

INCLUSION OF WET CORN GLUTEN FEED WITH PHYSICALLY EFFECTIVE
NEUTRAL DETERGENT FIBER AND THE RESULTING EFFECTS ON PRODUCTION
PARAMETERS

by

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Abstract

Wet corn gluten feed (WCGF) is commonly included in lactation rations for dairy cattle. Research at Kansas State University has shown that increasing WCGF inclusion decreased ruminal pH. Lack of adequate particle size may result in this decrease and can impact efficiencies, animal health and longevity. A study was conducted to look at the effects of feeding WCGF while maintaining $\geq 10\%$ of particles > 19 mm across diets. We hypothesized that as WCGF increased, DMI and milk yield would increase while ruminal pH would be maintained. Seven ruminally-cannulated, lactating Holstein cows were used in an incomplete 4×4 Latin square design with treatments of 0, 12.4, 24.5 or 35.1% WCGF across 4 periods of 21 d. Alfalfa hay was used to maintain particle size. All diets met particle size goals; however, as WCGF increased, the proportion of particles > 19 mm decreased ($P = 0.01$) and cows changed their sorting behavior in favor of particles > 19 mm ($P = 0.03$) and against particles on the bottom screen ($P < 0.01$) and pan ($P = 0.01$). As WCGF increased, ruminal pH and ECM/DMI were not affected, yet DMI ($P = 0.02$) and milk yield ($P = 0.02$) increased quadratically. Milk protein, lactose and fat concentrations were not affected; however, milk protein ($P = 0.004$; linear) and lactose ($P = 0.02$; quadratic) yields increased. In a separate study, active dry yeast (ADY) products, commonly used in the dairy industry to support ruminal health, were evaluated for product guarantees and effects of storage and storage medium. Few products received through normal distribution met product guarantees (1 of 6; experiment 1) and after 3 mo of storage cell viability dropped significantly ($P < 0.01$). In the second experiment, products were stored in ground corn or in a vitamin-trace mineral mix (VTM). Depressions in viability caused by high-temperature storage were partially mitigated when ADY products were stored with a

VTM. Although both mediums resulted in lower cell viabilities after storage at 40°C, VTM cell viabilities were significantly ($P = 0.02$) higher than ground corn.

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Dedication

It is with great pride that I dedicate this to an individual who has been a mentor to me over these past seven years. If it had not been for their love of teaching and Holsteins, I never would have had the opportunity to develop into a successful dairyman and scientist. This dedication cannot begin to express my gratitude for all this individual has done, and even if it could, he is too humble to accept it. With this, I dedicate this work to Mike Scheffel, my great mentor, friend and teacher.

Chapter 1 - Literature Review

Introduction

As dairy researchers, scientists, nutritionists, and producers, our goal is to create a profitable environment where production goals are met. For some operations, this focus is on milk production, for others milk components, but for all operations, healthy cows are essential. Many factors play into this parameter, including nutrition, handling, housing, vaccinations and treatment protocols. The role of nutrition in animal health and performance will be the main emphasis of this literature review and, more specifically, the fiber fraction called physically effective neutral detergent fiber (peNDF).

Physically effective NDF is defined as the fraction of fiber that is responsible for the stimulation of chewing (Mertens, 1997). It is typically referred to as particle size or scratch factor and often is associated with the forage portion of the diet. This differs from the effective neutral detergent fiber (eNDF) fraction which is defined by Krause et al. (2002b) as being “related to the total ability of a feed to replace forage in a ration so that milk fat percentage is maintained.” Unfortunately, these terms are often used interchangeably in the literature even though they differ largely in not only function, but in method of determination.

With today’s feeding systems that incorporate concentrates in diets at levels above 45% (DM basis), peNDF has become ever more important when formulating rations. With adequate peNDF delivered to the cow, the ability to maintain a normal ruminal environment is greater (Zebeli et al., 2008). Typical responses include increased total chewing time (TCT) and saliva production (Beauchemin and Yang, 2005; Kononoff et al., 2003), maintained ruminal motility (Ash, 1959), increased liquid passage rate (Yang and Beauchemin, 2007b) and maintenance or increase of milk fat yield (Einarson et al., 2004; Yang and Beauchemin, 2006c). It is also worthwhile to note the role of feed mixing and delivery, as well as housing, which may also impact the benefits seen with adequate peNDF as they relate to the reduction in particle size and the incidence of sorting (Arelovich et al., 2008; Enemark, 2009; Stone, 2004).

It has been suggested that peNDF_{>1.18} be included in the diet at levels of 30-33% (DM basis) for an optimal ruminal environment without impairing other production responses (Arelovich et al., 2008; Zebeli et al., 2008) when measured using the 3-screen Penn State Particle Separator. Other methods for peNDF determination may not be compatible with this suggested

level and will be discussed further later. Over the years, methods for determining peNDF have changed and improved and, in this review, these different methods will be discussed so as to identify ideal measurement techniques. Furthermore, the interaction between peNDF and diet fermentability will be addressed.

Current Feeding Systems

Presently in the United States, where cereal grains like corn and sorghum are readily available, diets incorporating substantial amounts of concentrates are commonly fed to dairy cattle. This feeding system is an effective way of delivering energy to the cow without the filling effects of forages that may limit dry matter intake (DMI; Allen, 2000; Arelovich et al., 2008; Yang and Beauchemin, 2007a). Moreover, there is a wide array of by-product feeds available as cheaper forms of energy that are often used as substitutes for corn grain, corn silage and alfalfa in the diets of lactating dairy cattle. Although these may meet the NDF requirements of the cow, they may fail to provide adequate physical effectiveness due to their small particle size and more rapid fermentation and passage from the rumen (Zebeli et al., 2008).

Diets containing higher levels of carbohydrates (over 65% of diet DM) can result in acid production and accumulation in the rumen (Allen, 1997). Utilization of diets high in carbohydrates with inadequate particle size is limited by a number of factors: decreased ruminal pH, depressed fiber digestion, milk fat depression (MFD), laminitis, subacute ruminal acidosis (SARA), and reduced DMI (Stone 2004; Yang and Beauchemin, 2006a; Zebeli et al., 2008). This is where a balance of forage and concentrate becomes important when formulating rations, particularly for lactating cattle. Moreover, the inclusion of peNDF in the diet has beneficial effects on ruminal pH, saliva secretion, rumination time, digestibility of NDF, and, in some instances, on DMI, fat corrected milk yield (FCM) and ultimately the decreased incidence of SARA (Adin, 2009; Allen, 1997; Beauchemin and Yang, 2005).

Neutral Detergent Fiber and peNDF

It is very typical for nutritionists to balance rations for NDF content. The NRC (2001) suggests a minimum of 28% NDF for 612 kg cows producing over 36.3 kg/d. Although diets may contain adequate levels of NDF, not all forms of NDF work the same in the rumen. This is why peNDF is important as it is directly linked to the portion of NDF that is responsible for the stimulation of chewing time and rumination. By thus doing so, it aids in the production of saliva,

ruminal contractions and fiber digestion (Ash, 1959) which all aid in the maintenance of a healthy rumen environment. Often referred to as the particle size of the diet, it is highly influenced by processing and mixing times of feed ingredients and total mixed rations (TMR).

By-product feeds that are used to replace forage fiber, like wet corn gluten feed or distillers grains, are a great example of the different functions that NDF and peNDF have in maintaining rumen health. When large quantities of by-products are fed in place of a forage source, often peNDF can become limiting. This limitation in peNDF has been shown to lead to depressed ruminal pH, shorter chewing time, less saliva production, and MFD (Galyean and Abney, 2006; Harvatine et al., 2002; Schingoethe et al., 2009). Schingoethe et al. (2009) stated that when distillers grains with solubles are fed to lactating cattle, adequate peNDF must be included in the diet to avoid MFD. Although distillers grains with solubles contains large amounts of NDF (39%) it only provides 3.4 to 19.8% peNDF due to its small particle size (Kleinschmit et al., 2007; Schingoethe et al., 2009).

Not only are there differences between NDF and peNDF, but between eNDF and peNDF as well. These terms are often used interchangeably in the literature and typically follow similar patterns, yet diets can be limiting in one and not the other. One example of such a situation is by Harvatine et al. (2002) who conducted a study looking at the effectiveness of whole linted cottonseed which was substituted for alfalfa hay in diets containing either ground corn or steam flaked corn. They found that NDF provided by whole linted cottonseed was only 84% as effective as forage NDF from alfalfa silage in maintaining milk fat concentration. Although particle size was not determined, TCT between treatments were not statistically different which would indicate no difference in peNDF between treatments.

As the mean particle size of a diet decreases, peNDF decreases while NDF can be maintained. Additionally, there may be differences in the effectiveness of fiber between two feedstuffs without any difference in the physical effectiveness. One caveat to this is the influence that other dietary factors have on the function of peNDF. The largest factor affecting the animal response to peNDF is the ruminally degradable organic matter (RDOM) content of the diet (Allen, 1997). As the portion of RDOM increases in the diet, the benefits of peNDF in the diet are magnified. Furthermore, in a diet with a larger quantity of RDOM, a small change in peNDF content of the diets can yield greater responses than in a diet that is less digestible (Krause et al., 2002a; Krause and Combs, 2003). Krause and Combs (2003) demonstrated these

interactions between forage source, fermentability, and peNDF on production parameters and ruminal pH in midlactation dairy cows. Cows were fed coarse or fine chopped alfalfa and corn silage with either high moisture corn or ground corn as the concentrate in a $2 \times 2 \times 2$ factorial design. Impacts of both particle size and RDOM, high moisture corn vs ground corn, were observed in this study. As forage particle size decreased, TCT decreased significantly ($P < 0.01$) from 678 min/d for the coarse alfalfa, ground corn diets to 530 min/d for the finely chopped alfalfa, ground corn diets, and from 702 min/d in the coarse alfalfa, high moisture corn diets compared to 575 min/d in the fine alfalfa, high moisture corn diets. There were no differences in TCT as a result of the RDOM ($P = 0.11$) of diets; however there was a significant effect of RDOM on ruminal pH with high moisture corn diets having a lower ($P = 0.03$) mean pH and ($P = 0.02$) minimum pH than those containing ground corn (Krause and Combs, 2003).

Methods for Measurement

Over the years multiple methods have been developed to measure peNDF. The main criteria used to evaluate different measuring techniques have been TCT, ruminal pH and milk fat concentration and yield. It is impractical to measure individual feed ingredients as they can be altered by mixing and delivery of the diet (Stone, 2004). Thus scientists have worked to develop methods which can be used to analyze the completed diets delivered to the animal. Originally, the American Society of Agricultural Engineers (ASAE) developed a standard method known as the S424 method. This is a laboratory scale particle separator which is comprised of 5 screens with openings of 19.0, 12.7, 6.3, 3.96, 1.18 mm and a pan. This standard was intended for both lab and field trials; however, it requires an oscillating machine which has limited its ease of use in field work. For this method, 9 to 10 L of a forage sample is utilized and shaken for 2 min, which yields a total of 288 ± 10 shakes. Forage particles retained on each screen are then weighed on a balance with at least 0.5 g accuracy so the percent retained on each screen can be calculated. The S424 method suggests repeating these steps 3 times for each sample and calculating the geometric mean for reporting results (ASAE, 1993). As the geometric mean particle size of the diet increases, so does the peNDF content which is measured as the NDF content of the diet multiplied by the DM retained on screens > 1.18 mm. This value has been adopted as it has been suggested that particles larger than this are too large to pass through the rumen and thus require further mastication and degradation (Mertens, 1997).

