

EFFECTS OF MIXER TYPE ON UTILIZATION OF DIETS WITH
INCREASING AMOUNTS OF MODIFIED WET DISTILLERS
GRAINS WITH SOLUBLES FOR LACTATING DAIRY CATTLE

BY

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THESIS

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ABSTRACT

Our objective was to determine if methods for preparing TMR [Keenan MechFiber (KMF) technology vs. vertical auger (VA) mixer] would alter physical form of the TMR and affect utilization of modified wet distillers grains with solubles (MWDGS). Holstein cows ($n = 24$ with 12 ruminally cannulated; $144 \text{ DIM} \pm 31 \text{ d}$ at start) were used in a split-plot design with mixer type as the whole plot and MWDGS concentrations as subplots in a 3×3 Latin square arrangement with 35-d periods. Inclusion rates of MWDGS were 10, 20, and 30% of dietary DM, primarily replacing corn, SBM, and whole cottonseed. Feed DMI was less for KMF ($P = 0.05$), but was unaffected by MWDGS concentration ($P = 0.39$). Milk production did not differ ($P = 0.75$) by concentration of MWDGS or by interaction of MWDGS \times mixer ($P = 0.18$). Milk protein content tended ($P = 0.09$) to decrease linearly with increasing MWDGS. Milk fat percentage declined with increasing MWDGS ($P = 0.003$) but the interaction between mixer wagon and MWDGS ($P = 0.006$) showed that decreases were larger with VA mixing. Cows fed the diet containing 30% MWDGS mixed with KMF averaged 3.45% (1.24 kg/d) milk fat; whereas, cows fed the same diet mixed with VA averaged 2.81% (1.10 kg/d) fat. Concentrations of CLA *trans*-10, *cis*-12 in milk likely explain the differences in milk fat; the concentration of CLA *t*-10, *c*-12 increased as MWDGS was increased ($P < 0.0001$) and the MWDGS \times mixer interaction ($P = 0.03$) showed that VA had greater concentrations. Greater mean particle size and variation with VA may partially explain differences in milk fat via increased sorting that allowed for an altered rumen environment and favored alternative biohydrogenation pathways. Feed conversion efficiency (FCE; energy-corrected milk/DMI) decreased linearly ($P = 0.007$) as MWDGS increased, but FCE tended to be maintained when higher MWDGS diets were mixed using KMF rather than VA (mixer, $P = 0.12$). Ruminal pH ($P = 0.05$) and ammonia

concentration ($P < 0.001$) decreased linearly as MWDGS increased. Using the KMF mixer wagon resulted in better FCE when higher amounts of MWDGS were fed, primarily because milk fat content and yield were not as depressed and DMI was lower.

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“Somewhere, something incredible is waiting to be known.” – Dr. Carl Sagan

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LIST OF ABBREVIATIONS

AA	amino acids
ADF	acid detergent fiber
ASAE	American Society of Agricultural Engineers
BCAA	branched-chain amino acids
BCS	body condition score
BCVFA	branched-chain volatile fatty acids
Ca	calcium
CLA	conjugated linoleic acid
Co-EDTA	cobalt ethylenediaminetetraacetate
CP	crude protein
Cr ₂ O ₃	chromic oxide
DDGS	dried distillers grains with solubles
DG	distillers grains
DGS	distillers grains with solubles
DIM	days in milk
DM	dry matter
DMI	dry matter intake
ECM	energy-corrected milk
EE	ether extract
FA	fatty acid
FAME	fatty acid methyl esters
FCE	feed conversion efficiency
Fe	iron
i.d.	internal diameter
IOFC	income over feed costs
h	hour(s)
K	potassium
KMF	Keenan MechFiber paddle mixer wagon
kg	kilogram(s)

MFD	milk fat depression
Mg	magnesium
Mn	manganese
Mo	molybdenum
MP	metabolizable protein
MWDGS	modified wet distillers grains with solubles
MUN	milk urea nitrogen
Na	sodium
NDF	neutral detergent fiber
NE _L	net energy for lactation
NFC	non-fiber carbohydrates
NRC	National Research Council
OM	organic matter
P	phosphorus
peNDF	physically effective neutral detergent fiber
PSPS	Penn State particle separator
PUFA	polyunsaturated fatty acids
R ²	coefficient of determination
RDP	rumen degradable protein
RUFAL	rumen unsaturated fatty acid load
RUP	rumen undegradable protein
S	sulfur
SAS	Statistical Analysis System
SCC	somatic cell count
TMR	total mixed ration
WDGS	wet distillers grains with solubles
VA	Kuhn-Knight vertical auger mixer wagon
VFA	volatile fatty acids
Zn	zinc

CHAPTER 1

INTRODUCTION

Background Information

Co-products from the ethanol industry such as modified wet distillers grain with solubles (MWDGS) are an attractive, abundant, and low-cost feed ingredient available to dairy producers. Most of the ethanol produced from corn comes from the process of dry grinding in which distillers grains with solubles (DGS) and carbon dioxide are produced as co-products (Rausch and Belyea, 2006). As the starch is fermented, the remaining nutrients become concentrated, making DGS high in protein, fat, and NDF. There has been extensive research on the appropriate level of inclusion DGS into dairy diets (Schingoethe, 1999; Leonardi et al., 2005; Kalscheur, 2005; Anderson et al., 2006; and Ranathunga et al., 2010) with recommendations as high as 30% of total dietary DM (Kalscheur, 2005). Use of DGS has been met with resistance in the field because of reports of lowered milk fat; however, a majority of the research conducted has not seen a reduction in milk fat.

The corn oil in MWDGS contains polyunsaturated fatty acids (PUFA), which have the potential to cause milk fat depression (MFD). Diets high in PUFA can overwhelm the biohydrogenation capacity of the rumen and lead to alternative biohydrogenation pathways that cause an increase in *trans*-10, *cis*-12 CLA, which is known to inhibit milk fat synthesis (Bauman and Griinari, 2001). Some research has indicated that adequate forage fiber (NDF) content in the ration will allow for greater inclusion of DGS. Increased effective NDF will minimize the impacts of the unsaturated oil on rumen fermentation (Schingoethe et al., 2009). Increasing DGS use in dairy diets from the current conservative norm depends on identification of approaches to overcome these concerns other than by altering other components of the ration. Producers often

may be unable or unwilling to make the large changes in feed ingredients needed to utilize these diets.

Research Purpose

Physical presentation of the diet has been shown to affect DMI, milk production, and milk components (Woodford and Murphy, 1988; Beauchemin et al., 2003; and Humphries and Reynolds, 2008). Although use of DGS has been studied extensively, we are not aware of studies that have evaluated the potential effects of mixer type when feeding diets with higher amounts of corn co-products. The Keenan MechFiber system for preparing total mixed rations (TMR) is hypothesized to produce a more uniform particle size that creates more homogeneous rumen digesta. The homogeneous rumen environment leads to a more optimal fermentation in the rumen.

The Keenan system has been shown to make improvements in rumen fermentation and feed conversion efficiency without large modifications of dietary ingredients used. The improved forage fiber structure and consistency in the rumen should make it possible to move to higher limits on DGS inclusion. Another advantage may be the improved rumen fermentation environment that is able to extract more energy from the feed, which might help maintain microbial protein production despite lower ration starch contents. There is little research available to support these projections at present and there is a potential large benefit to dairy producers if higher concentrations of MWDGS can be incorporated.

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CHAPTER 2

LITERATURE REVIEW

Introduction

With expansion of the ethanol industry, there has been an increase in ethanol co-products in the marketplace. Being able to effectively include co-products into dairy diets has been the focus of a substantial amount of research. Modified wet distillers grains with solubles (MWDGS) is a co-product with higher protein, fiber, and fat contents than the corn from which it is derived. The nature of the fermentation process to make ethanol removes the starch; therefore, the protein, fiber, and fat are concentrated. There has been concern about including high levels of MWDGS in dairy diets because of the risk of milk fat depression when PUFA are added to the diet. Additional research should help answer the question of how to best include MWDGS into dairy diets without the negative effects of milk fat depression.

Distillers Grains Products

Distillers grains (DG) are produced by the dry milling process for producing ethanol through fermentation of grains. In the United States, this is mostly corn; however, any grain containing starch can be effectively fermented to ethanol (Schingoethe, 2006). Distillers grains can be used as a partial replacement for both forage and concentrates in a dairy diet. Distillers grains are high in crude protein (CP), ruminally undegradable protein (RUP), and energy. These values have increased over the past decade because of improvements to the fermentation efficiency of ethanol plants (Spiehs et al., 2002), which removes more of the starch. From plant to plant the manufacturing processes of ethanol differ and variation occurs even within the same plant; therefore, the co-product of DG can vary because of plant and batch variations. Distillers

grains also can contain high amounts of phosphorus and sulfur; therefore, depending on feeding rate, DG may pose problems for the environment and animal health (Schingoethe et al., 2009).

As the solubles fraction of the fermentation process is added back to the DG the product becomes distillers grains with solubles (DGS). Much of the variation in nutrient content of DGS comes from the inclusion of differing levels of solubles. The solubles fraction contains much of the fat, phosphorus, and sulfur; adding larger amounts back to the DG results in a dilution of protein and an increase in fat, phosphorous, and sulfur. There have been various recommendations for inclusion rates of distillers solubles. The percentage of solubles added refers to the percent of solubles produced from a batch of distillers that is mixed with the DG. As levels of solubles were increased from 0 to 100%, the level of fat of DGS increased from 8.9 to 11.7% (Noll et al., 2007).

There is a concern with feeding dairy cows high amounts of DGS if phosphorus levels in the soil are already high. Including dry DGS (DDGS) in lactating dairy diets up to 10% did not sufficiently increase phosphorus levels in manure (Schmit et al., 2009). However, when dry cow or heifer diets contained DDGS, the concentration of P in manure was greatly increased. Lactating dairy diets containing 20% DDGS would result in significantly higher P in manure and would require different ways to incorporate P or an increase in acres to incorporate manure (Schmit et al., 2009).

Distillers products are normally high in RUP, with a range between 47% and 64% for DDGS and 42 to 58% for wet DGS. Values higher than this indicate heat damage where the epsilon amino group of lysine has reacted with reducing sugars through the Maillard reaction to create protein that is unavailable to the animal (Schingoethe et al., 2009). The first-limiting

amino acid in corn-based products is lysine. To have lysine further reduced through Maillard browning results in decreased available protein, decreased digestibility of protein, and decreased lysine availability in the small intestine. Variations in lysine concentration are a major concern when using DGS at high levels (Cromwell et al., 1993). Research has indicated that a golden-yellow color of DGS is usually but not always associated with protein quality (Kleinschmit et al., 2007).

The type of distillers product influences the ruminal degradability of protein. Compared to DDGS, MWDGS had a larger percent of rapidly degradable CP in the rumen. The differences in rapidly degradable fractions accounted for changes in RUP with DDGS containing 52.3% RUP compared to MWDGS at 38.3% RUP (Mjoun et al., 2010). Dried distillers products were more resistant to breakdown in the rumen and resulted in greater RUP. There is a concern with feeding DGS in high corn silage-based diets because of the amino acid profile. The lysine to methionine ratio of MWDGS is 0.79 compared with 3.53 for SBM, demonstrating a limitation of DGS. The optimal lysine to methionine ratio in metabolizable protein is 3:1 for milk and protein production (NRC, 2001). Thus, even though DG products provide a good source of RUP, there may be an inadequate amount of lysine available for absorption (Mjoun et al., 2010).

Distillers grains contain high amounts of NDF with relatively low amounts of lignin, making the NDF very digestible. The digestible NDF is what allows DGS to replace some forage in the diet. However, the small particle size of DGS makes very little of that fiber effective to stimulate mastication. Therefore, it is generally recommended that DGS replaces the concentrate portion in dairy diets. Because of the low starch contents in DGS, they would ferment differently in the rumen than a traditional concentrate and would result in a decreased ruminal acid load when compared to high starch feeds (Owens et al., 1998).

Milk Response to Distillers Grains

In a meta-analysis of 24 studies in which varying amounts of distillers products were fed to lactating dairy cattle, milk production was the same or greater when feeding DGS up to 30% of dietary DM (Kalscheur, 2005). Increased milk production could occur because of increased energy density of diets containing DGS (Sasikala-Appukuttan et al., 2008). Long-term effects of feeding DGS are a concern for dairy farmers; therefore a research trial was conducted to feed cows for an entire first lactation, dry period, and into a second lactation a diet containing 15% DGS or a control diet. There were no differences in milk production between the two diets but fat percentage, protein percentage, and feed efficiency were greater for cows fed DGS (Mpapho et al., 2006). Inclusion rates of DGS up to 15% of DM resulted in increased milk production when NDF remained constant (Leonardi et al., 2005). Inclusion of DGS at 20% was deemed acceptable if the total dietary fat was less than 7% because of a milk fat secretion response (Sasikala-Appukuttan et al., 2008). A meta-analysis measuring production response from 44 trials feeding 4.2 to 42% DG proposed that milk production in control cows is an indicator of how cows will respond to DG. A quadratic milk yield response occurred with a maximum increase of 1.2 kg/d at 21% DG with high producing cows (> 30 kg/d), but not with low producing cows (< 30 kg/d; Hollman et al., 2011).

