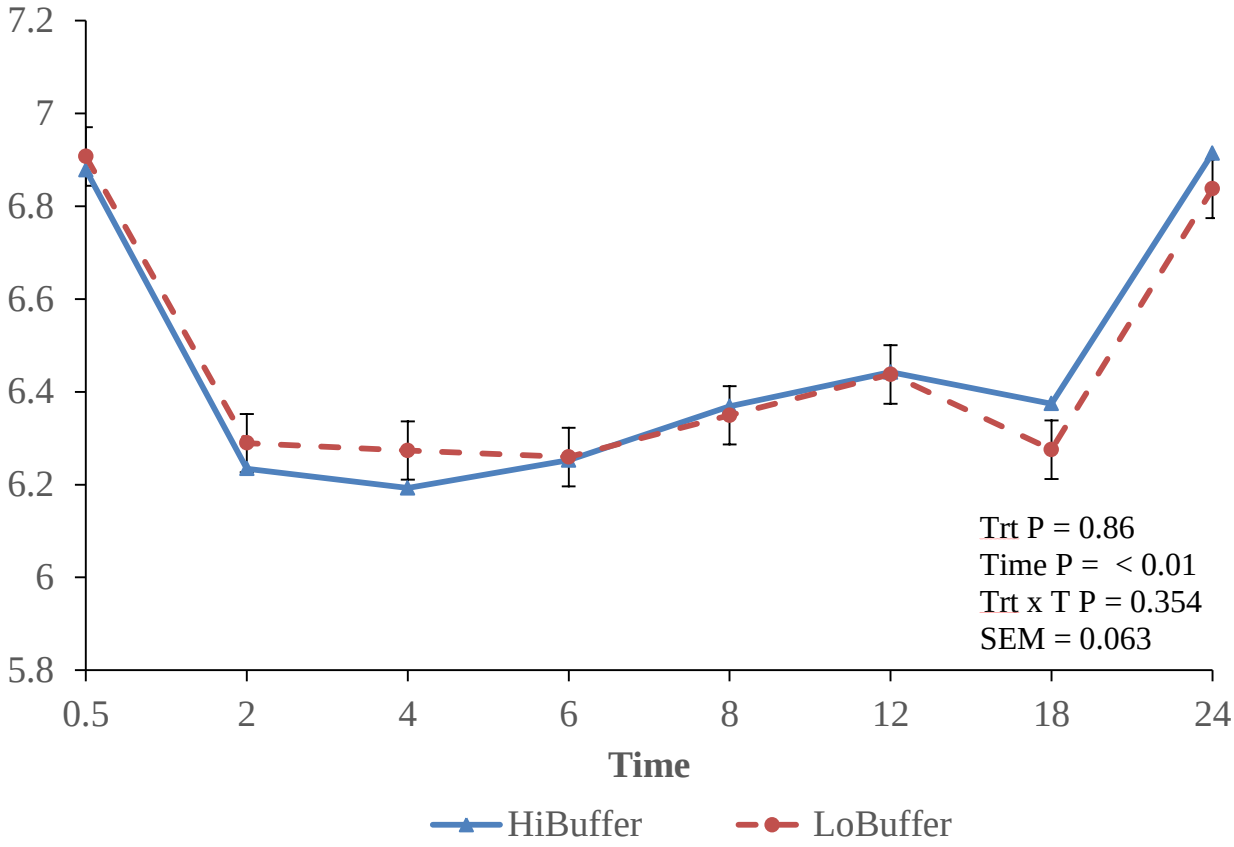


**Figure 2.** Ammonia production from dairy steers limit-fed buffer inclusion treatment diets with increased amounts of distillers dried grains with solubles (DDGS).