Due to the cumbersome nature of the S424 method, Lammers et al. (1996) set out to develop a simple method for particle size determination. This device would later be termed the Penn State Particle Separator (PSPS). The first PSPS had only 2 screens (> 19 mm, $19 - 8$ mm, and ≤ 8 mm) and was validated against the Standard S424 method. In order to obtain results with the 2 screen shaker box, screens were stacked and 1.4 L of wet sample was added to the top screen. The boxes were then shaken (forward and backward motion spanning 17 to 26 cm) 5 times in one direction, turned a quarter turn and shaken 5 more times. This was repeated for a total of 8 times and then feed retained on each screen was weighed so a percentage could be calculated for that screen. The percentage of particles, on a DM basis, greater than 8 mm (physical effective factor; pef) was then multiplied by the NDF content of the diet to yield the $\text{peNDF}_{>8.0}$ (Lammers et al., 1996). Due to the strong correlation between the 2-screen PSPS and the S424 method (the standard at that time for measuring particle size) and the simplicity of the PSPS, this technique has been widely adopted by nutritionists and dairymen to evaluate peNDF of diets.

More recently, the 3-screen PSPS was introduced. This method has been adapted over the years and is a simple on-farm test that generates results which are easy to interpret. The method is similar to the 2-screen PSPS with the exception of the pan which is separated further, resulting in four groups and the ability to capture fractions of particles even smaller than 8.0 mm. This PSPS is able to measure particles > 19 mm, 19 mm – 8 mm, 8 mm – 1.18 mm, and ≤ 1.18 mm. By multiplying the percent of particles > 1.18 mm (pef), on a DM basis, by NDF of the diet, $\text{peNDF}_{>1.18}$ can be determined. The term $\text{peNDF}_{>1.18}$ has been developed to differentiate the measurements from the 3-screen from the 2-screen which is represented as $\text{peNDF}_{>8.0}$. The range for pef is 0 to 1 with a pef of 0 suggesting no effect on stimulating chewing activity and 1 where the sample NDF is completely effective at stimulating chewing (Mertens, 1997). It has been suggested that particles greater than 1.18 mm in length are resistant to passage out of the rumen and thus play a greater role in stimulating chewing (Poppi et al., 1980).

In order to determine which method is ideal, much research has been conducted to evaluate these three methods of peNDF determination. Zebeli et al. (2008) used a meta analysis to evaluate particle size of TMR measured using $\text{peNDF}_{>8.0}$ and $\text{peNDF}_{>1.18}$ and its relation to ruminal pH and NDF digestibility along with the intake of ruminally degradable starch on ruminal pH and NDF digestibility. Physically effective $\text{NDF}_{>1.18}$ was more strongly correlated

($R^2 = 0.67$) with mean ruminal pH and NDF digestibility ($R^2 = 0.56$) than $\text{peNDF}_{>8.0}$ was ($R^2 = 0.27$ and 0.30 , respectively). Furthermore, they found that NDF digestibility was positively correlated with ruminal pH ($R^2 = 0.24$) and starch intake was negatively correlated with ruminal pH ($R^2 = 0.55$), which would suggest that $\text{peNDF}_{>1.18}$, NDF digestibility, and intake of ruminally degradable starch should all be used in developing models for predicting ruminal pH (Zebeli et al., 2008). Conversely, one study comparing $\text{peNDF}_{>8.0}$ and $\text{peNDF}_{>1.18}$ of TMR with varying particle sizes of corn silage suggested $\text{peNDF}_{>8.0}$ was a better predictor of particle size impacts on ruminal pH and chewing time. Researchers found that the magnitude of peNDF change between diets was greater using $\text{peNDF}_{>8.0}$ rather than $\text{peNDF}_{>1.18}$ values, yet they felt the new method ($\text{peNDF}_{>1.18}$) was more consistent in predicting chewing time (Yang and Beauchemin, 2006b).

In addition to studies evaluating the 3-screen and 2-screen PSPS methods for peNDF determination, others have been conducted to also evaluate the ASAE standard S424 method. Kononoff and Heinrichs (2003b) evaluated the effect of cottonseed hull inclusion and varying particle length on production responses in early lactation cows. There were no differences in $\text{peNDF}_{>1.18}$; however, mean forage particle length, determined using S424 method, decreased ($P = 0.05$) with decreasing particle size. Geometric mean particle lengths were 7.9 and 6.8 for the long forage length diets and 6.8 and 6.1 for the short. This study found no difference in TCT as mean particle length increased, which followed nicely with the lack of difference in $\text{peNDF}_{>1.18}$ between treatments. Although there are no published minimum required levels for geometric mean particle length to verify if adequate levels were utilized in this study, due to the lack of change in TCT, it would seem that $\text{peNDF}_{>1.18}$ was better at predicting chewing time than the S424 method. Conversely, Yansari et al. (2004) looked at the effects of particle size when alfalfa particle length was reduced. This study demonstrated significant decreases in $\text{peNDF}_{>8.0}$, $\text{peNDF}_{>1.18}$, and geometric mean particle size (S424 method) with decreasing alfalfa particle length. As forage particle size decreased, TCT significantly decreased as well. Although no one prediction method excelled over another, the pef from the $\text{peNDF}_{>1.18}$ and S424 methods were very similar while $\text{peNDF}_{>8.0}$ was consistently lower in predicting TCT.

Ruminal Environment

The effects of peNDF on animal health and performance are likely directly related to its impact on the ruminal environment. Increased secretion of saliva, stimulated by TCT, provides buffer in the rumen which aids in the maintenance of ruminal pH. In addition to this, larger particles stimulate reticulo-rumen contractions (Ash, 1959) which aid in mixing the rumen contents. This mixing brings VFA in the digesta into contact with the ruminal epithelium which thus increases the rate of VFA absorption and exchange for bicarbonate across the ruminal wall (Allen, 1997).

Saliva Production

Saliva, which contributes nearly half of the buffer in the rumen (Maekawa et al., 2002a) has the greatest role in buffering the rumen (Allen, 1997). Although it is constantly produced, it is secreted in greater quantities during rumination and mastication than during resting periods. Values for saliva flow rates vary between animal activities and between different studies with ranges from 196 to 253 mL/min during eating, 272 mL/min during rumination, and 91 to 151 mL/min when the animal is resting (Allen, 1997; Maekawa et al., 2002a). Although saliva flow rates vary between activities, they tend to be constant within each activity but the amount of time spent eating, ruminating and resting tends to vary between diets. It has been suggested that saliva production is most greatly influenced by forage fiber in the diet, which increases both eating and rumination time (Graf, 2005). Allen (1997) found that both NDF intake and particle length were positively related to TCT and explained a greater proportion of the variation in TCT than forage fiber, which was also found to be positively correlated. Additionally, parity has been shown to play a role in saliva production. This was demonstrated in a study conducted by Maekawa et al. (2002b), where it was found that multiparous cows spent more time eating and ruminating than primiparous cows, even after adjusting for DMI; however, salivation rate did not differ by parity, in agreement with other research (Beauchemin et al., 2008; Graf, 2005). Thus, if salivation rate is not directly affected by diet type or parity, the primary way to increase total saliva delivered to the rumen is for the animal to spend more time eating and ruminating instead of resting.

One method of altering the time an animal spends ruminating is by the inclusion of particles > 1.18. As mentioned previously, these particles are too large to pass through the

ruminal-omasal orifice (Mertens, 1997), which in turn leads to further mastication (by rumination) and microbial degradation in order for particle size to be broken down to a size that can pass out of the rumen. By including adequate peNDF, cattle are forced to ruminate and thus will produce more saliva than if at rest. Much research has been conducted feeding adequate (> 30%) and deficient (< 20%) levels of peNDF_{> 1.18}. In these studies, cattle consuming greater levels of peNDF had significantly greater TCT (Adin et al., 2009; Clark and Armentano, 2002; Beauchemin et al., 2008); however, other studies found no differences (Beauchemin et al., 1991; Kononoff and Heinrichs, 2003) or quadratic responses (Kononoff et al., 2003) in TCT. In studies that showed no differences, peNDF levels were greater than recommended levels and the magnitude of change in particle size between treatments was small. From this, it is evident that peNDF is required for greater TCT resulting in increased saliva production and consequently greater buffering in the rumen.

Acid Production and Neutralization

The purpose of salivary buffers in the rumen is to neutralize acid and help maintain ruminal pH. This action is in a constant balance between acid production, neutralization, absorption and passage and is also affected by dilution (Allen, 1997). Fermentation acids are naturally produced in the rumen as feeds are fermented or are derived directly from the diet. There are diurnal fluctuations in ruminal pH, with decreases observed following meals and the highest pH detected prior to meals (Allen, 1997; Zebeli et al., 2008). After meals, during bouts of high fermentation activity, acids accumulate in the rumen and pH drops to as low as 5.0 (Zebeli et al., 2008). One reason for this reduction in ruminal pH is that neutralization, absorption and passage of VFA do not occur as rapidly as production.

Typically, as meal frequency decreases and size increases, pH fluctuations after meals are larger, while the inverse is also true (Pitt and Pell, 1997). This is a result of the difference in fermentable organic matter load with cattle consuming fewer, larger meals having more rapid fermentation and thus having a greater impact on pH (Allen, 1997). Ruminal pH also decreases when dietary forage NDF concentrations are low, as a result of reduced TCT and saliva production (Allen, 1997; Owens et al., 1998; Pitt and Pell, 1997). Additionally, sources of NDF are typically slower to ferment and thus aid in reducing the fermentability of the diet. As acids

are produced, they have numerous fates, all of which affect ruminal pH. This literature review will focus primarily on neutralization, absorption and passage.

Neutralization

Hydrogen ions in the rumen are primarily neutralized by salivary buffers; however, alkalization and buffering by feed and products from feed break down are also important. Saliva contains bicarbonate and hydrogen phosphate ions which are responsible for the removal of hydrogen ions (Allen, 1997). The ability for these ions to adequately buffer the rumen is a function of their pK_a , which are 6.1 and 7.2 for bicarbonate and hydrogen phosphate, respectively. After a meal, fermentation in the rumen increases and pH begins to drop. As the pH reaches the pK_a of hydrogen phosphate it begins to convert to dihydrogen phosphate with the addition of a hydrogen ion. As noted earlier, the pK_a of hydrogen phosphate is 7.2 which is likely higher than the ruminal pH of a typical dairy cow, thus hydrogen phosphate is generally in the form of dihydrogen phosphate in the rumen. It is very common for pH to drop below 5.8 after a meal and as the pH of the rumen approaches 6.0, 94% of the hydrogen phosphate is used. At this point, bicarbonate begins to buffer the rumen and fortunately, the concentration of bicarbonate in the rumen is maintained fairly constant as pH declines (Allen, 1997).

As stated earlier, peNDF largely influences TCT and as it increases, more saliva is produced and thus more hydrogen phosphate and bicarbonate enter the rumen where they elicit neutralizing effects. Thus increasing peNDF should aid in buffering the rumen, although the direct relationship between peNDF and ruminal pH is not strong (Allen, 1997). It is important to recognize that saliva alone cannot fully buffer the fermentation acids produced after meals and thus other methods of acid neutralization are important as well.