Milk Fat Production in Response to Diets Containing DGS

Milk fat content was lower when DGS was supplemented to diets containing less than 50% forage and 22% forage NDF because of lack of physically effective fiber. A study that partially replaced corn silage with increasing amounts of DGS (0, 7, 14, and 21% DM) found a linear decrease in milk fat produced even as the amount of NDF remained the same (Cyriac et

al., 2005). The DGS was unable to stimulate chewing activity and altered the rumen environment, causing milk fat depression. A meta-analysis indicated that when the milk fat content was above 3.58% for control cows, DG decreased milk fat concentration. However, when milk fat was below 3.58% for control cows, DG increased milk fat concentration (Hollman et al., 2011). The mean milk fat content for the control diets analyzed was 3.42%, which is lower than industry averages. This may explain why research results may indicate positive effects on milk fat when DG is included in a diet while industry experience indicates negative results from DG inclusion (Hollman et al., 2011).

Dietary unsaturated fatty acids are antimicrobial because they penetrate into the cell, cause disorganization of the phospholipid membranes, and damage the cell (Jenkins, 2002). Some bacterial species are more susceptible to damage from unsaturated fatty acids, therefore there is a shift in microbial population as unsaturated fatty acids are fed in the diet. Adding corn oil to a diet increased propionic acid and decreased the acetate to propionate ratio compared to adding tallow (Jenkins, 1987). Feeding corn oil at levels above 2% decreased NDF digestibility (Jenkins, 1987). This shift can slow fermentation of carbohydrates and decrease the acetate to propionate ratio (Jenkins, 2002). The source, degree of saturation, and amount of fat added to the diet can affect rumen fermentation. Free fatty acids disrupt fermentation more than triglycerides and double bonds occurring in free fatty acids increase their antimicrobial activity (Chalupa et al., 1984).

Cottonseed, another source of fat added to the diet, contains 52.5% linoleic acid and 18.5% oleic acid (Rouse, 2003). Corn oil is typically more than 60% linoleic acid and less than 15% saturated fatty acids (Schingoethe, 2002). The fat from corn and cottonseed is 99% triglycerides and contains very little free fatty acids. However, the cottonseed oil is contained

inside the hull of the seed; whereas, corn oil is readily available in DGS because it is contained in the soluble fraction. When DGS are consumed, the triglycerides are quickly broken down into free fatty acids in the rumen. Rumen unsaturated fatty acid load (RUFAL) would increase when feeding corn-based co-products because of the large amount of PUFA (Lock et al., 2006). A combination of high availability and amount of PUFA contributes to a greater risk of MFD when feeding DGS (Jenkins and Lock, 2008).

The fat in DGS is high in PUFA and there is an association between the fat profile of the diet and the fat profile of the milk. One would expect to see higher levels of unsaturated fatty acid in the milk when feeding DGS. A few studies have looked at the fatty acid profile of milk from cows being fed DGS. From what has been reported, the levels of *cis*-9, *trans*-11 conjugated linoleic acid (CLA) have increased slightly when DGS levels increased in the diet. There were small, significant changes in the concentrations of *trans*-10, *cis*-12 CLA, which is associated with milk fat depression (Schingoethe et al., 1999, Leonardi et al., 2005; Anderson et al., 2006). Significant linear increases in several other 18:1 isomers and total linoleic acid also were reported (Leonardi et al., 2005).

Milk Protein Production in Response to Diets Containing DGS

If protein is limiting in the diet, feeding DGS may decrease the milk protein secreted. Feeding diets high in DGS may result in limiting amounts of lysine because the corn they are derived from is low in lysine and may have been subjected to Maillard browning during production of DGS. Consequently, DGS may decrease the amount of protein secreted if lysine is the limiting amino acid for milk protein. When DGS was fed with alfalfa instead of corn silage as the primary forage the amino acid balance was improved because lysine was no longer

limiting (Kalscheur, 2005). Diets with larger inclusion of DGS may need to be supplemented with blood meal or fish meal to supply the required lysine in the diet (Armentano, 1992). Replacing soybean meal with DDGS resulted in lowered MUN, likely because of less RDP in the diet (Kleinschmit et al., 2006).

Modified Wet Distillers Grains with Solubles

There is no current industry definition for what constitutes MWDGS. To some, MWDGS is a partially dried product of approximately 50% DM while others indicate that it includes varying amounts of solubles added back to the distillers grains. A major criticism of MWDGS is the variation because there is such ambiguity about what the term means. The amount of solubles added back often fluctuates from batch to batch meaning large variations in the end product (Schingoethe et al., 2009). Dry DGS are able to be stored for long periods of time because of low moisture content; whereas, MWDGS and WDGS can begin to mold and become unpalatable within 7 to 10 d because the high moisture content allows spoilage. Adding wet distillers products to the diet decreases DM content of the diet compared to adding DDGS. Rations below 50% DM may decrease DMI and, therefore, decrease production (NRC, 2001).

Distillers Products and Rumen Environment

Changes in VFA concentrations were seen as DG were included in diets for lactating dairy cows. Concentrations of acetate decreased as DDGS were included in the diet (Kelzer et al., 2009). Inclusion of DDGS at 15% of the diet resulted in lower acetate to propionate ratio than a diet containing no DDGS (Kelzer et al., 2009). Concentrations of non-fiber carbohydrates (NFC) are known to affect rumen pH, although there was no difference in pH when 15% DDGS was included in the diet (Kelzer et al., 2009). Decreased isovalerate concentrations were seen

when DDGS was included in a TMR (Anderson et al., 2006; Kelzer et al., 2009). Isobutyrate and isovalerate are produced by the microbial degradation of valine and leucine, respectively (Allison, 1978); therefore, the decrease in branched-chain AA (BCAA) available in the rumen could reduce the concentrations of isobutyrate and isovalerate. Decreased amounts of branched-chain VFA (BCVFA) could be the result of high corn diets containing lower concentrations of BCAA, the precursors for BCVFA synthesis (Kelzer et al., 2009).

Strains of several genera of rumen bacteria have specific nutritional requirements for BCVFA. *Ruminococcus flavefaciens* and *Bacteroides succinogenes*, two cellulolytic bacteria present in high populations under normal rumen conditions, have different requirements for growth. *R. flavefaciens* grows better in conditions containing both isobutyrate and isovalerate; whereas, *B. succinogenes* requires a branched-chain and a straight-chain VFA for optimal growth. *R. flavefaciens* is unable to effectively take up exogenous AA and must synthesize AA from BCVFA to meet requirements (Allison et al., 1962). *B. succinogenes* is one of the most common and important cellulolytic bacteria in the rumen. It is the most active strain against crystalline types of cellulose, but it is very sensitive to decreases in rumen pH (Russell, 1987). Small changes in the rumen environment can effectively shift rumen microbial populations. These shifts can cause changes in AA and VFA utilization and production. The rumen microbiota consists of complex symbiotic relationships where end products from one species are fuel for another species. Therefore, it is hard to predict how slight changes in rumen environment may impact microbial populations.

Milk Fat Depression

Milk fatty acids are produced by de novo synthesis in the mammary gland and the by uptake of preformed long-chain fatty acids from blood (Bauman and Davis, 1974). Short- and medium-chain fatty acids and a portion of C16 are primarily produced by de novo synthesis from the precursor acetate. All remaining fatty acids are from the absorption of dietary fats or mobilization of body reserves. Understanding the precursors of milk fatty acids led to the development of many theories involving the variability of milk fat production. Early research done in 1845 indicated that feeding different feedstuffs resulted in lower milk fat synthesis (Van Soest, 1994). Observations from 1939 saw a difference in milk fat produced depending on whether a feed was pelleted or remained intact (Erdman, 1996). This led to an understanding that physical form and composition of the diet influences the amount of milk fat produced.

Diet-induced milk fat depression (MFD) is described as a reduction as great as 50% in milk fat while milk yield and other milk components remain unchanged. During MFD milk fatty acid profile is altered. Two conditions are required to have MFD: 1) an alternate rumen fermentation pattern that is generally caused by a lowered rumen pH, and 2) unsaturated fatty acids must be included in the diet. Diets that are low in forage to concentrate ratio and contain high levels of PUFA have been shown to cause MFD. There have been several theories as to why MFD occurs in the dairy cow. These theories have been categorized as reducing the supply of milk fat precursors or inhibition of one or more steps of milk fat synthesis (Bauman and Griinari, 2001).

Unsaturated fatty acids are toxic to some rumen bacteria; therefore, a majority of dietary unsaturated lipids are biohydrogenated until a saturated fatty acid is produced (Palmquist et al.,

2005). Currently the widely accepted theory of altered biohydrogenation pathways is the focus of the majority of research done on MFD. The *trans*-10, *cis*-12 isomer of CLA was identified as a potent inhibitor of milk fat synthesis because infusion of small amounts of *trans*-10, *cis*-12 CLA resulted in a 50% reduction of milk fat yield (Baumgard et al., 2000). When cows are fed diets high in concentrates and PUFA, for example, an alternative pathway for biohydrogenation occurs and unique fatty acid intermediates are produced. If these FA intermediates contain the *trans*-10, *cis*-12 isomer of CLA then reduced milk fatty acid synthesis may result (Bauman and Griinari, 2001).

Other fatty acid intermediates, such as *cis*-8, *trans*-10 CLA isomer, have been shown to cause a less severe depression in milk fat synthesis (Chouinard et al., 1999). It is believed that several isomers may be responsible for the inhibition of milk fatty acid synthesis and that *trans*-10, *cis*-12 is just the first that was identified. There have been 14 CLA isomers identified in rumen fluid taken from cattle; however, the pathways for formation of all of these intermediates are not known (Jenkins et al., 2008). As the rumen environment is altered, increased *trans*-10 18:1 is found in the milk. This does not directly inhibit milk fat synthesis, but can be used as an indication of changes needed in the rumen to cause MFD (Lock et al., 2007).

Changes in rumen environment that may lead to MFD begin with a lowered ruminal pH. As rumen pH decreases the alternative biohydrogenation pathways are preferred. These changes can be slight and are not necessarily an indication of acidosis (Overton et al., 2006). Rates of fermentation of carbohydrates may impact MFD. More highly fermentable carbohydrates increase the acid load in the rumen (Oba and Allen, 2003). A reduction in particle size may also contribute to MFD (Grant et al., 1990). Monensin is a feed additive that can reduce milk fat synthesis by inhibiting complete biohydrogenation, resulting in absorption of intermediates that

reduce de novo synthesis of fatty acids in the mammary gland (Bauman and Griinari, 2003). Interactions between monensin and a lack of peNDF and NFC increased the severity of MFD (Dubuc et al., 2009). It is thought that it is not any one factor, but a combination of those mentioned that contributes to MFD.

Particle Size

The need for animals to reduce ingested particles to a size that can pass from the rumen is a limiting factor for intake of forages (Van Soest, 1994). Rumination time has an upper limit of no more than 10 h/d (Welch and Smith, 1982), indicating that the need to ruminate coarse digesta becomes a limiting factor for increasing DMI. As the particle size of a diet decreases, the cow spends less time chewing. Therefore, less saliva is produced that acts as a buffer to the rumen digesta, decreasing rumen pH (Grant et al., 1990). Reductions in DMI, milk production, and milk fat production occurred when alfalfa silage was decreased from 28 to 12% and replaced with alfalfa pellets in early lactation cows (Woodford and Murphy, 1988). Rumen fluid outflow and pH were reduced when alfalfa pellets replaced hay silage, indicating that particle size influences rumen pH. There is a positive correlation between particles over 19 mm consumed and rumination activity, and a negative correlation to the amount of time the rumen pH is below 5.8 (Krause et al., 2002; Kononoff and Heinrichs, 2003). However, the amount of additional saliva from increasing particle size is thought to be a 4% increase, which would not significantly alter rumen pH (Yang et al., 2001).

Particle size reduction may decrease the ruminal acetate to propionate ratio and decrease pH, which have been associated with low milk fat production (Shaver, 1990). Digestibility of certain nutrients also decreases as the particle size decreases because of increased passage rate

from the rumen. Reduced particle size can, therefore, lead to a reduction in microbial protein because of a shorter retention time in the rumen (Uden, 1987). However, increasing particle size increases the risk of sorting and, although mean pH values may be the same, the diurnal patterns can be drastically different. This leads to an inconsistent rumen environment; smaller particle sizes actually increased milk fat secretion compared to highly sorted diets (Bal et al., 2000). Excessive amounts of long, coarse particles can also limit intake and digestibility and have a negative impact on energy balance of the animal (Allen, 1997).