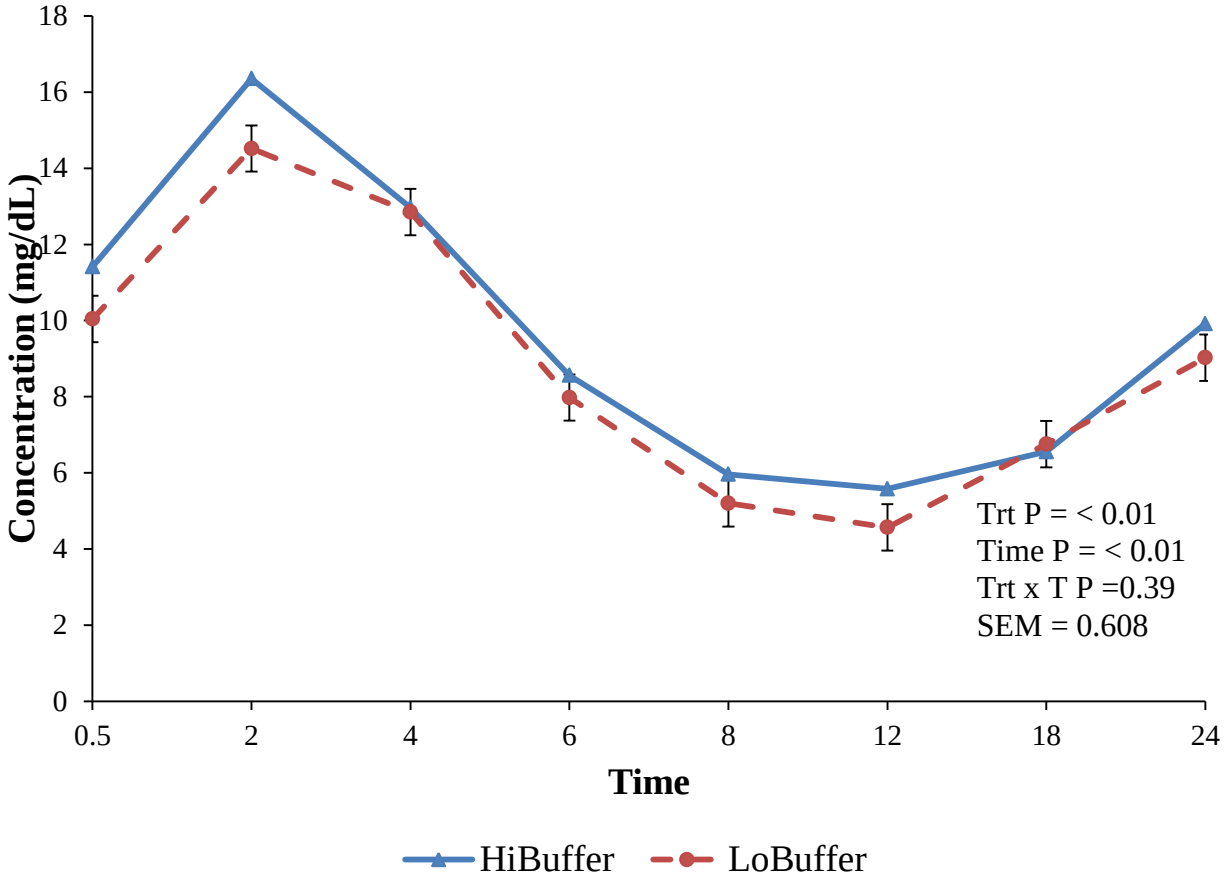


**Figure 3.** Total VFA production from dairy steers limit-fed buffer inclusion treatment diets with increased amounts of distillers dried grains with solubles (DDGS.