Absorption and Reticulo-rumen Motility

Approximately 53% of total hydrogen ions are removed from the rumen by absorption of VFA (Allen, 1997) and this process has been described as one of the keys in maintaining ruminal pH (Ash and Dobson, 1963). During absorption, bicarbonate is transferred into the rumen through protein dependent mechanisms (Aschenbach et al., 2010). Acetate absorption is responsible for contributing the most bicarbonate to the bicarbonate pool in the rumen (Ash and Dobson, 1963), which is absorbed across the ruminal apical surface in part via an acetate-bicarbonate exchange. Additionally, both propionate and butyrate absorption lead to increased

bicarbonate levels in the lumen of the rumen, although to a lesser extent than acetate (Aschenbach et al., 2010). This added buffer helps to neutralize acids and maintain pH.

Interestingly, absorption, passage and neutralization all can be impacted by reticulo-rumen motility. Mixing of ruminal contents plays a crucial role in bringing VFA into contact with ruminal epithelium for absorption from the rumen and in bringing VFA and buffers into contact for more neutralizing activity. Lastly, without contractions, fermentation decreases and nutrients, including VFA, do not pass out of the rumen.

In a review by Owens et al. (1998), it was suggested that as ruminal pH decreases during acidosis, rumen motility decreases. This is supported by a study by Ash (1959) which investigated reticulo-rumen contractions after infusions of acetic, propionic, butyric or lactic acids at different pH levels. Volatile fatty acids were either infused into the lumen of the rumen or sprayed directly onto the ruminal wall. These experiments demonstrated that as pH decreased with the infusion of acetic acid, the frequency and strength of reticulo-rumen contractions also decreased. When pH dropped to 3.6-4.0, contractions were completely inhibited. The infusions of propionic acid generated similar results, yet the infusion of butyric acid had much greater inhibitory effects than acetic acid. When lactic acid was infused, no effects were seen until pH of the infused lactic acid was 2.5, at which point all activity ceased. Interestingly, the infusion of buffered VFA into the carotid artery had much greater effects; yet, it is questionable whether the infused pH values are representative of physiological levels entering the blood stream through absorption from the rumen (Ash, 1959).

Gregory (1987) found similar results in a study using ruminally, abomasally, and duodenally cannulated sheep. When VFA were infused into the rumen, a decrease in the amplitude of contractions was observed with receptors being most sensitive to butyric acid followed by propionic, acetic and lactic acid. Eventually, as concentrations of VFA increased, motility ceased. The concentrations of VFA needed to affect reticulo-rumen motility when infused into the abomasum were much smaller than those infused into the rumen. As with ruminal infusions, butyric acid was the most potent VFA when infused into the abomasum. Duodenal infusions of 50 mM (5 ml/min) of VFA had no effect on reticulo-rumen contractions but did inhibit abomasal motility. At higher concentrations (100 mM; 5 ml/min), duodenal infusions significantly reduced reticulo-rumen motility and amplitude of contractions. Gregory

(1987) also found that as the proportion of undissociated acid increased, effects on motility were more pronounced.

Passage Rate and Retention Time

The third fate of acids in the rumen is passage out through the reticulo-omasal orifice. Allen (1997) summed up the flow of hydrogen ions from the rumen with 28% being incorporated into water, 9% as dihydrogen phosphate, and < 7% as VFA, ammonia, and particulate matter. This accounts for 44% of the hydrogen ions in the rumen. As saliva production increases, the amount of fluid entering the rumen increases and results in increased liquid passage rates. Some researchers feel that this increase is not large enough to truly affect fluid passage rates. Russell and Chow (1993) suggested that as buffers in the rumen increase, water intake increases and this is what leads to increases in liquid passage rates. Regardless, a large benefit of the increase in liquid passage rate is the movement of smaller starch particles out of the rumen prior to complete fermentation. Under this scenario, the site of starch fermentation would shift slightly and thus reduce acid production in the rumen. Fluid passage rate is also suggested to be influenced by osmolality of the rumen which draws water into the lumen (Owens et al., 1998), although the ruminal pH of dairy cattle typically does not promote significant increases in osmolality.

Alternatively, larger particles, which promote saliva secretion, move out of the rumen more slowly. This retention can elicit a filling effect in the rumen as well as mitigate the amount of fermentation taking place. Thus, as peNDF increases, not only can liquid passage rate increase, but particulate passage rate and DMI at each meal can decrease. This allows longer particles to aid in neutralization of fermentation acids for an extended time and also reduces the quantity of particles available for fermentation after each meal. This has been suggested to play an even greater role in the management of ruminal pH than saliva (Allen, 1997). One caveat to this method of stabilizing ruminal pH is that if too much peNDF is contained in the diet, DMI will significantly decrease as a result of the filling effect and animal performance will thus be limited (Mertens, 1997).

Microbial Population

To support optimal fiber digestion, ruminal pH should be maintained between 6.2 and 7.0, with an ideal pH of 6.5 (Shriver et al., 1986). Below this point, microbial attachment to feed particles, as well as fibrolytic bacteria numbers, are depressed (AlZahal et al., 2009; Hoover,

1986). This would have direct implications on fiber digestion and would suggest that if increasing peNDF increases the amount of saliva delivered to the rumen that it would also aid in maintaining fiber digestion. This idea has been demonstrated by Yansari et al. (2004) when comparing diets containing three different lengths of alfalfa hay. Physically effective NDF values were determined using the S424 method (utilizing particles > 1.18 mm) and were 23.4, 20.3, and 15.2% peNDF for the long, medium and short diets respectively. As particles size was reduced, ruminal retention time decreased, particulate passage rate increased and NDF digestibility decreased. Additionally, ruminal pH declined with decreasing peNDF with the short diet yielding a significantly ($P < 0.001$) lower pH of 6.12 compared to 6.58 and 6.59 for the long and medium diets. Not only do these results demonstrate the effects of peNDF on passage rate but demonstrate the impact ruminal pH has on fiber digestion. The pH values of 6.59 and 6.58 are very close to the ideal levels suggested by Shriver et al. (1986) for optimal fiber digestion and when the pH dropped below the lower limit of 6.2, digestion was significantly ($P = 0.051$) depressed.

In contrast, Yang and Beauchemin (2006b) found that NDF digestibility was depressed and that of starch and nitrogen not affected when barley silage particle length was increased. In this study, the 3-screen PSPS was used to determine $\text{peNDF}_{>1.18}$ values of 45.0, 41.8, and 39.0% for the long, medium and short treatment diets. Two factors can aid in explaining the disparity in these results from the expected. First, ruminal pH was 6.08, 6.06, and 5.99 for the long, medium and short diets, and were not different from each other. All these values are below the lower limit for optimal fiber digestion and would suggest that fiber digestion was depressed in all treatments. Secondly, this negative impact of peNDF on digestion can be attributed to the very high levels fed assuming an ideal range of 30-33% (Zebeli et al., 2008). Due to the larger peNDF values, it is also possible that sorting took place and that longer particles were not consumed. In summary, these studies demonstrate the positive role peNDF plays in fiber digestion when fed at adequate levels.

Production Parameters

Over the years, attention has been drawn to peNDF as a useful measurement for meeting nutritional requirements to improve or maintain various production parameters, including milk fat yield and feed efficiency. While some parameters are improved when excessive peNDF is

incorporated into a diet, negative effects on DMI and milk yield have been observed (Clark and Armentano, 1999). Much of the research regarding the effects of peNDF on production parameters is controversial, and discrepancies between studies may be due to differences in RDOM of the diets, levels of peNDF, method of determination, and sources of peNDF (Mertens, 1997).

In theory, milk fat yield should increase, or be maintained, with increasing peNDF of the diet. This is likely due to multiple factors, including reducing the risk of altered fermentation processes in the rumen associated with low pH and greater production of acetate which can be more readily used for *de novo* fatty acid synthesis in the mammary gland than propionate (Maxin et al., 2011). Ample research is available, particularly testing the effects of particle size modification of corn silage, alfalfa haylage, or alfalfa hay, and the results are conflicting. Kononoff and Heinrichs (2003a) evaluated the effects of reducing alfalfa haylage particle size for early lactation cows. Physically effective NDF, evaluated using the S424 method, was fed at levels of 25.7, 26.2, 26.4, and 26.7% for the short, medium short, medium long and long alfalfa hay treatments, respectively. They found no effects on milk fat yield, but did observe a decrease in fat concentration. This lack of effect on milk fat yield was likely the result of peNDF levels that were not different ($P = 0.52$) from each other. These findings are consistent with the findings of Yang and Beauchemin (2006c) who used $\text{peNDF}_{> 8.0}$ as their method of measurement feeding levels of 10.5, 11.8 and 13.8% $\text{peNDF}_{> 8.0}$ for the low, medium and high peNDF diets. Although in this study levels of peNDF increased with increasing particle size ($P = 0.01$), total chewing time was not reported and thus could not be used as an evaluation method. Milk fat yields, 0.94, 0.88, and 0.93 kg/d, respectively, were low for all treatments and not statistically different. These studies demonstrate the possible need for more adequate peNDF levels in these studies as well as the lack of effects observed when treatment peNDF values are too similar. Unfortunately, at this time there is no suggested level for $\text{peNDF}_{> 8.0}$, so verifying adequacy is challenging and heavily dependent on diet fermentability.

For studies utilizing corn silage, similar results have been demonstrated. Kononoff et al. (2003) found a significant quadratic ($P = 0.03$) milk fat yield response to corn silage particle size, with the diet containing the longest particles producing the lowest milk fat yield. Particle size was measured using the 3-screen PSPS and values were 31.7, 31.9, 32.0, and 32.1% $\text{peNDF}_{> 1.18}$ for short, mixed short, mixed long and long corn silage diets. Although peNDF was not

statistically different, the milk fat response could be related to a combination of lower DMI and possibly sorting, thus resulting in lower milk yields in the higher peNDF diets. This reduction in milk fat yield with increasing particle size of corn silage was also observed by Clark and Armentano (1999). Particle sizes of the TMR fed in this study were not reported but, as particle size of the forage sources increased, DMI decreased along with milk fat and protein yields and concentrations. There was a significant ($P < 0.05$) increase in TCT as alfalfa replaced corn silage in the diet and as particle length increased, although no effects on milk fat yield were observed.

When the source of peNDF was alfalfa hay, no effects of particle size on milk fat yields were observed when feeding peNDF_{>1.18} levels of 25.1, 22.6, and 17.2 for their long, medium and fine diets (Yansari et al., 2004). Although these levels are significantly different ($P < 0.001$) they are all below 30% which may possibly help explain the lack of effect. Other studies have demonstrated similar results when adjusting alfalfa hay particle length. Krause et al. (2002a) fed diets varying in fine or coarse alfalfa silage with either a high moisture corn or dry corn. Physically effective NDF was determined using the S424 method and calculated using particles > 8.0 mm in length. Diets containing fine silage had peNDF_{>8.0} of 3.72 and 2.57% whereas diets with coarse silage had 10.67 and 11.32% peNDF_{>8.0}. Although not significant, milk fat yields were greater for diets containing coarse silage compared to those with fine silage. Differences between treatments appear small, but the reported means reflected a numerical decrease of 5.3% in milk yield from diets containing dry corn and coarse corn silage compared with diets containing dry corn and fine corn silage. Other studies have also found increases in milk fat yield with increasing alfalfa silage particle length (Clark and Armentano, 2002).