Particles are reduced in size by four processes: mastication during eating, mastication during rumination, digestion in the rumen, and detrition in the rumen. After particles leave the rumen, it is assumed that there is no further reduction in particle size. Mastication of particles results in the greatest reduction in particle size; whereas, digestion and detrition in the rumen contribute a relatively small portion of particle size reduction (McLeod and Minson, 1988). Therefore, the upper critical size of particles leaving the rumen would correspond to the largest size of the particles in feces; 1.18 mm was deemed the appropriate critical size because less than 5% of fecal particles were above this size (Poppi and Norton, 1980).

Preparation of on-farm TMR impacts the size of particles that cows encounter. As mixing time increased the percentage of particles over 18 mm decreased and the mean particle size decreased (Heinrichs et al., 1999). Uniform particle size reduces sorting and limits the large variation in rumen fermentation and rumen pH. Analyzing both the TMR and refusals for particle size distribution can provide an indicator of sorting.

Measuring Particle Size

Murphy and Zhu (1997) indicated that particle size measurements are affected by the method, feedstuff, and interaction between method and feedstuff. Therefore, there was a need to develop a uniform way to measure particle size and express those measurements. The American Society of Agricultural Engineers (ASAE) developed a method to measure and express particle sizes of forages using a lognormal distribution (ASAE, 2001). The lognormal distribution provides the log mean particle size, which can be converted to mean particle size, as well as the log standard deviation of particle size. However, this complicated method was not able to translate to on-farm assessment and was not used for TMR.

On-farm assessment of TMR particle size and distribution can be estimated using the Penn State Particle Separator. The two screen sizes are 19 mm and 8 mm, consisting of round holes placed over a pan. The three-compartment separator was shown to closely follow the Weibull distribution with the lognormal distribution being the second best fit (Lammers et al., 1996). Modifications to the PSPS were developed to further split the smaller particle sizes by adding a sieve with a nominal aperture of 1.18 mm and diagonal aperture of 1.67 mm (Kononoff et al., 2003). This size was deemed relevant to retention time in the ruminoreticulum (Poppi and Norton, 1980; Mertens, 1997). In addition to dry- and wet-sieving with multiple screens to obtain a mean particle size, a prediction of mean particle size of TMR can be calculated from a single screen separation using a regression equation of:

mean particle length = $0.54 + 11.84 \times$ cumulative fraction of as-fed mass trapped on or above a 9-mm screen (Armentano and Taysom, 2005).

Physically Effective Fiber

Particle size alone does not alleviate milk fat depression. Polypropylene ribbon fed in combination with ground forages and concentrates increased rumination and saliva production without improving MFD (Welch and Smith, 1975). Both the physical size and the nutritional components that lead to acetate production need to be present to stimulate adequate milk fat production. Physically effective fiber is a method of measuring if dietary particles are able to stimulate mastication because it uses both the particle length and the NDF concentration of a diet (Mertens, 1997). The NRC (2001) indicates that an appropriate amount of peNDF is needed in the diet to reduce subacute ruminal acidosis. The NRC adjusts NDF recommendations based on the dietary forage NDF percentage, indicating that a minimum of 19% forage NDF should be maintained in the diet; however, this was based on rations with adequate particle size and fed as a TMR (NRC, 2001).

The concept of peNDF does not take into consideration the fermentability of carbohydrates in the rumen (Zebeli et al., 2008). Therefore, peNDF is not always correlated to ruminal pH. Intake of feed is an important factor that affects passage rate and digestion in the reticulorumen (Firkins et al., 2001; Stone, 2004). As more feed is consumed and moves through the reticulorumen faster, there is less acid buildup and a relatively higher ruminal pH. This confounds the effects of peNDF because a high intake of a diet lower in peNDF may have high ruminal pH because of faster passage rate. Therefore, fiber requirements for dairy cattle depend upon both the physical effectiveness of fiber and the production of fermentable acids in the rumen (Allen, 1997).

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CHAPTER 3

EFFECTS OF MIXER TYPE ON UTILIZATION OF DIETS WITH INCREASING AMOUNTS OF MODIFIED WET DISTILLERS GRAINS WITH SOLUBLES FOR LACTATING HOLSTEIN CATTLE

INTRODUCTION

Feeding high-fat co-products of the ethanol industry such as modified wet distillers grains with solubles (MWDGS) has been met with resistance in the dairy industry because of the potential for causing milk fat depression. Diets high in polyunsaturated fat such as that from corn oil in MWDGS have been linked to lower milk fat production because of alternative biohydrogenation pathways in the rumen that produce more *trans*-10, *cis*-12 CLA, a potent inhibitor of milk fat synthesis in the mammary gland (Bauman and Griinari, 2001).

The Keenan MechFiber system (Richard Keenan & Co., Borris, Ireland) for producing total mixed rations (TMR) is hypothesized to result in more optimal fermentation conditions in the rumen, although few data are available to document the mechanisms for such effects. There have been numerous studies that looked at performance of dairy cows fed increasing levels of distillers grains (Leonardi et al., 2005; Anderson et al., 2006; Schingoethe et al., 2009). However, we are aware of no studies that have evaluated potential mixer effects when feeding diets containing various concentrations of corn co-products.

The hypothesis of this study was that use of the Keenan mixer wagon would allow for greater inclusion rates of MWDGS than a vertical auger mixer without the negative effects of decreased milk fat content. The first objective of the study was to compare feed intake, milk production, milk composition, and feed conversion efficiency (FCE) for dairy cows fed diets containing increasing concentrations of MWDGS and mixed either in Keenan or a Kuhn-Knight vertical auger mixer. The second objective was to compare rumen fermentation, rumen digesta

structure, fractional passage rate of digesta, and total-tract nutrient digestibility among the different TMR.

MATERIALS AND METHODS

All procedures were conducted under protocols approved by the University of Illinois Animal Care Advisory Committee. Twenty-four lactating Holstein cows were enrolled in the study, 12 were ruminally cannulated. One cow did not complete the study due to chronic mastitis in two subsequent periods. Characteristics of the cows are shown in Table 3.1.

Cows were divided into two groups, one being fed with the Keenan MechFiber Klassic 140 paddle-type mixer with knives (KMF) and the other with the Kuhn-Knight VSL-142 vertical auger mixer (VA). Cows were blocked and enrolled in replicated 3 x 3 Latin squares in which diets were fed that contained 10, 20, or 30% MWDGS (on a DM basis) mixed with their respective mixer wagon. Primiparous cows formed one square for each mixer wagon. Cows rotated through all three concentrations of MWDGS in periods that lasted 35 d. All diets were formulated to meet National Research Council (NRC, 2001) requirements for lactating cows. All diets contained the same amounts of forages. The MWDGS replaced soybean meal, soy hulls, corn grain, cottonseed, and dicalcium phosphate (Table 3.2).

The diets were mixed once a day and fed as a TMR. Order of ingredients added to the TMR and length of mixing time varied with mixer and with concentration of MWDGS (Table 3.3). The Keenan system order of ingredients and extent of mixing was chosen using the Performance Acceleration and Control Enhancement (PACE) technology (Keenan System, Borris, Ireland). The ingredients were input and the PACE program supplied their optimal order and degree of mixing. The vertical mixer wagon provided very general recommendations in the

user manual and as a result the order of ingredients was selected to follow those recommendations and provide ease of loading based on location of ingredients on farm. The additional time of mixing was adjusted at the beginning of the trial to make a homogenous mix by visual assessment. Time of mixing differences for the varying concentrations of distillers was because of differences in load size and the location of the load in relation to the top of the vertical auger.

Cows were fed ad libitum to allow for 5 to 10% refusal. Treatments were mixed separately and delivered to two plastic tubs per cow by using a small drum mixer (Data Ranger, American Calan Inc., Northwood, NH). A majority (65 to 75%) of the feed was fed at 1200 h and the remaining feed was offered at 0400 h the following morning. Visual assessment oforts was used to score moisture content on a scale of 1 through 4, where 1 = similar to TMR offered and 4 = completely saturated with water. Orts were removed and weighed at 1100 h. Cows were housed in tie stalls throughout the experiment. Cows were milked three times daily (0400, 1200, 2000 h).

Data Collection, Sampling Procedures, and Analytical Methods

Intake from each cow was measured daily throughout the experiment. A portion of the ingredients was dried at 100°C in a forced-air oven for a minimum of 24 h for determination of DM on a weekly basis for diet formulation. Weekly samples of individual ingredients were frozen at -20° C then composited by period and analyzed at Dairy One Forage Analysis Laboratory (Ithaca, NY) for DM (AOAC, 2000; method 930.15), CP (AOAC, 2000; method 990.06), soluble protein (Roe and Sniffen, 1990), NDF and ADF (Van Soest et al., 1991; without sodium sulfate, using an Ankom Fiber Analyzer, Ancom Technology, Fairport, NY, with

100 μ L/0.50 g of sample heat-stable α -amylase, no. A3306, Sigma Chemical Co., St. Louis, MO), lignin (AOAC, 2000; method 942.05), NFC (by difference), ether extract (AOAC, 2000; method 2003.05), ash (AOAC, 2000; method 942.05), and minerals (Ca, P, Mg, K, Na, Fe, Zn, Cu, Mn, Mo, and S; Sirois et al., 1994). Composites of individual ingredients were also analyzed for total fatty acids (courtesy of A. Lock, Michigan State University). The fatty acids were separated on a gas chromatograph (Shimadzu, Kyoto, Japan) equipped with an auto sampler, a flame ionization detector, and a fused silica column (100 m \times 0.25-mm i.d. \times 0.2- μ m film thickness; Varian Inc., Lake Forest, CA).

Particle size distribution (Kononoff et al., 2003) was determined weekly for samples of TMR. The TMR were sampled weekly, frozen at -20° C, and wet sieved using 9500, 6300, 3350, 1180, 425, and 75 mm screen sizes. Samples of TMR were dry sieved using screen sizes of 9500, 6300, 3350, 1180, 425, and 75 mm to evaluate mean particle size. Orts were sampled daily during d 28 to 35 of each period and analyzed for DM content. Daily samples were frozen at -20° C; a portion was dried at 100°C, ground through a 1-mm screen in a Wiley mill (Arthur H. Thomas, Philadelphia, PA), composited on a proportional basis according to the amount of feed refused each day, and analyzed for the same nutrients as described for ingredients (Dairy One, Ithaca, NY). The remaining Orts samples were stored at -20° C and composited on a proportional basis according to the amount of feed refused each day before being wet sieved using the same sieves as those used for TMR samples.

Cows were dosed twice daily with 6 g of Cr₂O₃ via the rumen fistula or orally via a balling gun at 0700 and 1900 h during d 22 to 35 of each period. Fecal grab samples were taken twice daily at 0730 and 1930 h during d 28 to 35 of each period. Fecal samples were composited

and analyzed for nutrient composition as described for dietary ingredients (Dairy One, Ithaca, NY). Composited fecal samples were wet-sieved using 1180, 600, 425, 250, and 75 mm screens.

Cannulated cows were dosed with 5 g of cobalt-EDTA (Udén et al., 1980) through the rumen cannula at 0715 h on d 32 of each period. Rumen fluid samples (~200 mL) were taken through the rumen cannula on d 32 at 0700, 0800, 0900, 1000, 1100, 1300, 1500, 1700, 1900, and 2300 h and on d 33 at 0300, 0700, 1300, and 1700 h. Fluid samples from each time point were divided; one aliquot was acidified with 1 mL sulfuric acid for ammonia analysis (Chaney and Marbach, 1962; Cotta and Russell, 1982) and the second aliquot was used for VFA (courtesy S. Hansen, Iowa State University, Ames, IA) and cobalt analysis (University of Missouri Experiment Station Laboratory, Columbia, MO). Rumen fluid samples were frozen at -20° C until analyzed. Samples were thawed and centrifuged at 9000 g for 15 min before analysis. Rumen pH was determined at each time point using a pH meter with a glass electrode. Rumen mat samples were obtained at 0900, 1100, 1300, 1500, 1900, and 2300 h on d 32 and 0300, 0700, 1300, and 1700 h on d 33. Rumen mat samples were frozen at -20° C, composited by period, and analyzed for CP, NDF, ADF, lignin, NFC, crude fat, ash, Ca, P, Mg, K, Na, Fe, Zn, Cu, Mn, Mo, and S (Dairy One, Ithaca, NY) as described for feed ingredients.