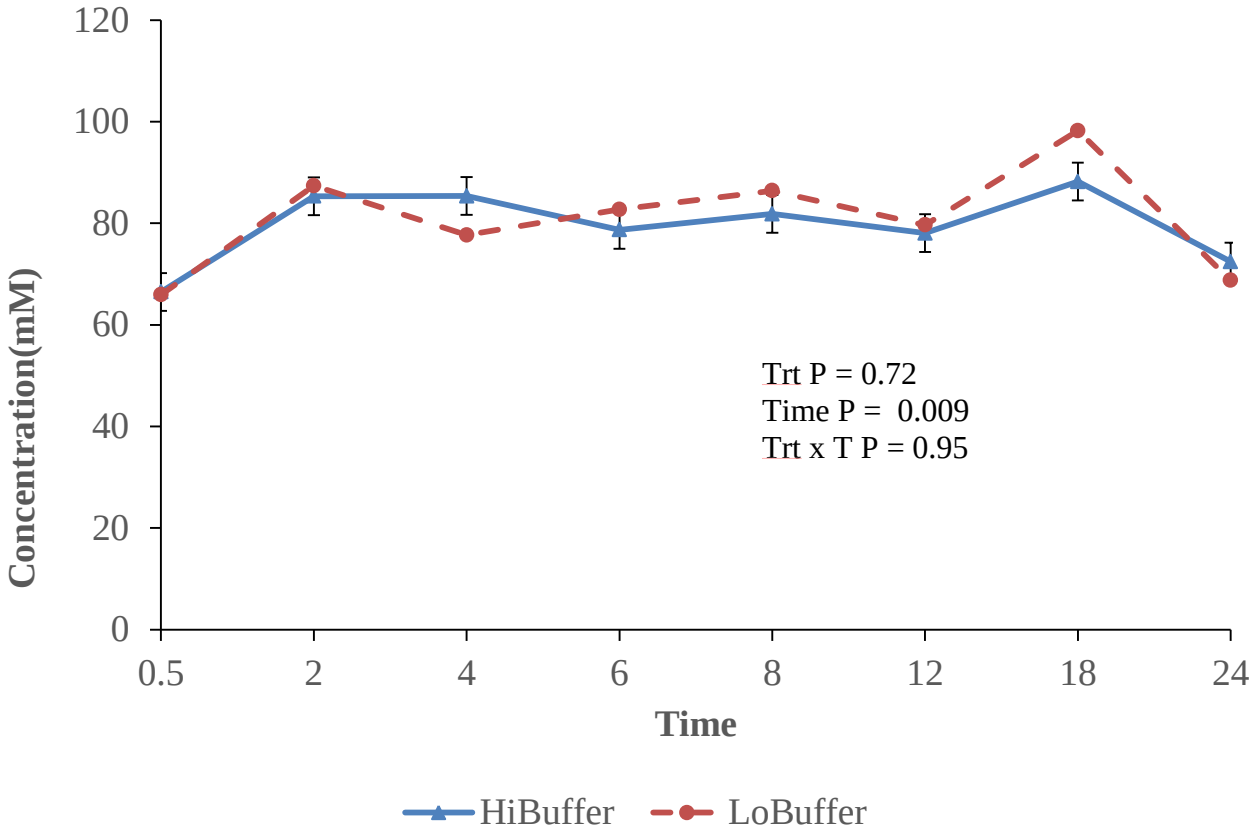


**Figure 4.** Cholesterol concentrations in dairy steers limit-fed buffer inclusion treatment diets with increased amounts of distillers dried grains with solubles (DDGS).