These results are highly variable yet tend to suggest that particle size, and consequently peNDF, have little effect on milk fat yield and in some cases may actually decrease it when peNDF is provided in excessive levels. In addition to the possible reasons for this explained earlier, another explanation for the lack of a benefit could be that control animals were not under extreme acid loads that would be indicative of MFD (mean pH < 5.8 ; Graf et al., 2005). Of the studies that reported mean ruminal pH values, only one study had a treatment which resulted in mean pH values below 5.8 (Krause et al., 2002b).

Another parameter that is largely influenced by particle size is dry matter intake, which can be limited by physical fill. As peNDF of the diet increases, the density of the TMR

decreases (Yansari et al., 2004). Thus, as the animal consumes a TMR of greater particle size, fill of the rumen can limit intake and, if the energy density is similar to a diet of smaller particle size, energy consumption is reduced. This is supported by multiple studies which found that DMI decreased with increasing corn silage particle length (Clark and Armentano, 1999; Kononoff and Heinrichs, 2003b; Kononoff et al., 2003). Similar results were observed with increasing alfalfa haylage length (Kononoff and Heinrichs, 2003a; Yansari et al., 2004).

Other research evaluating corn silage or alfalfa haylage showed no effects on DMI (Beauchemin and Yang, 2005; Clark and Armentano, 2002; Yang and Beauchemin, 2006a) which may be the result of multiple factors such as sorting, adequate or below adequate levels of peNDF in the diet and variations in peNDF between treatments. Two of these studies which detected no effects on DMI reported TMR peNDF levels. Of these, the lowest level of any treatment was only 30.8%, which is within the range (30-33% peNDF) suggested by Zebeli et al. (2008) for reducing the risk of SARA without hindering production responses. The third study that found no effects on DMI reported forage particle length but not TMR particle length. Although peNDF could be calculated, the value could possibly be confounded by mixing and delivery of TMR. In these cases, if peNDF was already adequate or slightly below adequate, physical fill is less likely to limit intake.

Consequently, if DMI is limited with increasing peNDF, it could be assumed that milk yield would follow a similar pattern, and Clark and Armentano (1999) observed this response when they increased corn silage particle size of the diet. Corn silage particle length was measured using the 3-screen PSPS, yet TMR particle size was not reported. This makes comparing these results to other studies more challenging. Surprisingly, many researchers have found no impact on milk yield when peNDF was increased with various forage sources (Clark and Armentano, 2002; Kononoff and Heinrichs, 2003; Yansari et al., 2004).

The general lack of an effect on milk yield while reducing DMI would suggest that production efficiencies of cows fed greater levels of peNDF would be more desirable. Interestingly enough, the research would imply just that. The following efficiencies were not reported but calculated from the published works and are reported here as energy corrected milk per unit DMI (ECM/DMI) for consistency. Kononoff and Heinrichs (2003) found that when corn silage particle size was increased, ECM/DMI increased from 1.68 in their short corn silage treatment to 1.83 in their long corn silage treatment. Yang and Beauchemin (2006a) found

similar improvements from 1.31 to 1.42 ECM/DMI with increasing corn silage particle size; however, Clark and Armentano (1999) had a numerical decrease in ECM/DMI with increasing particle size from 1.40 to 1.36 ECM/DMI. Studies utilizing alfalfa haylage as the source of peNDF also found increases in efficiency with increasing peNDF (Kononoff and Heinrichs, 2003; Yansari et al., 2004) while other studies showed no effects on efficiency (Clark and Armentano, 2002; Kononoff et al., 2003). These results would suggest that peNDF has a positive relationship with ECM/DMI which would be an advantage to producers looking to cut feed costs and maintain or improve production efficiencies. As the prices of commodities and fuel increase, this may be a method dairymen utilize to maintain profitability.

Metabolic Disorders

In addition to the benefits peNDF may have on certain production parameters it has also been suggested to aid in the reduction of metabolic disorders. Although the research is controversial over the roles that peNDF might play in certain disorders, it is generally recommended as a means of correcting or preventing disorders such as SARA, MFD, and laminitis. The occurrence of these may be linked with other factors aside from particle size of the diet, yet it has been associated as a contributing factor when at extremely low levels.

As of 2008, losses from SARA amounted to nearly \$1 billion dollars in the United States with estimates of up to 40% of US cattle suffering from the disorder (Enemark, 2009). Long term implications from SARA included MFD, compromised reproductive performance, reduced energy efficiency for milk production, laminitis and other health disorders (Graf et al., 2005; Nocek, 1997; Zebeli et al., 2008). The accepted causes of SARA are high starch diets, low peNDF, inadequate rumen adaptation and feeding practices resulting in large meal consumption (Enemark, 2009; Zebeli et al., 2008). Yang and Beauchemin (2007a) found that when forage to concentrate ratios were increased that TCT and ruminal pH were increased as well. Additionally, this increase was even greater when the particle size of the higher forage diet was increased, resulting in TCT of 670.7 and 739.8 min/d and mean pH values of 6.46 and 6.55 for the short and long diets, respectively. Although these pH values are above the 5.8 mark, the diets with lower forage to concentrate ratios and short particles sizes had a mean pH of only 5.86. These data demonstrate the role that forage concentration and particle size play in the prevention of SARA.

A parameter often measured to determine adequate fiber or the presence of SARA is milk fat yield. As stated previously, when SARA is present, MFD is often witnessed as well. This measure is directly related to peNDF of the diet, particularly when the fiber is from a forage source (Beauchemin et al., 1991). Several studies have demonstrated the benefits of peNDF inclusion, and its interaction with RDOM, when compared to controls that were fed below suggested levels (Allen, 1997; Harmison et al., 1997). Part of the reason for these positive impacts on milk fat yield when peNDF is included at adequate levels is the increase in acetate production, often associated with forages. Acetate is the primary fatty acid used for *de novo* synthesis of milk fatty acids. Thus, it is possible that as concentrations increase in the blood, acetate delivered to the mammary tissue for milk fat synthesis is greater as well, as long as blood flow to the udder is not affected. Furthermore, when peNDF levels are adequate, the incidence of incomplete biohydrogenation of unsaturated fatty acids to trans fatty acids is decreased (Grant et al., 1990). Zebeli et al. (2008) found that a peNDF of $31.9 \pm 1.97\%$ DM was optimal for reducing the risk of SARA and MFD although it is dependent on RDOM content.

Another condition often linked with SARA is laminitis, impacting over 10% of animals in herds diagnosed with SARA (Cook et al., 2004; Garret, 1996). Unfortunately, it often is overlooked as it is a long term result of SARA, unlike MFD, which can affect profitability more quickly. There is a direct correlation between starch level in the diet and laminitis, and it is believed that the link between the disorders is toxin absorption into the blood stream through the reticulo-rumen wall as pH declines. At this time the pathophysiological mechanism as it relates to ruminal pH is unknown, yet these toxins cause vasoconstriction of vessels in the foot and thus deterioration of the connective tissues in the hoof (Boosman, 1990; Enemark, 2009). Unfortunately, little research is available directly linking peNDF and SARA to laminitis. By including peNDF at adequate levels in lactating cow diets, low ruminal pHs can be avoided and thus may prevent the development of laminitis which has been shown to be induced when ruminal pH declines (Gozho and Plaizier, 2005).

Conclusion

From the available research, it is evident the role that peNDF plays in stimulating chewing activity of cattle and its positive relationships with ruminal pH, milk fat yield and animal health. Physically effective NDF_{> 1.18} levels of 30 - 33% have been suggested for

lactating cow diets and are supported by multiple studies; however, these suggested levels may not be ideal for $\text{peNDF}_{> 8.0}$ and may vary with differing diet fermentabilities. Although three methods of peNDF determination are available, it appears that the most successful method for predicting chewing activity is the 3 screen method of the PSPS yielding $\text{peNDF}_{> 1.18}$ values. Not only is this method the most strongly correlated with TCT predictions, but it is also an easy, on-farm method for evaluation.

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**Chapter 2 - Effects of Wet Corn Gluten Feed and Physically
Effective NDF on Ruminal pH and Productivity of Lactating Dairy
Cattle**

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Abstract

Wet corn gluten feed (WCGF), a by-product of the wet milling industry, is commonly substituted in lactating dairy rations for corn, corn silage, and alfalfa hay. Previous research at Kansas State University showed that increasing WCGF in the diet decreased ruminal pH. The objective of this study was to maintain at least 10% of particles > 19 mm in length across diets while increasing WCGF inclusion in the diet. We hypothesized that as WCGF increased, DMI and milk yield would increase while ruminal pH would be maintained. Seven ruminally-cannulated, lactating Holstein cows (4 multiparous, 3 primiparous) were used in an incomplete 4 × 4 Latin square design. Treatments included 0, 12.4, 24.5 or 35.1% WCGF while utilizing alfalfa hay to maintain particle size. Across treatments, CP and NDF concentrations were held relatively constant. Four 21-d periods were used with 17 d of adaptation and 4 d of sample collection. Free floating ruminal pH probes were utilized during sampling periods and recorded pH every 5 min. Particle size of TMR and orts were analyzed using a Penn State Particle Separator. Results were analyzed with mixed models to test the fixed effect of treatment. All diets contained ≥ 10% of particles > 19 mm; however, as WCGF increased, the proportion of particles > 19 mm significantly decreased ($P = 0.01$). Interestingly, with increasing WCGF, cows sorted for the particles > 19 mm ($P = 0.03$) while sorting against particles on the bottom screen ($P < 0.01$) and pan ($P = 0.01$). With increasing WCGF, ruminal pH was not affected, yet DMI ($P = 0.02$) and milk yield ($P = 0.02$) significantly increased in a quadratic fashion with the peak responses for the 24.5% WCGF diet. Milk protein, lactose and fat concentrations were not affected; however, milk protein ($P < 0.01$) and lactose ($P = 0.02$) yields increased as WCGF inclusion rate increased, driven by the increased milk yield. Additionally, production efficiency was not affected by the treatments as there were no differences in ECM/DMI. Thus it was demonstrated that if adequate particle size is maintained as WCGF increases in the diet, DMI and milk yield increase while maintaining production efficiency and ruminal pH.

Key words: wet corn gluten feed, ruminal pH, particle size, peNDF

Introduction

As the demand for cereal grains increases, the use of alternative by-product feeds becomes more appealing to dairy producers. One common by-product of the wet corn milling industry is wet corn gluten feed (WCGF). This is a high fiber, highly digestible, moderate energy and CP feedstuff (Schroeder, 2003) that is commonly used to replace corn silage, corn grain and/or alfalfa hay in lactating cow diets (Bernard and McNeill, 1991; Mullins et al., 2009). Increasing levels of WCGF in lactating diets have been shown to produce positive effects on dry matter intake (DMI) and milk production; however, it may result in decreased ruminal pH and efficiencies for milk production (Mullins et al., 2010). One reason for this could be the small particle size of the diet resulting in more rapid particulate passage rates, decreased saliva production, and decreased buffering in the rumen, thus decreasing ruminal pH.