Rumen contents were evacuated at 0900 h on d 34 of each period. Total rumen contents were weighed and a subsample of every tenth handful of rumen contents was saved for further analysis. The subsample of rumen contents was squeezed through a 1.18-mm screen to separate contents into primarily liquid and primarily solid subsamples (Volker Linton and Allen, 2007). The samples were frozen at -20° C and analyzed for DM by freeze drying. The freeze-dried samples were composited by proportion of rumen contents and analyzed for CP, NDF, ADF, lignin, NFC, crude fat, ash, Ca, P, Mg, K, Na, Fe, Zn, Cu, Mn, Mo, and S (Dairy One, Ithaca,

NY). Frozen rumen fluid and mat were thawed and combined using a weighted average and wet sieved through 9500, 2360, 1180, 850, 425, 250, and 75 mm screens.

Milk samples were taken from 6 consecutive milkings 4 weeks prior to the start of the trial. These samples were used as pretrial values. Samples were refrigerated until composited by day; two samples for each cow were analyzed for protein, fat, lactose, SCC, and MUN (Dairy Lab Services, Dubuque, IA).

Milk was sampled from 9 consecutive milkings during wk 4 and 5 of each period. Samples were refrigerated until composited by day; three samples for each cow per week were analyzed for protein, fat, lactose, SCC, and MUN (Dairy Lab Services, Dubuque, IA). Samples from the 9 milkings of wk 5 were preserved with Broad Spectrum Microtabs® II (D & F Control Systems Inc., Norwood, MA) and frozen at -20°C until analyzed for FA composition at Michigan State University. Milk lipids were extracted using the method of Hara and Radin (1978), and FA methyl esters (FAME) prepared by base-catalyzed transesterification (Christie, 1989). The FAME were quantified using a GC-2010 Plus gas chromatograph (Shimadzu, Kyoto, Japan) equipped with a split injector (1:100 split ratio) and a flame ionized detector using a CP-Sil 88 WCOT fused silica column (100 m × 0.25-mm i.d. × 0.2-µm film thickness; Varian Inc., Lake Forest, CA). Gas chromatographic conditions were as described by Kramer et al. (2002). The FAME were identified by comparison of retention times with known FAME standards [Supelco 37 component FAME mix, *cis/trans* FAME mix, bacterial acid methyl ester (BAME) mix, and polyunsaturated FA (PUFA) No. 3 mix from Supelco Inc., Bellefonte, PA; GLC reference standard 463 and conjugated linoleic acid (CLA) mixture #UC-59 M from Nu-Chek Prep, Elysian, MN]. Short-chain FAME were corrected for mass discrepancy using the correction factors published by Ulberth and Schrammel (1995).

Body weight and body condition scores (BCS; Wildman et al., 1982) were determined for each cow weekly during the trial. Body weights were taken at the same day and time each week. Three individuals assigned body condition scores independently at each time of scoring throughout the experiment.

Statistical Analyses

Cows were blocked by parity, days in milk, production, and whether they were ruminally cannulated. Within each block, half the cows were assigned to one 3 × 3 square fed with one mixer and the other half to a different 3 × 3 square fed with the other mixer. Performance, particle size, digestibility, and turnover data were analyzed as a replicated 3 × 3 Latin square within a split-plot arrangement using the MIXED procedures of SAS (Version 9.2, SAS Institute Inc., Cary, NC). Fixed model effects include mixer, concentration of MWDGS, and the interaction of mixer and MWDGS. Random model effects included square within group, cow within square, and period within group. Data for milk, fat, protein, and MUN were adjusted by analysis of covariance using the respective pretreatment measurement. The linear model was as follows:

$$y_{ijklmn} = \mu + M_i + D_j + (M * D)_{ij} + G_k + S(G)_{lk} + C(S)_{mk} + P(G)_{nl} + \varepsilon_{ijklmn}$$

where y_{ijkl} represents observation_{ijkl}; μ represents the overall mean; M_i represents the fixed effect of the i th mixer; D_j represents the fixed effect of j th concentration of MWDGS; $(M*D)_{ij}$ represents the fixed effect of the interaction between the i th mixer and the j th concentration of MWDGS; G_k represents the random effect of the k th group; $S(G)_{lk}$ represents the random effect of the l th square nested within the k th group; $C(S)_{mk}$ represents the random effect of the m th cow nested within the k th group; $P(G)_{nl}$ represents the random effect of the n th period nested within

the l th group. The residual term ε_{ijklmn} was assumed to be normally, independently, and identically distributed with variance σ_e^2 .

Rumen measurements for pH, ammonia, and VFA were analyzed as repeated measures by using the spatial power covariance structure in SAS (Version 9.2, SAS Institute Inc.). Fixed model effects include mixer, concentration of MWDGS, hour of sampling, and all interactions. The random model effect included cow within period, mixer, and concentration of MWDGS. The linear model was as follows:

$$y_{ijklm} = \mu + M_i + D_j + H_k + MD_{ij} + MH_{ik} + DH_{jk} + MDH_{ijk} + P_l + C(PMD)_{ijlm} + \varepsilon_{ijklm}$$

where y_{ijklm} represents observation $ijklm$; μ represents the overall mean; M_i represents the fixed effect of the i th mixer; D_j represents the fixed effect of the j th concentration of MWDGS; H_k represents the fixed effect of the k th hour; MD_{ij} represents the fixed effect of the interaction between the i th mixer and the j th concentration of MWDGS; MH_{ik} represents the fixed effect of the interaction between the i th mixer and the k th hour; DH_{jk} represents the fixed effect of the interaction between the j th concentration of MWDGS and the k th hour; MDH_{ijk} represents the fixed interaction between the i th mixer, j th concentration of MWDGS and k th hour; P_l represents the random effect of period, $NID(0, \sigma_p^2)$; $C(PMD)_{ijlm}$ represents the random effect of the i th cow nested within the l th period, i th mixer and j th concentration of MWDGS, $NID(0, \sigma_c^2)$. The residual term ε_{ijklm} was assumed to be normally, independently, and identically distributed, with variance σ_e^2 .

Statistical significance for all effects was declared when $P \leq 0.05$, and trends were discussed with $P \leq 0.10$. Treatment means are presented as least squares means, and the largest standard error of the means is reported.

RESULTS

Diet Composition

Several strategies could be used to incorporate greater amounts of MWDGS into dairy diets. Our approach was to maintain forages constant and attempt to minimize differences in diet composition across diets. We expected that this approach would maximize any effect of mixer in producing different effective fiber conditions in the rumen. To this end, we used MWDGS to replace cottonseed, soybean meal, corn grain, and soyhulls as sources of digestible fiber, protein, and fat. Chemical composition of the diets (Table 3.4) showed that we were reasonably successful in meeting our formulation objectives. The DM content of rations was equalized by addition of water to the 10% and 20% MWDGS rations. Contents of calcium decreased slightly and the content of lignin increased slightly as MWDGS increased from 10% to 30% of DM. Content of crude fat increased by more than one percentage unit as MWDGS was increased from 10% to 30% of dietary DM. Content of phosphorus increased from 0.36% of DM to 0.40% of DM despite the fact that no supplemental phosphorus was added to the 30% MWDGS diet. Sulfur also increased with the addition of MWDGS as expected.

The fatty acid profile (Table 3.5) indicates that the concentration of oleic acid increased with increasing MWDGS. Linoleic acid remained constant across all three concentrations of MWDGS. The concentration of linoleic acid decreased with increasing amounts of MWDGS; however, because of the increase in total fatty acids, the amount of each of the fatty acids increased in the diet. The total saturated, monounsaturated, and polyunsaturated fatty acids all increased as a percentage of DM of the diet (Table 3.6). The largest increase was for *cis*-configured monounsaturated fatty acids with an almost 20% increase over the 10% MWDGS.

DMI, Milk Production, and Composition

Dry matter intake was lower for cows fed with the Keenan mixer ($P = 0.05$, Table 3.7). Inclusion of MWDGS had no effect on milk yield. Milk fat percentage and yield decreased linearly as inclusion of MWDGS increased ($P < 0.0001$ and $P < 0.0003$, respectively). Therefore, energy-corrected milk [ECM, $(12.82 \times \text{milk fat kg}) + (7.13 \times \text{milk protein kg}) + (0.323 \times \text{milk kg})$ derived from Tyrrell and Reid, 1965] also decreased linearly as MWDGS was added to the diet ($P = 0.002$). There was a significant interaction of the linear effect of MWDGS by mixer for fat percentage ($P = 0.003$) and yield ($P = 0.006$), with the cows being fed with the Keenan mixer having higher fat percentage and yield as MWDGS increased. Milk protein content tended ($P = 0.09$) to decrease as MWDGS increased. MUN decreased as the concentration as MWDGS increased ($P = < 0.0001$) and there was no effect of mixer or increasing MWDGS on SCC ($P = 0.92$ and $P = 0.68$, respectively). Feed conversion efficiency (calculated as ECM divided by DMI) decreased linearly as MWDGS increased ($P = 0.007$).

Estimates Using NRC

Inputs from the cow performance were used to determine energy and metabolizable protein (MP) balance (Table 3.8). For the Keenan-fed cows, energy was the first limiting nutrient for milk production. Energy was first limiting for the VA-fed cows with the exception of the 30% MWDGS diet, where MP supply was lower than energy. The NRC (2001) model overestimated milk production; the overestimation was greatest for the cows fed 30% MWDGS. The lysine content as a percentage of MP decreased as concentration of MWDGS increased. In contrast, the methionine percentage of MP increased as the concentration of MWDGS increased; these changes made the ratio of lysine to methionine decrease as MWDGS increased.

Milk Fatty Acid Profile

Diet and mixer treatments significantly affected the fatty acid composition of milk fat (Table 3.9). Total saturated fatty acids (SFA) were reduced as concentration of MWDGS increased ($P < 0.0001$) while MUFA and PUFA increased as MWDGS was added to the diet ($P < 0.0001$ and $P < 0.0001$, respectively). The CLA isomer *trans*-10, *cis*-12, a potent milk fat inhibitor, increased as level of MWDGS increased ($P < 0.0001$) and was greater for the vertical mixer than Keenan mixer ($P = 0.01$). There was a significant interaction of the linear effect of MWDGS by mixer for CLA *trans*-10, *cis*-12 with the cows being fed with the Keenan mixer having lower CLA *trans*-10, *cis*-12 content as MWDGS increased. Both C18:1 *trans*-10 and C18:1 *trans*-11 increased as MWDGS were included in the diet ($P < 0.0001$ and $P < 0.0001$, respectively) with a significant interaction between level of MWDGS and mixer wagon ($P = 0.05$ and $P = 0.0002$, respectively) showing that rations mixed with the Keenan mixer had less 18:1 *trans*-10 and *trans*-11 isomers. Omega-3 and omega-6 fatty acids in milk fat increased as level of MWDGS increased ($P = 0.01$ and $P < 0.0001$, respectively). The CLA *cis*-9, *trans*-11 isomer, which is linked to health benefits in humans, increased as MWDGS increased ($P < 0.0002$), with an interaction between mixer wagon and increasing concentrations of MWDGS ($P = 0.02$).

A simple linear regression was used to determine the correlation between milk fat percentage and C18:1 *trans*-10 FA. The best fit line that resulted in the lowest total sum of squares residuals was $y = -0.45x + 3.77$, with a coefficient of determination of 0.32 for the cows fed with the Keenan mixer. The regression for cows fed VA diets was $y = -0.53x + 3.89$, with a coefficient of determination of 0.62 (Figure 3.1). When the same method was used to determine the correlation between milk fat yield (kg/d) and C18:1 *trans*-10 FA, the equation was $y = -0.14$

$x + 1.32$, with a coefficient of determination of 0.07 for the cows fed with the KMF mixer. The regression for cows fed VA diets $y = - 0.21 x + 1.55$, with a coefficient of determination of 0.42 (Figure 3.2). A simple linear regression was used to determine the correlation between milk fat yield (kg/d) and C18:2 *trans*-10, *cis*-12 FA with an equation of $y = - 47.69 x + 3.53$, with a coefficient of determination of 0.21 for cows fed KMF diets. The regression for cows fed with the VA mixer gave the equation $y = - 68.53 x + 3.71$ with a coefficient of determination of 0.58 (Figure 3.3). The large negative slope indicates that there was a drastic reduction in milk fat percentage as CLA *trans*-10, *cis*-12 increased. The correlation between milk fat yield (kg/d) and C18:2 *trans*-10, *cis*-12 FA resulted in an equation of $y = - 19.73 x + 1.25$ with a coefficient of determination of 0.07 for the Keenan diets. The regression equation for cows fed VA diets was $y = - 23.01 x + 1.46$ with a coefficient of variation of 0.30 (Figure 3.4).