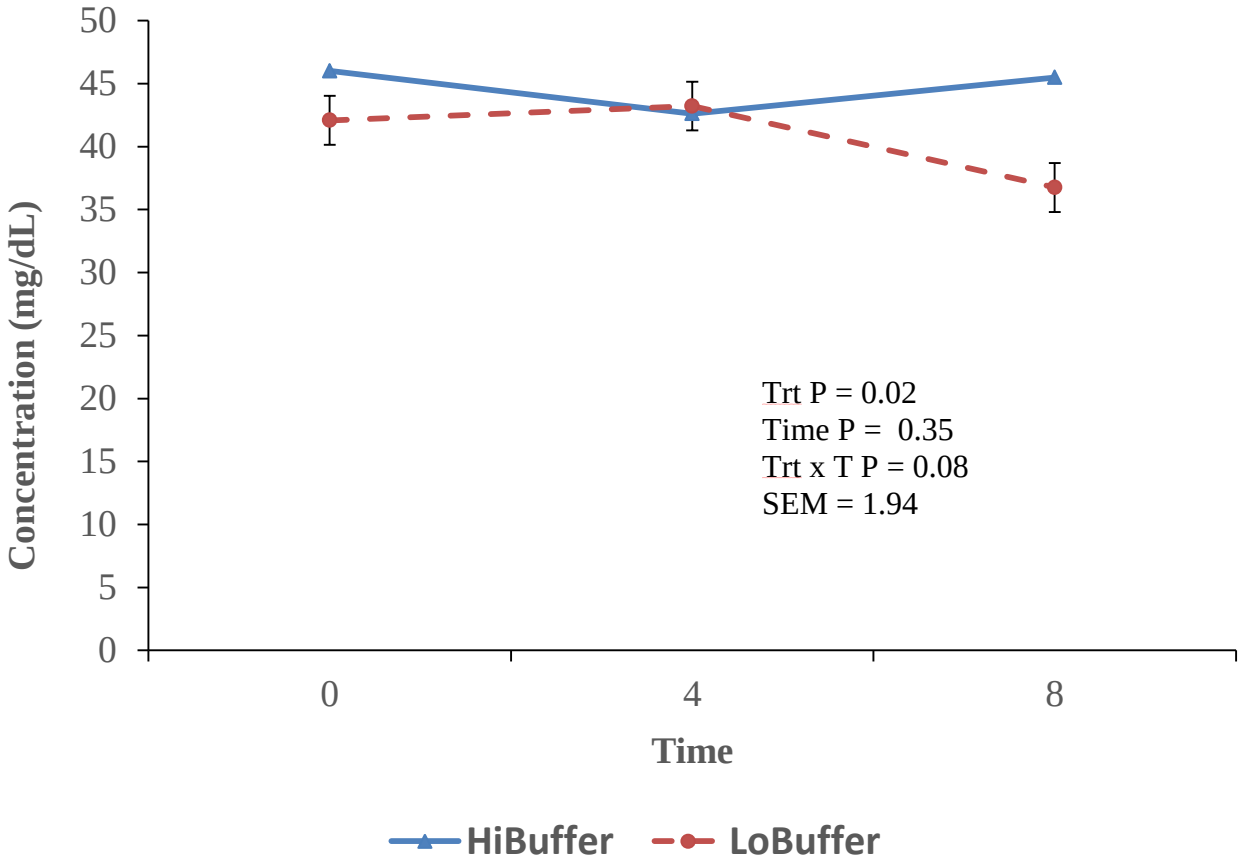


**Figure 5.** Glucose concentrations in dairy steers limit-fed buffer inclusion treatment diets with increased amounts of distillers dried grains with solubles (DDGS).

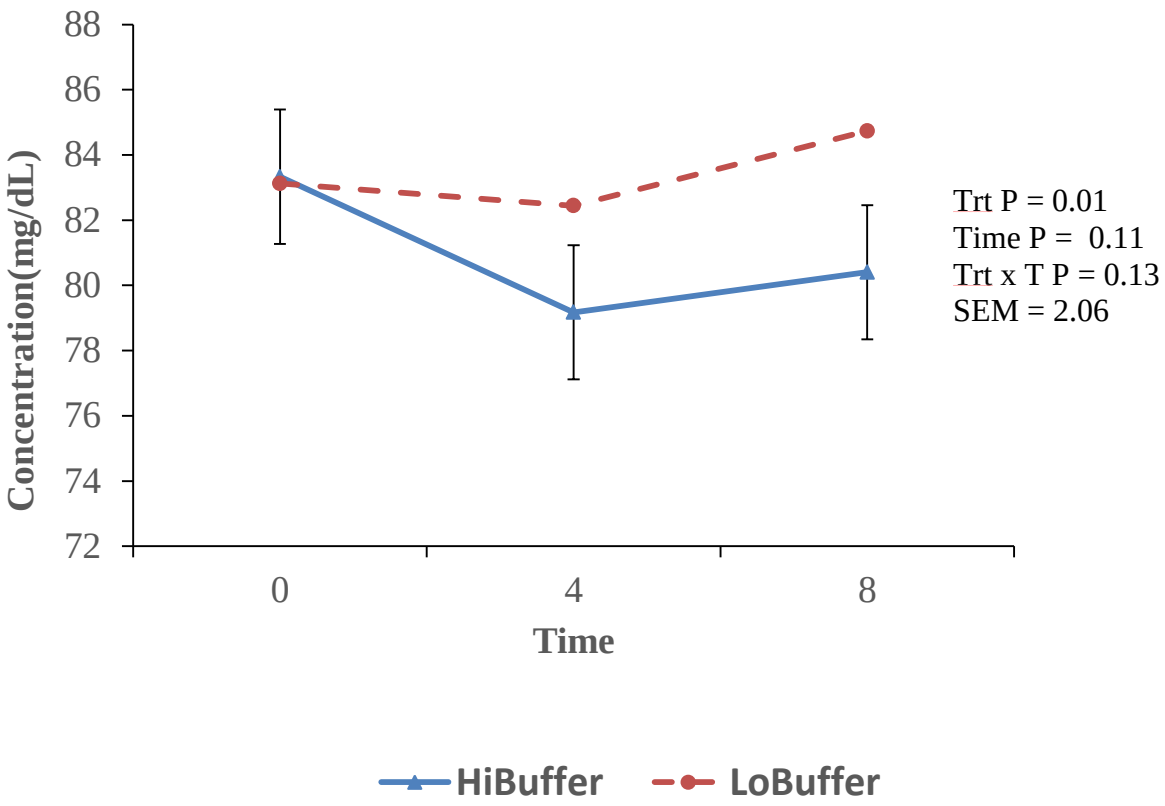
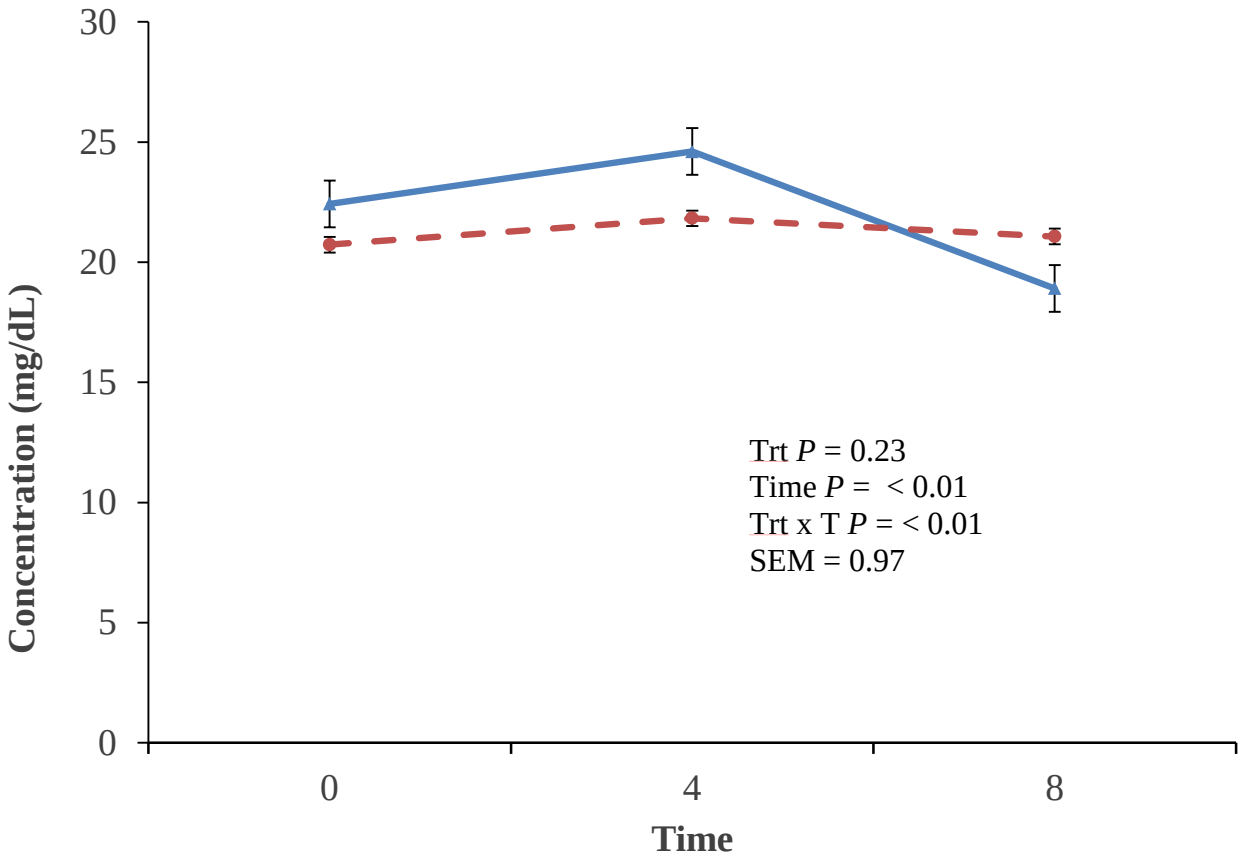