The importance of particle size in lactating cow diets has been summarized by Allen (1997) and is termed physically effective neutral detergent fiber (peNDF). This is a measure of a feedstuff's ability to stimulate chewing activity, which is the sum of time spent eating and ruminating (Beauchemin and Yang, 2005), often referred to as total chewing time (TCT). Studies have shown that increasing peNDF in lactating cow diets increases TCT, which results in increased saliva secretion due to mastication and thus greater saliva flow into the rumen (Beauchemin and Yang, 2005; Krause et al., 2002). Saliva contains buffers such as bicarbonate and hydrogen phosphate that function to remove hydrogen ions from the rumen and thus aid in maintaining ruminal pH (Allen, 1997). Additionally, increases in particle size have been shown to increase acetate to propionate ratios (A:P; Zebeli et al., 2008), but this response depends heavily on the fermentability of diet ingredients (Beauchemin and Yang, 2005; Krause and Combs, 2003).

No studies have been performed looking directly at the effects of feeding increasing levels of WCGF while maintaining a standard particle size measurement across treatment diets. The objective of this study was to evaluate the effects of WCGF inclusion (0, 12.4, 24.5, and 35.1% of diet DM) on ruminal pH, DMI, milk production and efficiency while maintaining a minimum dietary particle size. We hypothesized that by maintaining at least 10% of particles >

19 mm in all diets, ruminal pH and efficiency would be consistent across all diets while feed intake and milk production would increase as WCGF inclusion rate increased.

Materials and Methods

Experimental procedures were approved by the Institutional Animal Care and Use Committee at Kansas State University.

Design and Treatments

Four multiparous and 3 primiparous cannulated, lactating Holstein cows (132 ± 22 DIM; mean \pm SD) were randomly assigned to treatment sequence in an incomplete 4×4 Latin square design to evaluate the effects of WCGF on production parameters and ruminal pH. Initially, 8 cows were cannulated for the trial; however, one failed to transition in and was thus not used. One cow was removed from the study after the completion of the second period due to unrelated health problems. The study consisted of 4 periods, each lasting a total of 21 d with 17 d of adaptation and 4 d for sample collection. Diets were designed to include WCGF (Sweet Bran®; Cargill, Blair, NE) at 0, 12, 24, or 36% of diet DM and were formulated and mixed to have at least 10% of particles > 19 mm. The purpose of fixing the particle size was to maintain a minimum peNDF across all diets. Particle size goals were achieved by grinding alfalfa hay for 2 min in a horizontal mixing wagon (Roto-mix, Dodge City, Kansas) prior to mixing the TMR in a stationary batch mixer. The formulations of the 4 diets, which were identical to those used in Mullins et al. (2010), are shown in **Table 1**, and the initial corn silage, alfalfa hay and WCGF analyses are shown in **Table 2**. Although diets were designed to include 0, 12, 24 and 36% WCGF, actual concentrations fed were 0, 12.4, 24.5 and 35.1 % (DM basis).

Cows were fed twice daily at 1100 and 1500 h to provide 110% of expected intake and were milked 3 times daily at 200, 1000, and 1800 h. Animals were housed in a tie stall barn with an evaporative cooling system from June to August 2010 at the Kansas State University Dairy Teaching and Research Facility.

Data and Sample Collection

Feed intakes were recorded daily as the difference between feed offered and refused. Throughout the treatment periods, corn silage and WCGF samples were analyzed twice weekly for DM and diets were adjusted accordingly. During the sampling periods, individual ingredient

samples as well as TMR and ort samples were collected daily. Water intakes were recorded daily during the sampling periods.

On d 18-21 of each period, milk samples were collected and yields recorded at each milking. At 9 h increments on d 18-20, ruminal fluid samples for VFA and NH₃ determination were collected as grab samples from multiple locations in the ventral sac, composited, strained through 8 layers of cheese cloth, and stored at -20°C. Ruminal pH was recorded every 5 min during the sample period by the use of free floating ruminal pH probes (Rumen Sensors, Kahne Limited, Auckland, New Zealand). Probes were calibrated and inserted into the rumen through the cannula on d 17, removed and recalibrated on d 19, and then placed back in the rumen until the completion of the sample period. Data from the first 24 h after each calibration (2 sets/period) were used for analysis.

Feed and Milk Analyses

Milk samples were analyzed for fat, true protein, lactose (B-2000 Infrared Analyzer; Bentley Instruments, Chaska, MN), urea nitrogen (MUN spectrophotometer, Bentley Instruments), and somatic cells (SCC 500, Bentley Instruments) by the Heart of America DHIA (Manhattan, KS). Energy-corrected milk (ECM) was calculated as follows: $0.327 \times \text{milk yield} + 12.86 \times \text{fat yield} + 7.65 \times \text{protein yield}$, and 3.5% fat-corrected milk (3.5% FCM) was calculated as: $(\text{milk yield} \times 0.432) + (\text{fat yield} \times 16.216)$ (DHI glossary, 2010). For the evaluation of production efficiency, ECM was divided by DMI.

Samples of TMR and Orts were evaluated for particle size using the 3-sieve Penn State Particle Separator (Kononoff et al., 2003). A sorting index was calculated for TMR and ort samples for each cow \times period using the procedure outlined by Silveira et al. (2007). Separated samples as well as ingredient samples were dried in a 55°C forced-air oven for 72 h and DM was determined. Samples were ground to pass through a 1 mm screen in a Wiley mill (Arthur H. Thomas, Philadelphia, PA) and then composited by period. True DM was determined by drying samples at 105°C in a forced-air oven for 24 h and nutrient concentrations are reported relative to this value. The concentration of ash was determined by oxidizing samples at 500°C for 8 h in a muffle furnace. NDF concentrations were determined (Van Soest et al., 1991) in the presence of α -amylase and sodium sulfite with an Ankom Fiber Analyzer (Ankom Technology, Fairport, NY). Starch was determined by digesting samples in α -amylase and glucoamylase and then

determining glucose quantities with a commercial kit (Autokit Glucose; Wako Chemicals USA, Richmond, VA). Crude protein was determined by oxidation and detection of N₂ (Leco Analyzer; Leco Corp., St. Joseph, MI). Ether extract was used to determine crude fat concentrations (AOAC, 2000, method 920.9).

Ruminal VFA and NH₃ Analyses

Ruminal fluid samples were thawed and composited by cow period to obtain an 8 mL sample. Samples were then mixed with 2 mL of 25% metaphosphoric acid and were centrifuged at 30,000 x g for 20 min at room temperature. The supernatant was collected for VFA and NH₃ determination. Ammonia was determined colorimetrically with an autoanalyzer (Technicon Analyzer II; Technicon Industrial Systems, Buffalo Grove, IL). Ruminal VFA were measured with a gas chromatograph (Model 5890; Hewlett-Packard, Avondale, PA) with a flame ionization detector. The chromatograph was fitted with a 1.82 m x 6.35 mm inside diameter glass column packed with GP 10% SP-1200/1% H₃PO₄ (Supelco #1-1965, Bellefonte, PA). The column was maintained at 130°C, the detector and injector were maintained at 250°C, and carrier gas (N₂) flow was 80 mL/min. Run time was approximately 5 min.

Statistical Analysis

Data were analyzed by JMP 8.0 (SAS Institute, Cary, NC) with cow and period as random effects. Parity, treatment and parity by treatment interaction were included as fixed effects. When the *P*-value for the parity by treatment interaction was > 0.6, the term was removed from the model. Responses to WCGF inclusion rate were tested with linear and quadratic contrasts, which were declared significant at *P* < 0.05 and tendencies if 0.05 < *P* < 0.10.

Results

Particle Size

Diets were designed to maintain at least 10% of particles > 19 mm to help ensure consistent and adequate peNDF across diets (**Table 3**). Although all diets contained at least 10% of particles on the top screen, there was a significant (*P* < 0.01) linear decrease in particles > 19 mm as WCGF increased in the diet. Conversely, there was a significant (*P* < 0.01) linear

increase in particles on the bottom screen (1.18 – 8 mm) as WCGF increased. This can be explained by the reduction in corn silage and alfalfa as WCGF was increased in the diet, resulting in a decrease in the number of large particles. In order to determine if cows actually consumed the TMR as fed, a sorting index was employed as described by Silveira et al. (2007). The calculated values are shown in **Table 3**. A value of 1.00 indicates that no sorting occurred, whereas values < 1.00 indicate animals sorted against the fraction and values > 1.00 indicate animals selected for the fraction. Surprisingly, as WCGF increased in the ration, selection for larger particles significantly ($P = 0.04$) increased in a quadratic fashion while selection of particles on the bottom screen (1.18 – 8 mm) and pan (< 1.18mm) linearly decreased ($P < 0.01$).

Ration Composition, Feed Intake, and Milk Production

Intake and milk components are presented in **Table 4**. There was a significant quadratic effect ($P = 0.02$) on DMI with the highest intake for the 24.5% WCGF diet. Milk production responded similarly to intake with a significant quadratic increase ($P = 0.02$) with the 24.5% WCGF treatment again yielding the greatest response with production of 41.4 ± 3.6 kg/d. There were no differences in 3.5% FCM, BCS or BW between diets; however, there was a tendency ($P = 0.08$) for ECM to increase linearly with increasing levels of WCGF. When evaluating efficiency (ECM/DMI), no differences were detected between treatments.

Across all treatments, milk fat concentration, lactose concentration, and fat yield were similar (**Table 5**); however, there was a tendency for a quadratic increase ($P = 0.09$) in milk protein concentration as WCGF inclusion rates increased and a significant quadratic response ($P = 0.04$) was observed for MUN. Furthermore, milk protein yield increased linearly ($P < 0.01$) with WCGF inclusion while milk lactose yield showed a significant quadratic ($P = 0.02$) response. The quadratic increase in milk lactose yield is likely a result of the increase in milk production, shown in **Table 3**, as there were no differences in lactose concentration among diets.

Ruminal pH and VFA

Mean ruminal pH ranged from 6.00 to 6.13 across treatments (**Table 6**); neither time under nor area under pH 5.8 or 5.6 were affected by treatment. Total ruminal VFA concentration was not affected by treatment (**Table 7**); however, acetate concentration linearly decreased ($P < 0.01$) with increasing levels of WCGF. Propionate, butyrate and lactate concentrations were not affected by WCGF inclusion level. As WCGF increased in the diet, isobutyrate and isovalerate

concentrations linearly decreased ($P < 0.01$) while valerate concentrations linearly increased ($P < 0.001$). Although propionate concentration was not affected, the decrease in acetate levels was great enough to lead to a significant decrease in the A:P ratio ($P < 0.001$).

Discussion

This study was successful in meeting particle size goals across all diets and the quantity of particles on each screen differed greatly from those reported by Mullins et al. (2010). The present study maintained consistently higher levels of particles > 19 mm with ranges of 10.1 – 15.0% compared to 2.36 – 3.85% in the previous study (Mullins et al., 2010). Another large difference between the two was the proportion of particles < 8 mm, with the present study providing fewer particles below this level than did Mullins et al. (2010). These differences in particle size may play a crucial role in the differences seen in intake, yields, efficiency, and ruminal pH.

Aside from the differences in particle size, treatment diets were relatively similar between the two experiments. In the present experiment, NDF and CP concentrations were slightly lower across all treatment diets, yet followed similar trends as those fed by Mullins et al. (2010). In both studies, starch decreased with increasing WCGF, although the decline was sharper in the present study.