The same method of simple regression was used to evaluate the change between 10% MWDGS, which was used as the control, and both 20 and 30 % MWDGS for milk fat and milk fatty acid isomers. When milk fat percentage and C18:1 *trans*-10 FA were graphed, the equation for the difference between the 10 to 20% MWDGS was $y = 0.02 x - 0.53$ with a coefficient of determination of 0.46. The difference between the 10 to 30% MWDGS had an equation of $y = - 0.06 x - 0.39$ with a coefficient of variation of 0.46 (Figure 3.5). The simple regressions of change in milk fat yield (kg/d) using C18:1 *trans*-10 FA from the 10% MWDGS for each cow as a control were calculated for 20% and 30% MWDGS rations. The equation for the change of 10 to 20% MWDGS was $y = 0.04 x - 0.32$ with a coefficient of determination of 0.54. The regression for the change of 10 to 30% MWDGS $y = 0.06 x - 0.21$ with a coefficient of variation of 0.38 (Figure 3.6). Using simple regression to evaluate the change in milk fat percentage by change in C18:2 *trans*-10, *cis*-12 FA resulted in the equation $y = - 0.10 x - 45.91$ with a

coefficient of determination of 0.29 for comparing 10 to 20% MWDGS. The regression for the change of 10 to 30% MWDGS was $y = -0.04x - 60.45$ with a coefficient of variation of 0.53 (Figure 3.7). The best fit equation for change in milk fat yield (kg/d) by change in C18:2 *trans*-10, *cis*-12 FA was $y = -0.03x - 27.59$ with a coefficient of determination of 0.35 for comparing 10 to 20% MWDGS. The change of 10 to 30% MWDGS had the equation $y = 0.11x - 32.08$ with a coefficient of variation of 0.44 (Figure 3.8).

Particle Size of Rations, Diet Consumed, and Digesta Fractions

To evaluate sorting, the chemical composition of orts was determined (Table 3.10). There was a mixer effect for percentage of CP of orts with VA having higher CP than KMF ($P = 0.04$). The crude fat of orts increased as the concentrations of MWDGS increased ($P = 0.08$), which would be expected because of increases in crude fat in the TMR as MWDGS increased. However, the highest level of crude fat in orts was for 20% MWDGS; therefore, there was a quadratic effect of MWDGS ($P = 0.02$). The phosphorus and sulfur contents of orts increased linearly as MWDGS increased ($P = 0.001$ and $P < 0.0001$, respectively).

Samples of the TMR were separated weekly using a Penn State particle separator (Table 3.11). The amount on the top tray (largest particles) was significantly different between mixer wagons ($P < 0.0001$) with the ration mixed in the vertical mixer wagon having more particles on the top tray. The mixer effect was significant for the amount of material on the tray containing particles 8 to 19 mm, with the Keenan mixer wagon having a larger percentage in the middle tray. There was a trend for a greater percentage of particles contained in the bottom tray for rations mixed in the vertical mixer wagon ($P = 0.08$). The percentage of particles on the middle tray decreased linearly as the concentration of MWDGS was increased ($P < 0.0001$). The lower tray had a linear increase in the percentage of particles as level of MWDGS increased ($P < 0.0001$).

These data indicate that the type of mixer resulted in a different physical presentation of the same ration.

The TMR was wet sieved for every period and the results (Table 3.12) indicated a trend for a mixer effect ($P = 0.06$), with Keenan diets having a smaller mean particle size. The mixer effect for the log standard deviation of particle size was significant ($P = 0.03$) with the Keenan rations having less variation than the vertical rations.

To determine particle size consumed (Table 3.12), the weighted average of the refusals on each of the screens was subtracted from the amount of material on each of the screens used for determination of TMR particle size. For consumed particles, there was a quadratic effect of MWDGS ($P = 0.01$) and an interaction of the linear effect of MWDGS by mixer ($P = 0.04$) for log mean particle size. The Keenan rations had a reduction in mean particle size as MWDGS increased as would be expected by changes in diet formulation; whereas, the vertical rations with 20% MWDGS had the largest particle size. The interaction of the quadratic effect of MWDGS by mixer was significant for the standard deviation of particle size, in which the Keenan ration had the least deviation at 20% MWDGS and the vertical ration had the greatest deviation at 20% MWDGS.

Differences seen in particle size of consumed feed were not observed in the rumen, where particle size measurements did not differ among treatments (Table 3.12). For fecal particle size, the quadratic effect of MWDGS ($P = 0.05$) showed that the 20% MWDGS diet had the smallest mean particle size for both mixers (Table 3.12).

The TMR was dry sieved (Table 3.13) and there was a quadratic effect of MWDGS ($P = 0.004$) for mean particle size with the 20% MWDGS diet having the greatest mean particle size.

The effect of mixer was significant for log standard deviation ($P = 0.001$) with Keenan rations having less variation. A trend for a quadratic effect of MWDGS ($P = 0.06$) for log standard deviation showed that 20% MWDGS tended to result in the largest distribution for both mixers. Physically effective NDF (peNDF) was not affected by treatment; however, peNDF consumed (kilograms/day) tended to increase linearly with greater inclusion of MWDGS ($P = 0.07$) and the interaction of quadratic concentration of MWDGS by mixer tended to be significant ($P = 0.10$), with Keenan at 20% MWDGS having the lowest peNDF consumed and Vertical at 20% having the highest peNDF consumed. Results of the regression analysis from wet sieving are shown in Table 3.14 (TMR consumed) and Table 3.15 (rumen particles).

Results of this extensive characterization of particle size in feed and throughout the digestive tract showed that differences in physical characteristics of the rations and particle size consumed did not result in any detectable changes in the particle size profile of rumen digesta or feces.

Rumen Measurements

Rumen fluid was sampled and pH of rumen fluid determined from the rumen-cannulated cows at 14 time points over 2 consecutive days. Rumen pH (Table 3.16) decreased linearly as MWDGS increased ($P = 0.05$). The minimum pH was not affected by treatment whereas the maximum rumen pH decreased as MWDGS was added to the diet ($P = 0.05$). The range of rumen pH was greatest at 10% MWDGS and smallest at 30% MWDGS ($P = 0.04$), indicating that the pH was lower and more consistent for the highest concentration of MWDGS. Area under pH 6.0 was unaffected by treatment. The area under pH 6.0 for 10% MWDGS showed the reduction in pH within hours of the major feeding event (Figure 3.9). Figure 3.10 shows area

under pH 6.0 for 20% MWDGS, with a nonsignificant greater time spent below pH 6.0 for VA. The area under pH 6.0 for 30% MWDGS was lower after both feeding events (Figure 3.11). Rumen pH was affected by hour ($P < 0.0001$) and the interaction of hour and MWDGS ($P = 0.01$, Figure 3.12).

The concentration of ammonia in the rumen decreased linearly as MWDGS increased ($P < 0.0001$). The mixer type and the interactions of mixer by MWDGS were not significant for these variables.

Weights of rumen contents and nutrient fractions in rumen digesta determined from evacuation were not different (Table 3.17). There was a trend for an increase in the primarily fluid portion of rumen digesta as MWDGS increased ($P = 0.08$); however, the DM of rumen contents, both in kilograms and percentage of total, increased as MWDGS increased ($P = 0.05$ and $P = 0.03$, respectively). Fat percentage in the rumen digesta decreased linearly as level of MWDGS increased ($P = 0.03$).

Rumen Volatile Fatty Acids

Total VFA concentration was similar for all treatments (Table 3.18); however, there were differences in individual VFA concentrations and proportions. Acetate concentration and percentage decreased linearly as level of MWDGS increased ($P = 0.02$ and $P = 0.0002$, respectively). Propionate concentration tended to increase as level of MWDGS increased ($P = 0.06$) and the percentage of propionate also increased as MWDGS was added to the diet ($P = 0.005$). The shifts in acetate and propionate concentrations resulted in a lowered acetate to propionate ratio as concentrations of MWDGS increased ($P = 0.001$). Isobutyrate concentration and percentage decreased as MWDGS increased ($P < 0.0001$ and $P < 0.001$, respectively). The

concentration and percentage of isovalerate decreased as MWDGS was added ($P = 0.03$ and $P = 0.04$, respectively). There were no differences in concentrations of butyrate and valerate ($P = 0.23$ and $P = 0.21$, respectively); however, the percentages of butyrate and valerate increased as concentrations of MWDGS increased ($P = 0.006$ and $P = 0.02$, respectively). There were significant effects of time and the interaction of time and treatment for butyrate and isobutyrate concentrations. Butyrate concentrations differed by hour ($P < 0.0001$) and by hour \times MWDGS ($P = 0.03$) (Figure 3.13). Isobutyrate concentrations differed by hour ($P < 0.001$) and by hour \times mixer ($P = 0.02$) (Figure 3.14).

Apparent Digestibility of Nutrient Fractions in the Total Digestive Tract

Apparent digestibility of nutrients was calculated from feed consumed and fecal samples that were collected during the last 6 d of each period (Table 3.19). Digestibility of NDF tended to increase linearly as MWDGS increased ($P = 0.09$). Crude fat digestibility increased linearly as MWDGS increased ($P = 0.0003$). The interaction of the linear effect of MWDGS by mixer ($P = 0.03$) showed that this increase in fat digestibility occurred mainly in cows fed the VA rations. No significant differences among treatments were observed for any other nutrient fraction.

Turnover of Nutrients in the Rumen per Hour

Turnover of nutrients in the rumen indicates the rate of disappearance from the rumen by either passage or digestion. There were no differences in turnover rates for liquid, DM, NDF, crude fat, or ash among treatments (Table 3.20). Turnover of CP in the rumen tended to decrease as MWDGS increased ($P = 0.09$), which could be due to the lower rumen degradability of protein in MWDGS. Turnover of NFC in the rumen decreased as MWDGS increased ($P = 0.02$).

Correlations and Regression Analysis

Correlations among variables related to production of milk and milk fat are shown in Table 3.21. Multiple regression analysis used a number of these variables to attempt to explain the variation of milk fat secretion among treatments (Table 3.22). The most important factor explaining milk fat production was milk yield ($r^2 = 0.539$). Three other factors were included in the final model: CLA *trans*-10, *cis*-12; days in milk (DIM); and mean rumen pH all contributed significantly to explaining the variation. When the four factors were combined, they explained 83% of the variation ($r^2 = 0.826$).

In an attempt to explain the variation of milk fat percentage among treatments, multiple regression analysis was used (Table 3.23). The most important factor explaining milk fat percentage was concentration of CLA *trans*-10, *cis*-12 ($r^2=0.405$). Days in milk was the only other factor that was included in the final model. When the two factors were combined, they explained 61% of the variation in milk fat percentage ($r^2 = 0.607$).

Economic Analysis

Feed costs and milk prices at a fixed time (March, 2011) were used to calculate income over feed cost as affected by mixer type and diet (Table 3.24). The value of the milk produced decreased with the inclusion of MWDGS because of the decrease in milk fat as MWDGS increased. The cost of the feed consumed also decreased as more MWDGS was included in the diet because MWDGS replaced more expensive ingredients. The income over feed cost (IOFC) was greatest for Keenan 20% MWDGS because of the lowered DMI and less of a reduction in milk fat compared to the vertical mixer. The vertical 10% MWDGS had the second highest IOFC because of the high milk production.

DISCUSSION

Dry Matter Intake

Differences in DMI between mixer wagons may be partially explained by differences in the sortability of the diet. As a diet is able to be sorted, the cow will preferentially select for the concentrate portion and leave the longer particles (Mulfair et al., 2010). The long particles are what may most limit DMI as they contribute to physical fill and take longer for particle size to be decreased. Therefore, because the VA diets had a larger mean particle size and larger variation, it likely was easier to sort and DMI was increased. Evidence of sorting can be seen by looking at the difference between the TMR offered and the consumed particle size and distribution between mixers, as well as the orts composition data.

The nutrient composition of the diets indicates that the phosphorus and sulfur content increased as MWDGS increased because of the high levels of sulfur and phosphorus in the soluble fraction. While this is consistent with the composition of the diet, the fat concentration was not what was expected. Orts from both diets containing 20% MWDGS has the highest concentration of fat, which is different from the composition of the diets. We would expect the amount of fat to still be in the same proportion with the 30% diet having the highest fat in the refusals. Greater than 60% of the fat in the 30% MWDGS diet is from the distillers grains with solubles, indicating that cows possibly were able to sort out the MWDGS better as the concentration increased past 20%.

Milk Production

Using the NRC (2001) model, potential milk production was not reached. This could be a result of the lysine being limiting when concentrations of MWDGS increased. Data published

in the NRC (2001) indicate that the optimal lysine concentration is 7.2% of MP with a lysine to methionine ratio of 3:1. While our ratio was always greater than 3:1, lysine was significantly less than 7.2% of MP.