**Figure 6.** Plasma urea nitrogen concentrations in dairy steers limit-fed buffer inclusion treatment diets with increased amounts of distillers dried grains with solubles (DDGS).



# SUMMARY AND CONCLUSIONS

The research presented met our overall objectives, which were to make recommendations on increasing dairy production through accurate prediction of forage quality and evaluate diet buffer supplementation as a way to improve nutrient utilization of DDGS. Results indicate that sample preparation and method used to evaluate forage quality has a profound impact on accurate prediction of animal performance as described in Chapter 2. The rumen degradability of fiber was different for the five test forages. This proves our original hypothesis that different method will vary in digestibility results but difference among treatments will follow similar patterns among method. Also samples from different growing systems (conventional versus hybrids) were also different. The result from the study showed that there is significant effect of sample preparation and the methods used to evaluate forage quality. Thus, an accurate prediction of animal performance from forage analysis will require an improved standardization of procedures.

In Chapter 3, it was observed that buffer inclusion at two amounts in the current study did not affect the digestibility of fiber or dry matter if diets containing large inclusion rates of DDGS fed to steers. It was found that both treatments maintained a consistent pH, thereby facilitating steady conducive environment for rumen microorganisms and promoted rumen fermentation through the release of VFA.

In conclusion, these results demonstrated that there is need to standardize procedures for evaluating forage quality and amount of buffer in diets with high DDGS had minimal impacts, although both treatment diet contain a large proportion of grass hay which may have stimulated rumination and saliva production and minimized the impacts of the two treatments. Future research should focus on precise rate of buffer inclusion to improve the utilization of DDGS as a feedstuff for dairy heifers.

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