Dietary NDF concentrations were relatively low in this study. The NRC (2001) provided recommendations for NDF as a function of NDF derived from forage sources, as forage sources of NDF are more effective in maintaining milk components. For example, the minimum NDF recommended for a diet containing 19% NDF from forage is 25%, while a diet containing only 15% NDF from forage requires at least 33% NDF (NRC, 2001). In the 0% and 12.4% diets, NDF requirements were satisfactory in regards to requirements outlined by the NRC; however, the 24.5 and 35.1% diets are both limiting in NDF, as the NRC would require a minimum of 31 and $> 33\%$ NDF for these levels of forage NDF. The lack of a negative effect on performance from these 2 diets, especially in light of the decrease in efficiency observed in the previous study (Mullins et al., 2010) highlights the important role of particle size in the present study.

Both studies found similar responses in multiple production parameters, demonstrating increases in DMI, milk yield, ECM, milk protein yield, and milk lactose yield as WCGF inclusion rates increased. These results parallel those of VanBaale et al. (2001) who fed 0 or

20% WCGF to cows averaging 147 ± 55 DIM (mean \pm SD). Additionally, neither study reported any changes in milk fat or lactose percentages with increasing WCGF. In the study by Mullins et al. (2010) milk fat yield increased while there was no change in milk protein concentration. This differs from the present study where milk fat yields were not statistically different between diets and milk protein concentration tended to increase quadratically. Other differences occurred between the two studies, including a decrease in efficiency and an increase in BCS in the study by Mullins et al. (2010) suggesting energy was directed toward adipose accretion in diets containing high levels of WCGF. In the present study, efficiencies were maintained across all treatments along with BCS, which would suggest that energy consumed in the present study was partitioned in a consistent manner across diets. One possible reason for this observed difference in the partitioning of energy could be the result of the proportions of acetate and propionate. Van Kneysel et al. (2007) determined that diets designed to be glucogenic resulted in decreased milk fat yields and less body fat mobilization suggesting an alteration of the energy balance between body reserves and milk fat yields with varying isocaloric diets in early lactation cows. These results may not hold true for later lactation animals.

Ruminal levels of acetate significantly decreased in both the present study and that of Mullins et al. (2010); however, in the present study, there was only a trend for a quadratic increase in propionate while Mullins et al. (2010) found a significant increase in propionate levels with increasing WCGF. These changes are also seen in the A:P of the two studies. At the highest inclusion of WCGF, A:P for the present study was 2.0 while Mullins et al. (2010) yielded 1.96. These results differ largely from those of Schroeder (2003) who found no differences in DMI, milk yield, milk fat and milk protein concentrations, or efficiencies when feeding WCGF at levels between 0 and 45% DM in a corn silage and alfalfa haylage diet. In this study, ruminal pH along with A:P were much higher than those observed by Mullins et al. (2010) or in the present study and there were no significant changes to ruminal concentrations of acetate or propionate.

Another area of interest between these studies is the effects of WCGF and particle size on ruminal parameters. In the study by Mullins et al. (2010), ruminal pH declined with increasing WCGF; however, in the present study, there were no statistical differences in ruminal pH between treatments. Despite the changes across treatments in particles > 19 mm in the present study, the relative abundance of large particles in all treatments may help to explain the

consistency of ruminal pH by stimulating chewing activity, increasing salivary secretions and ultimately increasing buffers delivered to the rumen. Another possible explanation could be that the large particles slowed passage rates and thus decreased feed intake and the rapid fermentation that can occur after large meals. Although DMI was slightly lower in the present study than those in Mullins et al. (2010), it is difficult to compare these values to determine if this difference was enough to affect ruminal pH.

Under extreme conditions such as sub-acute ruminal acidosis (SARA), modifications to ruminal fermentation can arise and often lead to milk fat depression and depressed DMI (Kleen et al., 2003). Kleen et al. (2003) define SARA as present when mean ruminal pH is ≤ 5.5 and absent when mean ruminal pH is ≥ 5.8 . Zebeli et al (2008), who conducted a meta analysis of 100 studies, concluded that mean ruminal pH should not drop below 6.16 and that time under pH 5.8 should not exceed 5.24 h/d in order to minimize the risk of SARA. Based on these criteria, when using mean pH as a predictor for SARA, it was highly unlikely that SARA was present in any of the treatment diets; yet, time under pH 5.8 was greater than 5.24 h/d for all treatment diets except the 12.4% WCGF diet. This does not verify the presence or lack of SARA in these treatments, yet it may help to explain the low milk fat concentrations in this study. Krause and Oetzel (2006) determined that pH can range between 0.5 – 1.0 pH units around the mean and, along with diurnal pH fluctuations, mean pH values should be used with caution (Palmonari et al., 2010). In this study, pH data was collected continuously, which adds confidence to the mean pH values reported here.

Another interesting observation from the present study was the finding that as particle size decreased, cows selected for larger particles. This increased selection for large particles, which may aid in maintaining ruminal pH and function, is consistent with the theory presented by Cooper et al. (1996). This theory states that when given the opportunity, ruminants will sort in favor of particles that aid in maintaining a healthy rumen environment. These results are similar to those of DeVries et al. (2008) who utilized mid-lactation (low-risk) and early lactation (high-risk) cows to evaluate the effects of acidosis on sorting. When cows were not challenged with acidosis, they tended to sort for medium length particles and against the longest and shortest particles. When high-risk cows were challenged with acidosis, they demonstrated the greatest degree of sorting and sorted in favor of longer particles. Beauchemin and Yang (2005) found similar patterns in sorting for longer particles when cows had low ruminal pH. Although

multiple studies have demonstrated this behavior, the level of ruminal pH at which this behavior is observed is not known.

Conclusion

When WCGF was increased to 24.5% of diet DM, DMI, milk yield, and protein yield were increased while ruminal pH and production efficiencies were maintained, likely in part because of adequate particle size. The study was successful in maintaining at least 10% of the diet as particles > 19 mm, although there was a linear decrease in particles > 19 mm as WCGF inclusion increased. The quadratic responses seen in this study would suggest that, although milk protein yield increased linearly, including WCGF at 24.5% of diet DM resulted in the most desirable production responses while still maintaining consistent ruminal pH. Additionally, results from this study support the theory that ruminants, when given the opportunity, will sort for particles that help to maintain a healthy ruminal environment.

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Table 2.1. Ingredient and nutrient composition of dietary treatments

		Treatment			
		0%	12.4%	24.5%	35.1%
% of DM	Corn silage	19.9	23.0	19.8	16.3
	WCGF	---	12.4	24.5	35.1
	Alfalfa	24.5	24.8	21.1	17.4
	Cottonseed	6.4	6.5	6.4	6.2
	Ground corn grain	26.5	21.8	18.8	15.9
	Soybean meal 48	8.6	4.9	2.2	2.2
	Molasses	0.4	0.4	0.4	0.4
	Soybean hulls	5.0	---	---	---
	Limestone	1.0	1.1	1.3	1.4
	Expeller soybean meal	3.3	3.7	4.0	3.6
	Magnesium oxide	0.26	0.24	0.21	0.17
	Micronutrient premix	1.3	1.3	1.3	1.3
Nutrients ¹					
	Dry matter, % as-fed	63.1	60.3	60.2	60.8
	Crude protein	18.1	17.5	17.1	17.6
	Neutral detergent fiber	27.4	28.4	29.8	30.6
	NDF provided by forage	18.5	18.7	16.0	13.2
	peNDF _{>1.18}	23.9	25.7	27.1	28.2
	Starch	27.2	25.9	24.2	22.2
	NFC	41.9	41.5	40.5	39.3
	Ether extract	4.4	4.3	4.2	4.1
	Ash	8.2	8.3	8.4	8.4

¹Nutrients other than DM expressed as a percent of diet DM.

Table 2.2. Composition of corn silage, alfalfa hay, and wet corn gluten feed (WCGF)

Nutrient ¹	Corn Silage	Alfalfa Hay	WCGF
DM, % as-fed	33.0	84.7	59.4
NDF	37.3	40.7	37.9
CP	8.0	20.3	19.0
Starch	36.7	1.6	13.5
EE	3.6	2.4	2.1
Ash	5.5	9.9	5.5

¹Nutrients other than DM expressed as a percentage of diet DM.

Table 2.3. Particle size distribution and sorting index

	Treatment				SEM	<i>P</i> value		
	0%	12.4%	24.5%	35.1%		Linear	Quadratic	
TMR, % of DM	> 19 mm	15.0	13.6	12.3	10.1	1.88	0.01	0.71
	8-19 mm	25.8	27.3	30.6	27.2	1.95	0.45	0.24
	1.18-8 mm	47.0	49.4	48.0	54.2	1.50	0.01	0.28
	< 1.18 mm	12.6	9.5	8.9	7.8	1.23	< 0.01	0.01
Sorting Index ¹	> 19 mm	0.95	0.90	0.95	1.04	0.04	0.04	0.04
	8-19 mm	0.99	0.99	0.99	1.01	< 0.01	0.27	0.35
	1.18-8 mm	1.01	1.02	1.00	0.98	0.01	< 0.01	0.09
	< 1.18 mm	1.04	1.03	1.02	1.00	0.01	0.01	0.75

¹Calculated from methods outlined by Silveira et al. (2007). >1.0 = sorting in favor, <1.0 = sorting against.

Mean refusals as percent of feed offered: 0% = 14.6%, 12.4% = 13.6%, 24.5% = 12.4%, 35.1% = 16.0%

Table 2.4. Effects of treatments on intake and performance of lactating cows

	Treatment				SEM	<i>P</i> value	
	0%	12.4%	24.5%	35.1%		Linear	Quadratic
DMI, kg/d	25.2	26.6	27.0	26.5	1.17	0.02	0.02
Water intake, gal/d	34.1	34.1	37.1	36.7	2.08	0.11	0.88
Milk, kg/d	37.7	40.8	41.4	40.7	3.64	0.01	0.02
3.5% FCM	34.8	37.3	36.7	36.8	3.06	0.19	0.18
ECM, kg/d	35.3	37.9	37.6	37.7	3.03	0.08	0.14
ECM/DMI	1.39	1.43	1.39	1.42	0.08	0.60	0.92
BW change, kg/21 d	-2.7	-11.1	-13.8	-6.3	8.80	0.58	0.14
BCS change/21 d	-0.10	-0.05	-0.08	0.03	0.05	0.14	0.56

Table 2.5. Effects of treatments on milk components

	Treatment				SEM	<i>P</i> value	
	0%	12.4%	24.5%	35.1%		Linear	Quadratic
Milk fat, %	3.05	2.99	2.83	2.97	0.16	0.24	0.17
Milk protein, %	2.94	2.92	2.93	3.01	0.12	0.10	0.09
Lactose, %	4.84	4.87	4.86	4.84	0.07	0.93	0.29
MUN, mg/dL	13.2	12.6	12.9	13.6	0.82	0.30	0.04
Yield, kg/d							
Milk fat	1.14	1.21	1.16	1.18	0.11	0.73	0.53
Milk protein	1.09	1.18	1.20	1.21	0.09	< 0.01	0.09
Milk lactose	1.82	1.98	1.99	1.96	0.16	0.03	0.02