Milk Fat

Physical presentation of the diet influenced milk fat production. The mean particle size of both the TMR and consumed particles partially explains the milk fat production. There was a significant linear MWDGS by mixer interaction indicating that the particle size influenced the reduction in milk fat. Although smaller particles have been linked to a reduction in milk fat secretion (Woodford and Murphy, 1988; Grant et al., 1990; Krause et al., 2002), larger particles can increase the amount of sorting. The VA mixer had a larger log standard deviation of particle size, indicating more variation. Larger particles with more variation of particles can increase sorting behavior. Increased sorting contributes to reduced milk fat (Bal et al., 2000). The diets mixed with the VA mixer contributed to more sorting, which likely contributed to reduced milk fat secretion. The differences in mixing order may have contributed to the particle size and distribution differences.

Altered rumen environment as concentration of MWDGS increased can be seen by the reduction of rumen pH. Bauman and Lock (2006) indicated that lowered pH may be an indicator of MFD because of bacterial populations favoring alternative biohydrogenation pathways. While the area under pH 6 was not significantly different between treatments, Overton et al. (2006) indicated that acidosis is not a prerequisite for MFD; therefore, small changes in the rumen environment can lead to MFD.

Milk fat CLA *trans*-10, *cis*-12 was greater as MWDGS increased, particularly when the TMR were mixed with the vertical mixer wagon. The presence of this isomer indicates that there was an altered rumen environment causing the production of the alternative biohydrogenation pathway. Other trials including DGS also reported elevated CLA *trans*-10, *cis*-12 when increasing amounts of DGS were added to the diet of dairy cows (Schingoethe et al., 1999; Leonardi et al., 2005; Anderson et al., 2006).

Results for VFA indicate that there was a reduction in acetate concentration and molar percentage as concentration of MWDGS increased, similar to what was reported by Sasaikala-Appukuttan et al. (2008) for DDGS. Acetate is the precursor for de novo synthesis of milk fatty acids. The acetate to propionate ratio decreased as DDGS was added to the diet, which was also reported by Kelzer et al. (2009). If acetate is reduced, usually because of increased production of propionate, the amount of saturated short and medium fatty acids is reduced (Sutton et al., 1988; Doreau et al., 1999). Diets with lower acetate had a reduction in milk saturated fatty acids.

Milk Protein

A significant interaction of mixer by the linear effect of MWDGS for milk protein yield showed that protein yield decreased as MWDGS increased for cows fed with the Keenan mixer, but not for the vertical mixer. Lowered isovalerate concentrations in the rumen are a potential indicator of reduced rumen degradable protein, specifically leucine. Reductions in isovalerate were also seen by Anderson et al. (2006) and Kelzer et al. (2009). Lowered protein levels with 30% MWDGS might be attributed to the lowered lysine levels in corn-based DGS. The differences between the Keenan and vertical diets may be explained by differences in DMI. The

branched-chain FA in milk decreased as MWDGS increased possibly because of the reduction in branched-chain VFA and branched-chain precursors entering circulation.

As level of MWDGS increased, the MUN and ammonia concentrations decreased because of the reduction in RDP. The MWDGS had more RUP than the feedstuffs it replaced, which would reduce the amount of ammonia that is produced in the rumen. These results are similar to results reported by Sasikala-Appukuttan et al. (2008) and Kleinschmit et al. (2006). As RDP in the diet increases, more nitrogen needs to be cleared from the rumen in the form of ammonia. There may be a reduction in microbial protein because of the reduction in rumen degradable AA.

Digestibility and Turnover

Fat digestibility increased as MWDGS was added to the diet likely because of the source and availability of the fat. The soluble fraction of MWDGS contains most of the lipid fraction, which is readily available in the rumen. As more MWDGS were added to the diet in replacement for cottonseed and corn grain, more of the lipid was readily available which may have led to increased apparent total tract fat digestibility. The increased lipid turnover in the rumen may indicate that the PUFA did not have enough time to become fully biohydrogenated as more MWDGS was added to the diet. Digestibility of NDF increased as MWDGS increased, because the amount of forage NDF remained constant; this would indicate that the NDF in MWDGS is more digestible than the NDF of the concentrates that were replaced.

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Table 3.1 Cow characteristics of 24 lactating Holsteins enrolled in study.

Item	Mean		Range		SD	
	Keenan	Vertical	Keenan	Vertical	Keenan	Vertical
Parity	1.83	1.83	1 to 3	1 to 3	0.56	0.56
BW ¹ , kg	677	666	562 to 770	577 to 754	44.6	42.8
BCS ²	3.05	2.98	2.7 to 3.73	2.27 to 3.85	0.24	0.30
DIM ³	146.9	140.9	96 to 196	72 to 192	29.8	32.7
DCC ⁴	65.2	37.7	8 to 106	1 to 78	33.6	29.7

¹ Body weight taken as an average of each period.

² Body condition score taken as an average of each period.

³ Days in milk at the beginning of the trial.

⁴ Days carried calf at the beginning of the trial.

Table 3.2. Ingredient composition of the diets (% of DM).

Ingredient	Diet % DM		
	10% MWDGS	20% MWDGS	30% MWDGS
Alfalfa silage	10.0	10.0	10.0
Alfalfa hay	5.0	5.0	5.0
Corn silage	30.0	30.0	30.0
Wheat straw	2.0	2.0	2.0
Cottonseed	5.0	2.5	----
Soy hulls	5.13	3.59	2.05
Soybean meal	10.0	6.9	3.8
Dry Ground corn	20.0	17.1	14.2
MWDGS ¹	10.0	20.0	30.0
Blood meal	0.50	0.55	0.60
Rumensin	0.007	0.007	0.007
Sodium bicarbonate	0.75	0.75	0.75
Limestone	0.66	0.77	0.88
Dicalcium phosphate	0.22	0.11	----
Magnesium oxide	0.10	0.09	0.08
UI Dairy Minerals/Vitamins ²	0.17	0.17	0.17
Biotin	0.36	0.36	0.36
Salt	0.10	0.10	0.10

¹ MWDGS from Archer Daniels Midland Co., Decatur, IL plant.

² Composition: Mg, 5% DM; S, 10% DM; K, 7.5% DM; Fe, 2% DM; Z, 3% DM; Mn, 3% DM; Cu, 5000 ppm; I, 250 ppm; Co, 40 ppm; Se, 150 ppm; Vitamin A, 450,000 IU/kg; Vitamin D₃, 136,000 IU/kg; Vitamin E, 4,500 IU/kg.

Table 3.3 Conditions for preparation of the TMR.

	Keenan ¹ 10%	Keenan ¹ 20%	Keenan ¹ 30%	Vertical ¹ 10%	Vertical ¹ 20%	Vertical ¹ 30%
Order	Ingredient	Ingredient	Ingredient	Ingredient	Ingredient	Ingredient
1	grain mix	grain mix	grain mix	grain mix	grain mix	grain mix
2	water	water	alfalfa hay ²	water	water	alfalfa silage
3	alfalfa hay ²	alfalfa hay ²	wheat straw	alfalfa silage	alfalfa silage	wheat straw ²
4	wheat straw	wheat straw	corn grain	wheat straw ²	wheat straw ²	alfalfa hay
5	cottonseed	cottonseed	MWDGS	alfalfa hay	alfalfa hay	corn grain
6	soy hulls	soy hulls	alfalfa silage	cottonseed	cottonseed	MWDGS
7	corn grain	corn grain	corn silage	soy hulls	soy hulls	12 minutes ³
8	MWDGS	MWDGS	8 revolutions ³	corn grain	corn grain	corn silage
9	alfalfa silage	alfalfa silage		MWDGS	MWDGS	
10	corn silage	corn silage		5 minutes ³	10 minutes ³	
11	8 revolutions ³	8 revolutions ³		corn silage	corn silage	
Mixing time, min ⁴	45	33	27	49	36	36
Load size, kg ⁵	2907	1460	1019	3205	1529	1064

¹ Keenan - 8-9 revolutions per minute of paddle mixer from when power take off (PTO) was engaged except when alfalfa silage was added, PTO disengaged; Vertical – tractor run at 750 revolutions per minute (RPM) constantly running from when PTO was engaged.

² Indicates when PTO was engaged.

³ Additional mixing duration.

⁴ Average total mixing time (minutes) of diet.

⁵ Average kilograms of TMR mixed.

Table 3.4. Nutrient composition of total diets.

Nutrient	10% MWDGS	20% MWDGS	30% MWDGS
DM, % of as fed	47.2	47.0	47.3
NDF, % of DM	35.19	35.55	36.36
ADF, % of DM	23.57	23.01	22.75
Lignin, % of DM	3.68	3.88	3.99
CP, % of DM	17.54	17.66	17.61
Soluble protein, % of CP	38.24	38.02	37.94
Crude fat, % of DM	4.41	4.95	5.47
Fatty acids, % of DM	3.68	3.88	4.10
NFC ¹ , % of DM	37.68	37.00	36.45
Ash, % of DM	7.62	7.73	7.57
Calcium, % of DM	0.87	0.85	0.79
Phosphorus, % of DM	0.38	0.39	0.40
Sulfur, % of DM	0.23	0.27	0.31
Sodium, % of DM	0.32	0.37	0.38
Magnesium, % of DM	0.26	0.27	0.26
Potassium, % of DM	1.17	1.16	1.15
Iron, ppm of DM	339.2	336.6	318.6
Zinc, ppm of DM	93.1	90.4	80.4
Copper, ppm of DM	15.5	15.5	14.0
Manganese, ppm of DM	84.3	89.7	78.3
Molybdenum, ppm of DM	1.1	1.0	1.0

¹ 100 – [CP – (NDF – NDICP) – crude fat – ash]

Table 3.5. Fatty acid profile of total diets and MWDGS (grams/100 gram fatty acid methyl esters).

Fatty acid	Diet 10% MWDGS	Diet 20% MWDGS	Diet 30% MWDGS	Ingredient MWDGS
C14:0	0.29	0.28	0.26	0.05
C16:0	18.13	18.04	17.33	15.14
C18:0	3.01	2.89	2.77	2.00
C18:1 <i>cis</i> -9	15.90	16.55	17.25	22.59
C18:2 <i>cis</i> -9, <i>cis</i> -12	47.08	47.08	47.06	55.35
C18:3 <i>cis</i> -9, <i>cis</i> -12, <i>cis</i> -15	9.58	9.21	8.89	1.79
C22:0	0.63	0.62	0.61	0.20
C24:0	0.69	0.69	0.69	0.28
Others	4.69	4.64	4.57	2.59
SFA	24.11	23.85	23.55	18.17
MUFA <i>cis</i>	17.96	18.56	19.22	24.29
PUFA <i>cis</i>	56.72	56.36	56.00	57.18
C18:1 <i>trans</i>	0.010	0.008	0.009	0.00
BCFA	0.19	0.19	0.19	0.00
Total FA content, % of DM	3.68	3.88	4.10	8.72

Table 3.6. Fatty acid composition of total diets (% of DM).

Fatty acid	Diet 10% MWDGS	Diet 20% MWDGS	Diet 30% MWDGS
SFA, % of DM	0.89	0.93	0.97
MUFA <i>cis</i> , % of DM	0.66	0.72	0.79
PUFA <i>cis</i> , % of DM	2.09	2.19	2.30

Table 3.7. DMI, milk production, milk composition, and feed conversion efficiency (FCE).

Item	Treatments ¹						SEM ²	Mixer ³	Contrasts ⁴			
	K10	K20	K30	V10	V20	V30			C1	C2	C3	C4
DMI, kg/d	24.0	23.1	24.2	25.2	26.0	26.0	0.89	0.05	0.39	0.55	0.62	0.14
Milk yield, kg/d	36.7	37.0	35.9	37.5	37.3	39.0	2.26	0.58	0.75	0.83	0.18	0.24
ECM ⁵ , kg/d	32.9	32.5	31.5	35.1	33.3	32.2	1.99	0.55	0.002	0.98	0.24	0.58
Fat, %	3.60	3.49	3.45	3.64	3.26	2.81	0.19	0.15	< 0.0001	0.97	0.003	0.71
Fat, kg/d	1.28	1.25	1.24	1.38	1.25	1.10	0.11	0.91	0.0003	0.90	0.006	0.82
Protein, %	3.05	3.03	2.97	2.98	2.98	2.93	0.09	0.36	0.09	0.54	0.67	0.97
Protein, kg/d	1.12	1.11	1.03	1.10	1.09	1.13	0.06	0.85	0.34	0.79	0.05	0.27
Lactose, %	4.74	4.62	4.56	4.69	4.69	4.69	0.12	0.58	0.15	0.76	0.16	0.78
Lactose, kg/d	1.70	1.70	1.60	1.82	1.78	1.89	0.11	0.27	0.79	0.77	0.12	0.20
MUN, mg/dL	11.5	11.5	10.3	11.6	10.9	10.0	0.49	0.87	< 0.0001	0.29	0.41	0.18
SCC (× 1000)	101	117	152	80	183	85	72	0.92	0.68	0.43	0.74	0.34
FCE ⁶ , kg/kg	1.43	1.45	1.36	1.38	1.27	1.24	0.07	0.12	0.007	0.72	0.40	0.13

¹ K indicates Keenan mixer, V indicates vertical mixer; 10, 20 and 30 indicate concentration (%) of modified wet distillers grains with solubles.

² Largest standard errors among treatments.

³ Mixer is the *P* value associated with the main effect of mixer.

⁴ C1=linear effect of MWDGS, C2=quadratic effect of MWDGS, C3=linear effect of MWDGS by mixer, C4=quadratic effect of MWDGS by mixer.

⁵ Energy Corrected Milk = [(12.82 × kg milk fat) + (7.13 × kg milk protein) + (0.323 × kg milk)] (Tyrrell and Reid, 1965)

⁶ Feed Conversion Efficiency (ECM/DMI).

Table 3.8. Inputs used to estimate energy balance and MP in the NRC (2001) model and estimates of energy and MP balance; lysine and methionine supply for 24 Holstein cows fed three concentrations of MWDGS.

Item	Treatment ¹					
	K 10	K 20	K 30	V 10	V 20	V 30
Inputs ²						
DMI, kg	24.0	23.1	24.2	25.2	26.0	26.0
BW, kg	677	677	677	666	666	666
DIM	210	210	210	204	204	204
Day of gestation	128	128	128	101	101	101
Milk, kg	36.7	37.0	35.9	37.5	37.3	39.0
Milk fat, %	3.60	3.49	3.45	3.64	3.26	2.81
Milk protein, %	3.05	3.03	2.97	2.98	2.98	2.93
Milk lactose, %	4.74	4.62	4.56	4.69	4.69	4.69
Estimates						
DMI- predicted, kg/d	25.35	25.23	24.77	25.55	24.69	24.27
NE _L allowable milk, kg/d	38.9	38.6	42.1	41.5	45.9	50.0
MP allowable milk, kg/d	40.0	40	44.7	43.5	46.7	49.0
NE _L balance, Mcal/d	1.5	1.1	4.2	2.8	5.7	6.8
Days to gain one condition score	> 305	> 305	124	184	89	75
RDP balance, g/d	318	225	139	321	230	135
MP balance, g/d	151	136	391	269	417	436
Diet NE _L , Mcal/kg DM	1.58	1.60	1.61	1.57	1.58	1.59
Diet CP, % DM	17.6	17.7	17.9	17.6	17.7	17.8
Lysine, % of MP	6.26	5.99	5.69	6.23	5.92	5.64
Methionine, % of MP	1.80	1.81	1.82	1.79	1.80	1.81
Lysine/Methionine ratio	3.48:1	3.31:1	3.13:1	3.48:1	3.29:1	3.12:1

¹ K indicates Keenan mixer, V indicates vertical mixer; 10, 20 and 30 indicate concentration (%) of modified wet distillers grains with solubles.

² Inputs of DMI, milk production and components from treatment LSMeans. Mature body weight 700 kg. DIM and day of gestation taken as an average throughout the trial.

Table 3.9. Milk fatty acid (grams/100 gram fatty acids methyl esters).

Fatty Acid	Treatments ¹						SEM ²	Mixer ³	Contrasts ⁴			
	K10	K20	K30	V10	V20	V30			C1	C2	C3	C4
C4:0	3.22	3.16	3.16	3.22	3.15	3.20	0.21	0.97	0.69	0.62	0.86	0.92
C6:0	2.05	1.98	1.86	2.10	1.98	1.90	0.17	0.84	0.01	0.93	0.90	0.76
C8:0	1.17	1.15	1.04	1.23	1.16	1.10	0.11	0.65	0.01	0.67	0.99	0.54
C10:0	2.99	2.90	2.52	3.11	2.88	2.71	0.29	0.66	0.001	0.56	0.79	0.42
C12:0	3.36	3.27	2.89	3.46	3.28	3.13	0.27	0.53	0.002	0.52	0.54	0.44
C14:0	11.56	10.91	10.13	11.43	10.97	10.36	0.44	0.83	<0.0001	0.74	0.49	1.00
C14:1 <i>cis</i> -9	0.59	0.64	0.72	0.62	0.74	0.82	0.09	0.47	0.001	0.96	0.45	0.65
C16:0	30.84	27.87	27.00	30.68	28.36	25.86	0.87	0.77	<0.0001	0.15	0.20	0.09
C16:1 <i>cis</i> -9	0.83	0.85	0.96	0.89	1.01	1.10	0.09	0.27	0.01	0.79	0.55	0.60
C18:0	12.41	12.77	12.29	12.08	11.87	11.44	0.72	0.32	0.36	0.46	0.53	0.66
C18:1 <i>trans</i> -4	0.016	0.020	0.022	0.017	0.019	0.023	0.001	0.52	<0.0001	0.49	0.53	0.08
C18:1 <i>trans</i> -5	0.013	0.015	0.017	0.015	0.015	0.018	0.001	0.43	<0.0001	0.12	0.48	0.24
C18:1 <i>trans</i> -6-8	0.25	0.32	0.37	0.26	0.33	0.42	0.03	0.32	<0.0001	0.99	0.32	0.52
C18:1 <i>trans</i> -9	0.21	0.26	0.28	0.22	0.27	0.31	0.02	0.33	<0.0001	0.33	0.51	0.53
C18:1 <i>trans</i> -10	0.42	0.73	1.09	0.55	1.06	1.94	0.27	0.08	<0.0001	0.49	0.05	0.61
C18:1 <i>trans</i> -11	0.81	1.08	1.36	0.91	0.96	1.08	0.08	0.10	<0.0001	0.69	0.0002	0.67
C18:1 <i>trans</i> -12	0.42	0.50	0.52	0.46	0.51	0.55	0.03	0.44	<0.0001	0.13	0.64	0.22
C18:1 <i>cis</i> -9	19.15	21.03	22.83	18.52	20.85	22.75	1.26	0.81	<0.0001	0.75	0.55	0.83
C18:2 <i>cis</i> -9, <i>cis</i> -12	2.44	2.91	3.23	2.60	2.96	3.54	0.14	0.28	<0.0001	0.73	0.27	0.13
C18:3 <i>cis</i> -6, <i>cis</i> -9, <i>cis</i> -12	0.026	0.026	0.024	0.028	0.024	0.021	0.002	0.05	0.001	0.94	0.06	0.38
C18:3 <i>cis</i> -9, <i>cis</i> -12, <i>cis</i> -15	0.27	0.28	0.29	0.28	0.29	0.31	0.01	0.38	<0.0001	0.35	0.62	0.32
CLA <i>cis</i> -9, <i>trans</i> -11	0.30	0.44	0.59	0.33	0.41	0.53	0.05	0.73	<0.0002	0.39	0.02	0.73
CLA <i>trans</i> -10, <i>cis</i> -12	<0.001	0.001	0.005	0.003	0.005	0.013	0.002	0.01	<0.0001	0.15	0.03	0.53
Σ Others	6.67	6.89	6.79	6.96	6.89	6.86	0.15	0.49	0.86	0.31	0.16	0.22
Σ SFA	69.42	65.80	62.60	69.26	65.45	61.45	1.87	0.76	<0.0001	0.93	0.51	0.82
Σ MUFA	23.04	25.30	27.32	22.61	25.33	27.46	1.44	0.95	<0.0001	0.66	0.60	0.85
Σ <i>cis</i> PUFA	3.11	3.58	3.90	3.28	3.63	4.20	0.15	0.32	<0.0001	0.72	0.35	0.10
Σ <i>cis</i> CLA	0.30	0.44	0.61	0.34	0.42	0.56	0.05	0.88	<0.0001	0.33	0.06	0.72
Σ BCFA	1.46	1.37	1.33	1.51	1.40	1.36	0.03	0.37	<0.0001	0.11	0.63	0.78
Σ C18:1 <i>trans</i>	2.13	2.93	3.65	2.43	3.16	4.32	0.36	0.20	<0.0001	0.61	0.38	0.49
Σ n-6	2.77	3.23	3.54	2.94	3.28	3.84	0.14	0.30	<0.0001	0.76	0.34	0.10
Σ n-3	0.33	0.34	0.35	0.34	0.34	0.36	0.01	0.62	0.01	0.32	0.73	0.20

¹ K indicates Keenan mixer, V indicates vertical mixer; 10, 20 and 30 indicate concentration (%) of modified wet distillers grains with solubles.

² Largest standard errors among treatments.

³ Mixer is the *P* value associated with the main effect of mixer.

⁴ C1=linear effect of MWDGS, C2=quadratic effect of MWDGS, C3=linear effect of MWDGS by mixer, C4=quadratic effect of MWDGS by mixer.

Table 3.10. Nutrient composition of refusals.

Item	Treatments ¹						SEM ²	Mixer ³	Contrasts ⁴			
	K 10	K 20	K 30	V 10	V 20	V 30			C1	C2	C3	C4
NDF, % of DM	41.82	41.50	38.92	40.47	40.20	40.75	1.84	0.85	0.30	0.74	0.21	0.48
ADF, % of DM	25.78	26.37	25.58	26.97	25.78	27.10	1.16	0.51	0.96	0.66	0.82	0.14
Lignin, % of DM	6.08	5.75	6.13	6.23	5.98	5.65	0.38	0.92	0.42	0.58	0.34	0.48
CP, % of DM	16.23	16.30	16.38	17.13	16.98	16.95	0.42	0.04	0.96	0.91	0.62	0.93
Soluble Protein, % of CP	28.33	26.17	28.83	29.00	31.00	29.17	1.58	0.14	0.83	0.86	0.92	0.12
Crude fat, % of DM	3.70	4.17	4.03	3.45	4.32	3.90	0.27	0.66	0.08	0.02	0.79	0.37
NFC, % of DM	30.32	31.52	33.52	31.80	30.78	31.25	1.79	0.66	0.14	0.45	0.04	0.82
Ash, % of DM	7.90	6.52	7.15	7.10	7.71	7.19	0.51	0.76	0.50	0.61	0.39	0.08
Calcium, % of DM	1.24	0.66	0.82	0.81	1.02	0.86	0.21	0.96	0.37	0.59	0.24	0.11
Phosphorus, % of DM	0.42	0.44	0.46	0.44	0.44	0.47	0.02	0.19	0.001	0.45	0.42	0.23
Sulfur, % of DM	0.23	0.27	0.30	0.24	0.27	0.31	0.01	0.28	< 0.0001	0.63	1.00	0.81
Sodium, % of DM	0.28	0.30	0.35	0.31	0.35	0.36	0.01	0.02	0.0001	0.70	0.46	0.22
Potassium, % of DM	1.38	1.37	1.40	1.42	1.40	1.41	0.04	0.28	0.80	0.46	0.68	0.99
Iron, ppm of DM	444	368	437	433	577	376	72	0.46	0.64	0.39	0.71	0.05
Zinc, ppm of DM	74	70	87	84	86	85	6	0.11	0.22	0.37	0.33	0.28
Copper, ppm of DM	16	13	14	16	16	15	0.5	0.003	0.003	0.36	0.59	0.003
Manganese, ppm of DM	84	65	75	84	87	77	6	0.16	0.14	0.42	0.84	0.03
Molybdenum, ppm of DM	0.80	0.78	0.77	0.90	0.87	0.77	0.07	0.25	0.13	0.72	0.35	0.72

¹ K indicates Keenan mixer, V indicates vertical mixer; 10, 20 and 30 indicate concentration (%) of modified wet distillers grains with solubles.

² Largest standard errors among treatments. ³Mixer is the *P* value associated with the main effect of mixer.

⁴ C1=linear effect of MWDGS, C2=quadratic effect of MWDGS, C3=linear effect of MWDGS by mixer, C4=quadratic effect of MWDGS by mixer.

Table 3.11. Distribution of ration feed particles using the Penn State particle separator.

Particle size	Treatments ¹						SEM ²	Mixer ³	Contrasts ⁴			
	K10	K20	K30	V10	V20	V30			C1	C2	C3	C4
>19.0 mm (top)	11.29	10.73	10.33	13.40	13.42	13.63	0.71	< 0.0001	0.61	0.89	0.40	0.99
8 to 19 mm (middle)	41.06	39.43	35.52	38.59	35.57	32.80	0.65	< 0.0001	< 0.0001	0.37	0.85	0.26
1.18 to 8 mm (lower)	36.27	38.66	42.97	35.80	38.52	41.67	0.52	0.14	< 0.0001	0.20	0.43	0.41
< 1.18 mm (bottom)	11.38	11.18	11.19	12.21	12.49	11.89	0.66	0.08	0.70	0.77	0.92	0.64

¹ K indicates Keenan mixer, V indicates vertical mixer; 10, 20 and 30 indicate concentration (%) of modified wet distillers grains with solubles.