Table 2.6. Effects of treatments on rumen pH

	Treatment				SEM	<i>P</i> value	
	0%	12.4%	24.5%	35.1%		Linear	Quadratic
pH	6.05	6.13	6.00	6.00	0.07	0.26	0.45
Standard Deviation	0.42	0.40	0.40	0.44	0.02	0.58	0.19
Time under 5.8 (min/d)	434	304	467	465	72.9	0.31	0.24
Area under 5.8 (pH x min/d)	130	88	144	138	28.5	0.50	0.46
Time under 5.6 (min/d)	257	175	290	293	56.4	0.33	0.39
Area under 5.6 (pH x min/d)	62	39	73	61	16.6	0.65	0.74

Table 2.7. Effects of treatments on ruminal VFA

	Treatment				SEM	<i>P</i> value	
	0%	12.4%	24.5%	35.1%		Linear	Quadratic
Total VFA, mM	129.3	135.7	128.1	123.1	5.65	0.20	0.20
Acetate, mM	77.4	78.3	71.9	66.8	2.83	<0.01	0.26
Propionate, mM	31.2	35.1	34.6	33.9	2.45	0.18	0.08
Butyrate, mM	14.8	15.7	15.3	15.8	0.71	0.37	0.75
Isobutyrate, mM	1.04	1.02	0.91	0.86	0.05	<0.01	0.78
Isovalerate, mM	0.91	0.86	0.74	0.62	0.09	<0.01	0.58
Lactate, mM	0.18	0.91	0.87	0.51	0.33	0.57	0.15
Valerate, mM	2.58	3.08	3.23	3.68	0.24	<0.01	0.92
A:P Ratio	2.58	2.26	2.08	2.00	0.11	<0.01	0.03
NH ₃ , mM	6.44	7.66	7.23	6.81	0.57	0.76	0.10

Chapter 3 - Viable Cell Yield from Active Dry Yeast Products and Effects of Storage Temperature and Diluent on Yeast Cell Viability

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Abstract

Active dry yeast (ADY) products are commonly fed in the dairy industry, but research regarding quality control for such products is limited. The objectives of this study were to determine yeast viability in field samples relative to manufacturers' guarantees (experiment 1), measure the effects of high-temperature storage on yeast viability (experiment 1), and determine the effect of vitamin trace mineral (VTM) premix on yeast viability (experiment 2).

Commercially available ADY products were acquired in triplicate through normal distribution channels and stored at 4°C upon receipt. Initial samples were evaluated for colony forming units and compared with product label guarantees. Only 1 of the 6 products sampled in experiment 1 met product guarantees for all 3 samples. To determine effects of storage temperature and duration on viability, ADY samples were stored in an incubator at 40°C with ambient humidity for 1, 2, and 3 mo. High-temperature storage significantly decreased viability over the 3-mo period; approximately 90% of viable cells were lost each month. Three of the 5 products sampled in experiment 2 met product guarantees. Fresh samples of 4 of these 5 ADY products were mixed in duplicate with ground corn (GC) or a VTM premix to achieve a target concentration of 2.2×10^8 cfu/g. For each product, GC and VTM samples were stored at ambient temperature (22°C) and at an elevated temperature (40°C) for 2 wk. There were no differences in viable yeast count between GC and VTM samples immediately after mixing or after storage at ambient temperature. Yeast viability in GC and VTM samples decreased during storage at an elevated temperature. There also was a significant interaction of diluent and storage temperature; VTM samples had higher cell viability than GC samples when subjected to high-temperature storage. Results suggest that (1) ADY products failed to consistently meet product guarantees; (2) viability of ADY products was greatly diminished during storage at 40°C for 2 wk; and (3) the loss in viability at elevated temperatures may be attenuated when ADY products are diluted with a premix containing VTM.

Key words: active dry yeast, storage, viability, vitamin trace mineral

Introduction

Several active dry yeast (ADY) products are marketed for use in diets for lactating dairy cattle. These products are included in rations because they are thought to alter ruminal fermentation and increase milk production, although in vivo studies conducted to investigate these claims have shown inconsistent results (Swartz et al., 1994; Putnam et al., 1997; Robinson and Erasmus, 2009). Proposed modes of action for ADY products include release of growth factors and removal of oxygen from the rumen, each of which could stimulate growth of anaerobic bacteria (Newbold et al., 1996). If utilization of oxygen by yeast is required, or if a yeast product is intended to produce bacterial growth factors after it reaches the rumen, viable yeast cells are required for the ADY product to have any efficacy. Production, storage, and delivery protocols for yeast products should be designed to maintain yeast cell viability.

Companies marketing ADY products generally establish product guarantees that specify the quantity of viable yeast cells provided. In the United States, viable cells are currently enumerated on the basis of colony-forming units. Although these guarantees are routinely used to determine suggested feeding rates for ADY products, little independent research has been conducted to determine whether such guarantees are valid. This is of critical importance because losses in cell viability may occur during the distribution process. Temperatures above 48°C consistently decrease yeast cell viability in a matter of minutes (Bayrock and Ingledew, 1997). However, little is known about long term effects of moderately high temperatures on yeast cell viability of ADY products. Therefore, this study was designed to determine if storing ADY products in conditions similar to those found in summer warehouse storage would influence yeast viability and to assess whether ADY products acquired via commercial distribution channels consistently meet viable cell guarantees.

In addition, it has become common practice to combine yeast supplements with vitamin-trace mineral (VTM) packs. The effects of minerals on the viability of lyophilized yeast cells have not been widely studied. Some minerals act as oxidizing agents, and storing yeast in the presence of these minerals may compromise viability. To determine the effects of such conditions, we assessed the viable cell count of blended ADY products with or without VTM in the diluent after storage at ambient and elevated temperatures.

Materials and Methods

Products

Eight commercial ADY products were obtained through normal distribution channels. Not all products were used for both experiments because of variation in regional availability at each sampling time. The number of products tested was somewhat limited by difficulty in identifying multiple product distributors without involving the yeast manufacturers. The limited sample size increases the risk of samples not being representative of ADY products as a whole; however, multiple lots were sampled for each product to minimize the risk of bias in this analysis. Few product labels contained expiration dates, but products were analyzed before expiration dates if they were present.

Active dry yeast products used in this study and their guaranteed viable cell counts were as follows: product A: Yea-Sacc¹⁰²⁶ (Alltech, Nicholasville, KY), 2.50×10^8 cfu/g and 5.00×10^9 cells/g (US product label); product B: Yea-Sacc¹⁰²⁶ Farm Pak 2X (Alltech), no cfu/g guarantee but 8.81×10^8 cells/g; product C: Biomate Yeast Plus (Chr. Hansen, Hørsholm, Denmark), 5.00×10^9 cfu/g; product D: Levucell SC FarmPak (Lallemand Animal Nutrition, Blagnac, France), 2.00×10^8 cfu/g; product E: Procreatin-7 (SAF Agri, Milwaukee, WI), 1.50×10^{10} cfu/g; product F: FermaStar (Animal Science Products, Nacogdoches, TX), 2.20×10^9 cfu/g; product G: Biosaf (SAF Agri), 1.0×10^{10} cfu/g; and product H: Levucell SC20 (Lallemand Animal Nutrition), 2.00×10^{10} cfu/g.

Experiment 1: Product Survey and High-Temperature Storage

In experiment 1, 3 samples for each of 6 ADY products sampled were acquired from 2 distributors in 2007. Repeat purchases from a single source occurred at least 3 mo apart to ensure that samples represented different lots of the product. Products were acquired in the spring (March through April) and summer (July through August). Upon receipt, approximately 400 g of each shipped sample was collected into opaque Whirl-Pak bags (Fisher Scientific, St. Louis, MO), sealed, and stored at 4°C until analysis. Samples were analyzed to determine viable cell count within 3 wk of receipt. After initial analysis, a subsample (approximately 50 g) of each product acquired in the spring was placed in an incubator (Isotemp Incubator, Thermo Fisher Scientific, Waltham, MA) for storage at 40°C with ambient humidity. Samples stored in high-

temperature conditions were analyzed to determine viable cell count after 1, 2, and 3 mo of storage.

Experiment 2: Product Survey and Preparation of Diluted ADY Mixtures

Samples were collected for 5 products in 2009; products chosen for this portion of the study were selected on the basis of commercial availability at the time and the colony forming units concentration needed to allow for blending. Blended products (e.g., products B and D) were excluded from experiment 2 to achieve necessary initial colony forming units concentrations for the dilution experiment. Upon receipt, approximately 400 g of each shipped sample was collected into opaque Whirl-Pak bags (Fisher Scientific), sealed, and stored at 4°C until analysis. Samples were analyzed to determine viable cell count within 3 wk of receipt. Four of the 5 products were determined to have a minimum viable yeast concentration of 2.20×10^9 cfu/g, which would allow them to be blended with at least 90% diluent to a desired final concentration of 2.20×10^8 cfu/g. The desired final concentration was based on a target feeding rate of 1×10^{11} cfu/cow per day, supplemented at a rate of 454 g/cow per day; this is a relatively common strategy in the US dairy industry. To assess the effect of VTM inclusion on yeast cell viability, ADY products were blended with a VTM premix or with ground corn (GC) that matched the particle size of corn in the premix. The VTM premix (Table 1) was formulated to provide supplemental vitamins and minerals at recommended concentrations for lactating dairy cattle (NRC, 2001) when fed at 0.45 kg/cow per day along with a limestone supplement.

To enable small batches to be mixed to homogeneity, a Daisy II in vitro incubator (Ankom Technologies, Macedon, NY) was used with the heat turned off. Before mixing ADY products with diluents, the appropriate mixing protocol was determined by blending 2 g of chromium into 454 g of ground corn grain. Four separate batches were mixed, and 5 samples were collected from each batch after 10, 20, 30, 60, and 120 min of mixing (25 samples total) for analysis of chromium concentration (Williams et al., 1962). The within-batch coefficient of variation for chromium concentration decreased through the 120 min sample, at which point it was equivalent to the across batch coefficient of variation (data not shown). Therefore, ADY products were mixed with the respective diluents for 120 min before storage and analysis. Samples of the remaining products were diluted in GC and VTM. Diluted ADY products were analyzed for viable cell count immediately after mixing. Remaining mixes were stored at 4°C for approximately 2 wk before the second phase of the study.

To determine effects of storage temperature on cell viability, ADY blends were split 4 ways and assigned to either ambient (22°C) or elevated temperature (40°C) storage for 2 wk. Thus, for each product, duplicate samples diluted in GC or VTM were stored at each of the 2 temperatures.

Colony-Forming Unit Enumeration

For experiment 1, colony forming units were quantified according to AOAC method 997.02 (AOAC, 2002) with minor modifications. Specifically, 1 g samples were diluted in 100 mL of 0.31 mM phosphate buffer (pH 7.2) and homogenized for 5 min by using a magnetic stirrer. This solution was serially diluted (1:10), and appropriate dilutions were inoculated onto Petrifilm plates (Petrifilm yeast and mold count plates, 3M Microbiology Products, St. Paul, MN). Plates were incubated at 25°C for 5 d before yeast colonies were counted. Only plates with total counts between 15 and 300 colonies were used for data analysis, and each diluted sample was plated and counted in triplicate. The mean value from the triplicate analysis was used for subsequent statistical analysis.

For experiment 2, samples were shipped on ice to Medallion Labs (Minneapolis, MN) for analysis. Colony forming units were quantified according to AOAC method 997.02 (2002), and each sample was quantified in duplicate. Water activity (a_w) was evaluated on an AquaLab Series 3 (Decagon Devices, Inc., Pullman, Washington) machine by using the chilled-mirror dew point method (Fontana, 2001).

Statistical Analysis

Mean colony-forming units per gram and standard error of the mean were determined for each of the first 6 products collected. The effect of storage time was assessed using 4 values (0, 1, 2, and 3 mo of storage) for each of 6 products and analyzed in an ANOVA model including the fixed effect of storage time and random effect of product. Effects of season of purchase (spring, $n = 6$; summer, $n = 12$) were analyzed by ANOVA with the fixed effect of season and random effect of product included in the model. For the blended products, postmixing effects were analyzed separately from results after 2 wk of storage. Effects on viable cell count were analyzed in a mixed model including fixed effects of diluent, storage temperature, and their interaction and the random effect of product. Significance and tendencies were declared at $P < 0.05$ and $0.05 < P < 0.10$, respectively.

All values were \log_{10} transformed before analysis to achieve a normal distribution of residuals. For comparisons with product guarantees, \log_{10} means were back transformed to derive the geometric mean, which is different from the mean of the raw values (Bland and Altman, 1996).

Results and Discussion

The concentration of colony forming units in commercial yeast products was highly variable. Within individual products, the colony-forming units concentration of samples purchased at different times varied by as much as 4,000-fold. Of the products analyzed in experiment 1, only product E contained the guaranteed concentration of viable cells in each of the 3 samples collected, and product A and product F were the only other products with mean colony forming units concentrations greater than the product claim (Table 2).

Cell viability was decreased ($P < 0.001$) by high temperature storage (Table 3); mean colony forming units per gram decreased by approximately 90% with each month of storage at 40°C. These data align with those presented by Reed and Chen (1978), which showed a mean reduction of 99.1% in viability of active dry wine yeast products stored at 40°C for 6 mo. This was noticeably less than the mean reduction of 38.8% when the products were stored at 5°C for 6 mo, but the authors provided no statistical analysis.

The results of the high temperature storage test led us to consider the effect of season on the viability of product samples. Products acquired in the summer were compared with those acquired in the spring, but season did not affect ($P = 0.31$) viable cell count (9.88 and 9.16 \log_{10} cfu/g for spring and summer, respectively). The limited number of samples analyzed and the variability in viable cell count within seasons made it unlikely that we would detect a significant effect of season. The study was blind to the length of storage of each product at the distributor level. Differences in total storage time may have contributed to the variability in viable yeast count of samples at initial sampling time.

Two of the five commercial ADY products in experiment 2 did not contain the concentration of viable yeast cells guaranteed by the manufacturer (Table 4). Given that the target final concentration for the diluted products was 2.2×10^8 cfu/g, product A could not be used because the final diluted product would have been 77% product A and only 23% diluent. Therefore, the remaining 4 products were used in the premix experiment.

After products were blended with GC and VTM diluents, the mean colony forming units for GC and VTM mixes were 9.80×10^7 cfu/g and 8.49×10^7 cfu/g, respectively; neither was significantly different from the target concentration (Figure 1). After samples had been stored for 2 wk at ambient temperature, no differences were observed in viable yeast counts between mixes (1.11×10^8 cfu/g for GC mixes and 9.80×10^7 cfu/g for VTM mixes, Figure 1). Yeast viability decreased ($P < 0.001$) when mixes were stored at 40°C and was affected by treatment; means at the warmer temperature were 9.65×10^6 cfu/g for GC mixes and 2.56×10^7 cfu/g for VTM mixes. Surprisingly, yeast viability in the VTM mix was greater than that in the GC mix when samples were held at 40°C for 2 wk (treatment \times time interaction, $P = 0.02$, Figure 1).

We initially hypothesized that corrosive effects of minerals in VTM would decrease cell viability. At ambient temperatures, cell viability was virtually the same for both diluents; at high temperatures, however, viability was significantly lower in the GC treatment. Walker et al. (2006) demonstrated that although heavy metals may be toxic to yeast, some bulk and trace minerals, such as magnesium, zinc, and manganese, may help sustain yeast viability by maintaining cell wall structure and integrity. Walker et al. (2006) showed that the availability of zinc and magnesium increased yeast viability in a brewing medium subjected to heat stress. Although these effects were observed in media common to commercial fermentation and not in a dry environment, they may help explain the increased yeast viability in VTM samples in the present experiment.

Moisture content of the diluents is another potential reason for the reduction in colony-forming units concentration in GC mixes at elevated temperatures. Yeast cells in ADY products are in a dormant state, but as temperature and a_w increase, cells can become activated and die because of inadequate available nutrients. Mugnier and Jung (1985) defined a_w as the partial pressure of water in the product divided by the partial pressure of pure water. This value is a measure of the water available in a sample to do work, which distinguishes it from a DM value typically measured in animal feedstuffs. Water activity of the GC and VTM diluents was 0.506 and 0.448, respectively. Mugnier and Jung (1985) found that viability of *Saccharomyces cerevisiae* rapidly declined at a_w values > 0.40 when samples were stored for 15 d at 25°C. Although a_w values for both GC and VTM were greater than this value, the high storage temperature (40°C) used in this experiment may have amplified the response to a slightly higher

a_w in GC. This could also explain why no difference in yeast viability was observed in GC or VTM samples stored at ambient temperature.

Finally, it is possible that vitamins included in the VTM could aid in maintaining cell viability because of their antioxidant characteristics. Although our initial hypothesis focused on oxidizing minerals in the premix, the VTM diluent also included vitamins A and E, as well as selenium, which are potent antioxidants. It is possible that these antioxidant components of VTM could counteract any potential harmful effect of oxidizing minerals.

In experiment 2, ADY product dilution was adjusted to meet a target colony forming units per gram value to better assess the effects of GC and VTM on viability. This adjustment would not typically occur in the industry, and given the initial variability in products along with storage effects, there is the potential for huge variability in the colony forming units that cows would consume. For example, product H arrived at 48% of its product guarantee. If this product were blended with GC under the assumption that it met its guarantee and then stored in a warm warehouse, a cow that consumed 454 g of the blend would have received 2.1×10^9 cfu. Conversely, product G surpassed its product guarantee by 440%. If this product were blended with GC and stored in a cool environment, a cow that consumed 454 g of the blend would have received 2.7×10^{11} cfu, which is more than the target feeding rate and 129 times the colony-forming units delivered in the first scenario. This example demonstrates the dramatic variability that likely exists on dairy farms that use ADY products and emphasizes the importance of quality control for improving consistency in feeding rates. These results may also help to explain why previous studies have shown such variability in results with the inclusion of ADY products (Kung et al., 1997; Wohlt et al., 1998).

Conclusion

Samples of ADY products collected via normal distribution channels revealed highly variable concentrations of viable cells within and across products. Within individual products, viable cell counts of samples purchased at different times varied by as much as 4,000-fold. Even when samples were stored in an airtight, ambient humidity environment, the high-temperature storage condition (40°C) decreased viable cell counts of ADY products by approximately 90% per month. Similarly, yeast viability of products blended with GC or VTM decreased during storage at elevated temperatures, although diluting with VTM attenuated this loss of viability

compared with diluting with GC. Active dry yeast products should be stored at cool temperatures and used as quickly as possible if yeast cell viability is to be maintained until the product is fed. Also, blending such products in a VTM premix may provide partial protection from the harmful effects of high temperature storage.

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Table 3.1. Ingredient and nutrient composition of vitamin/trace mineral Premix

Ingredient	% of DM
Ground corn grain	56.4
Trace mineral salt premix	22.0
Magnesium oxide	11.0
4-Plex [†]	4.0
Selenium premix	3.0
Vitamin A,D,E premixes	3.6
<hr/>	
Nutrients	
Ca (%)	1.55
P (%)	0.17
Mg (%)	6.26
K (%)	0.21
Na (%)	8.58
Cl (%)	13.5
S (%)	0.07
Co (ppm)	76.1
Cu (ppm)	391
Fe (ppm)	441
I (ppm)	15.5
Mn (ppm)	969
Se (ppm)	18.0
Zn (ppm)	1705
Vitamin A (KIU/kg)	61.7
Vitamin D (KIU/kg)	86.6
Vitamin E (IU/kg)	291

[†] Zinpro Corporation. Eden Prairie, Minnesota

Table 3.2. Colony-forming units observed upon receipt in samples of commercial active dry yeast products (experiment 1)

Product	Season Acquired	Colony forming units (g ⁻¹)	Mean (Log ₁₀ CFU/g)	SEM	Geometric mean ¹ (CFU/g, % of guarantee)
Yea-Sacc ¹⁰²⁶	Spring	2.15×10^{10}	8.78 × 0.78		6.04×10^8
	Summer	6.83×10^7			242%
	Summer	1.50×10^8			
Yea-Sacc ¹⁰²⁶ FarmPak 2X	Spring	4.70×10^8	8.87 × 0.69		7.38×10^8
	Summer	6.10×10^7			
	Summer	1.40×10^{10}			
Biomate Yeast Plus	Spring	3.62×10^{10}	9.60 × 1.07		3.97×10^9
	Summer	2.93×10^7			79%
	Summer	5.87×10^{10}			
Levucell SC FarmPak	Spring	3.04×10^9	7.63 × 1.05		4.26×10^7
	Summer	7.33×10^5			21%
	Summer	3.47×10^7			
Procreatin-7	Spring	1.11×10^{11}	12.08 × 0.61		1.21×10^{12}
	Summer	1.11×10^{12}			8,052%
	Summer	1.43×10^{13}			
FermaStar	Spring	1.46×10^9	9.43 × 0.13		2.71×10^9
	Summer	3.77×10^9			123%
	Summer	3.63×10^9			

¹ Back transformed from the log₁₀ mean after distribution analysis.

Table 3.3. Effect of storage at 40°C on viable cell count in commercial active dry yeast products (experiment 1)

Item	Months of Storage				SED	P-value
	0	1	2	3		
Mean cfu/g (log ₁₀)	9.88 ^a	7.37 ^b	6.28 ^{bc}	5.43 ^c	0.86	<0.001

^{a-c}Values without like superscripts are significantly different ($P < 0.05$).

Table 3.4. Colony-forming units observed upon receipt in samples of commercial active dry yeast products (experiment 2)

Product	Colony forming units (g ⁻¹)	Colony forming units (% of guarantee)
A	2.85×10^8	114% ^a
C	3.55×10^9	71%
E	1.80×10^{10}	120%
G	5.40×10^{10}	540%
H	9.50×10^9	48%

^aRelative to guaranteed CFU/g. Analyzed value is 6% of guaranteed cells/g listed on US product label.

Figure 3.1. Viable yeast cell count after mixing with corn (GC) or a vitamin-trace mineral (VTM) premix and after 2 wk of storage at ambient or high temperature. The target viable yeast count after mixing was 2.2×10^8 cfu/g. No difference in colony-forming unit concentration was found between GC and VTM immediately after mixing ($P = 0.47$). A significant effect of temperature was observed ($P < 0.001$) as well as a diluent \times temperature interaction ($P = 0.02$) after 2 wk of storage. Values are means \pm SED ($n = 4$); note the \log_{10} scale on the figure.