² Largest standard errors among treatments.

³ Mixer is the *P* value associated with the main effect of mixer.

⁴ C1=linear effect of MWDGS, C2=quadratic effect of MWDGS, C3=linear effect of MWDGS by mixer, C4=quadratic effect of MWDGS by mixer.

Table 3.12. Particle size of wet-sieved samples.

Table S.12. Particle size of wet sieved samples.												
Item	Treatments ¹						SEM ²	Mixer ³	Contrasts ⁴			
	K10	K20	K30	V10	V20	V30			C1	C2	C3	C4
TMR												
Log10 mean	3.47	3.45	3.41	3.44	3.51	3.47	0.03	0.06	0.41	0.04	0.03	0.23
Log10 SD	0.68	0.68	0.70	0.72	0.72	0.71	0.02	0.03	0.63	0.84	0.35	0.56
Mean, μm	3000	2876	2543	2792	3336	2999	186	0.06	0.41	0.04	0.03	0.20
Consumed												
Log10 mean	3.46	3.44	3.40	3.41	3.52	3.45	0.03	0.17	0.70	0.01	0.04	0.06
Log10 SD	0.68	0.67	0.71	0.67	0.73	0.70	0.02	0.43	0.15	0.44	0.99	0.02
Mean, μm	2921	2825	2527	2628	3414	2884	199	0.14	0.69	0.01	0.06	0.06
Rumen												
Log10 mean	3.22	3.23	3.17	3.20	2.18	3.19	0.03	0.56	0.25	0.51	0.49	0.28
Log10 SD	0.47	0.50	0.49	0.53	0.52	0.51	0.03	0.49	0.96	0.54	0.44	0.54
Mean, μm	1643	1735	1476	1583	1528	1548	109	0.56	0.29	0.39	0.48	0.19
Fecal												
Log10 mean	2.85	2.82	2.86	2.85	2.84	2.86	0.02	0.61	0.68	0.05	0.79	0.25
Log10 SD	0.44	0.42	0.42	0.43	0.44	0.41	0.01	0.87	0.11	0.75	0.69	0.08
Mean, μm	717	662	732	718	703	719	27	0.61	0.75	0.05	0.76	0.25

¹ K indicates Keenan mixer, V indicates vertical mixer; 10, 20 and 30 indicate concentration (%) of modified wet distillers grains with solubles.

² Largest standard errors among treatments.

³ Mixer is the *P* value associated with the main effect of mixer.

⁴ C1=linear effect of MWDGS, C2=quadratic effect of MWDGS, C3=linear effect of MWDGS by mixer, C4=quadratic effect of MWDGS by mixer.

Table 3.13. Dry-sieved particle size and physically effective fiber (peNDF).

Item	Treatments ¹						SEM ²	Mixer ³	Contrasts ⁴			
	K10	K20	K30	V10	V20	V30			C1	C2	C3	C4
Log mean	3.37	3.39	3.35	3.36	3.41	3.39	0.01	0.15	0.77	0.005	0.18	0.71
Log SD	0.38	0.37	0.34	0.37	0.40	0.38	0.01	0.001	0.17	0.06	0.01	0.18
Mean, μm	2346	2476	2276	2321	2597	2439	74	0.15	0.74	0.004	0.21	0.68
peNDF, % ⁵	29.7	30.1	30.4	29.7	30.5	30.4	0.73	0.83	0.33	0.72	0.97	0.75
peNDF, kg ⁶	7.16	6.96	7.47	7.45	7.88	7.88	0.39	0.19	0.07	0.68	0.77	0.10

¹ K indicates Keenan mixer, V indicates vertical mixer; 10, 20 and 30 indicate concentration (%) of modified wet distillers grains with solubles.

² Largest standard errors among treatments.

³ Mixer is the *P* value associated with the main effect of mixer.

⁴ C1=linear effect of MWDGS, C2=quadratic effect of MWDGS, C3=linear effect of MWDGS by mixer, C4=quadratic effect of MWDGS by mixer.

⁵ peNDF % = (Total NDF concentration) – ((fraction passing through the 1.18-mm sieve) × (<1.18-mm composite sample NDF concentration)).

⁶ peNDF kg = peNDF*intake.

Table 3.14. Particle size analysis of consumed particles by wet-sieving.

Item	Treatments ¹						SEM ²	Mixer ³	Contrasts ⁴			
	K 10	K 20	K 30	V 10	V 20	V 30			C1	C2	C3	C4
Log mean	3.46	3.43	3.40	3.39	3.50	3.44	0.03	0.45	0.84	0.03	0.02	0.06
Log SD	0.68	0.66	0.70	0.65	0.71	0.69	0.02	0.77	0.09	0.76	0.68	0.02
Percentage												
> 9.5 mm, %	22.15	20.37	20.44	18.25	24.84	21.64	1.94	0.65	0.60	0.15	0.11	0.04
> 6.3 mm, %	30.55	28.60	28.30	26.25	33.06	29.81	2.01	0.68	0.69	0.14	0.08	0.04
> 3.35 mm, %	45.69	43.87	42.66	41.47	47.67	44.68	1.79	0.65	0.95	0.09	0.04	0.06
> 1.18 mm, %	71.17	70.36	67.66	68.62	72.06	70.16	0.92	0.36	0.18	0.006	0.001	0.18
Kilograms												
> 9.5 mm, kg	5.45	4.77	5.17	4.63	6.51	5.53	0.51	0.49	0.49	0.25	0.19	0.01
> 6.3 mm, kg	7.51	6.69	7.14	6.65	8.67	7.66	0.56	0.53	0.53	0.32	0.17	0.02
> 3.35 mm, kg	11.19	10.27	10.67	10.48	12.47	11.52	0.62	0.62	0.62	0.38	0.14	0.02
> 1.18 mm, kg	17.29	16.41	16.64	17.26	18.68	18.06	0.77	0.88	0.88	0.58	0.14	0.07

¹ K indicates Keenan mixer, V indicates vertical mixer; 10, 20 and 30 indicate concentration (%) of modified wet distillers grains with solubles.

² Largest standard errors among treatments.

³ Mixer is the *P* value associated with the main effect of mixer.

⁴ C1=linear effect of MWDGS, C2=quadratic effect of MWDGS, C3=linear effect of MWDGS by mixer, C4=quadratic effect of MWDGS by mixer.

Table 3.15. Particle size analysis of rumen particles from wet-sieving.

Item	Treatments ¹						SEM ²	Mixer ³	Contrasts ⁴			
	K 10	K 20	K 30	V 10	V 20	V 30			C1	C2	C3	C4
Log mean	3.19	3.21	3.16	3.20	3.18	3.18	0.03	0.99	0.28	0.56	0.78	0.20
Log SD	0.49	0.51	0.50	0.54	0.53	0.49	0.03	0.52	0.46	0.36	0.16	0.91
Percentage												
> 9.5 mm, %	5.39	7.16	5.11	7.44	6.78	5.88	1.76	0.70	0.44	0.32	0.59	0.38
> 2.36 mm, %	35.43	36.65	33.34	37.53	35.73	34.38	2.90	0.82	0.22	0.57	0.80	0.49
> 1.18 mm, %	59.81	60.36	57.14	59.62	57.87	58.45	1.72	0.81	0.22	0.57	0.62	0.24
> 0.85 mm, %	70.47	70.80	67.86	69.45	67.94	69.45	1.40	0.57	0.26	0.78	0.26	0.12
> 0.425 mm, %	87.42	87.44	85.54	85.68	84.90	87.10	0.89	0.34	0.74	0.65	0.02	0.04
> 0.25 mm, %	94.64	94.51	93.48	93.22	92.87	94.51	0.63	0.31	0.89	0.51	0.02	0.09
Kilograms												
> 9.5 mm, kg	0.63	0.82	0.63	0.88	0.86	0.79	0.23	0.57	0.79	0.41	0.79	0.55
> 2.36 mm, kg	4.17	4.38	4.15	4.41	4.58	4.62	0.47	0.52	0.80	0.64	0.75	0.79
> 1.18 mm, kg	6.93	7.21	7.00	7.00	7.41	7.87	0.64	0.61	0.21	0.72	0.29	0.66
> 0.85 mm, kg	8.15	8.49	8.31	8.14	8.70	9.36	0.74	0.64	0.08	0.74	0.16	0.62
> 0.425 mm, kg	10.09	10.53	10.46	10.01	10.87	11.76	0.91	0.65	0.02	0.73	0.11	0.71
> 0.25 mm, kg	10.91	11.39	11.41	10.89	11.89	12.75	0.97	0.62	0.02	0.70	0.14	0.84

¹ K indicates Keenan mixer, V indicates vertical mixer; 10, 20 and 30 indicate concentration (%) of modified wet distillers grains with solubles

² Largest standard errors among treatments.

³ Mixer is the *P* value associated with the main effect of mixer.

⁴ C1=linear effect of MWDGS, C2=quadratic effect of MWDGS, C3=linear effect of MWDGS by mixer, C4=quadratic effect of MWDGS by mixer.

Table 3.16. Rumen pH and ammonia concentration.

Item	Treatments ¹						SEM ²	Mixer ³	Contrasts ⁴			
	K10	K20	K30	V10	V20	V30			C1	C2	C3	C4
pH, Mean	6.17	6.13	6.03	6.12	6.04	6.01	0.06	0.26	0.05	0.93	0.80	0.57
pH, Min	5.61	5.69	5.65	5.54	5.56	5.60	0.07	0.16	0.44	0.70	0.88	0.61
pH, Max	6.68	6.61	6.45	6.60	6.52	6.48	0.09	0.49	0.05	0.83	0.53	0.67
pH, Range	1.07	0.93	0.80	1.06	0.96	0.87	0.11	0.72	0.04	0.94	0.68	0.99
pH _{6.0 hr} ^e	2.43	2.87	2.50	2.69	3.18	2.98	0.65	0.68	0.63	0.28	0.76	0.93
Ammonia, mg/dL	7.90	6.10	4.78	7.56	6.13	4.75	0.49	0.77	<0.0001	0.76	0.75	0.80

¹ K indicates Keenan mixer, V indicates vertical mixer; 10, 20 and 30 indicate concentration (%) of modified wet distillers grains with solubles.

² Largest standard errors among treatments.

³ Mixer is the *P* value associated with the main effect of mixer.

⁴ C1=linear effect of MWDGS, C2=quadratic effect of MWDGS, C3=linear effect of MWDGS by mixer, C4=quadratic effect of MWDGS by mixer

⁵ Area under pH 6.0.

Table 3.17. Measurements of rumen characteristics from rumen evacuations.

	Treatments ¹								Contrasts ⁴			
Item	K10	K20	K30	V10	V20	V30	SEM ²	Mixer ³	C1	C2	C3	C4
Weight in rumen												
Digesta, kg	82.7	82.1	82.4	79.4	83.8	85.3	5.4	0.95	0.31	0.82	0.27	0.69
Primarily fluid, kg	46.6	49.4	49.0	45.5	50.3	51.6	4.1	0.88	0.08	0.38	0.42	0.97
Primarily solid, kg	41.2	32.5	33.1	33.3	33.4	33.5	4.2	0.62	0.21	0.36	0.19	0.37
DM, kg	11.5	12.2	12.4	11.4	12.6	13.2	0.94	0.76	0.03	0.50	0.39	0.94
CP, kg	2.13	2.13	2.30	1.97	2.03	2.30	0.24	0.73	0.07	0.39	0.53	0.93
NDF, kg	6.53	7.05	7.06	6.53	7.27	7.35	0.85	0.88	0.13	0.43	0.74	0.92
Fat, kg	0.29	0.26	0.23	0.23	0.30	0.26	0.04	0.38	0.56	0.27	0.11	0.25
Ash, kg	0.95	1.26	1.15	.94	1.05	1.20	0.15	0.73	0.05	0.31	0.75	0.24
NFC, kg	2.12	2.63	2.54	2.44	2.36	2.78	0.28	0.76	0.06	0.85	0.83	0.10
Composition of rumen digesta												
DM, %	14.1	14.9	15.0	14.3	15.0	15.4	0.57	0.70	0.03	0.60	0.83	0.73
CP, %	18.04	16.98	18.06	17.14	16.42	17.73	1.07	0.54	0.65	0.09	0.67	0.97
NDF, %	56.05	56.73	56.83	58.07	57.60	56.00	3.39	0.85	0.79	0.83	0.55	0.95
Fat, %	2.50	2.00	1.82	2.03	2.30	1.97	0.22	0.96	0.03	0.61	0.07	0.11
Ash, %	8.11	10.20	8.97	8.31	8.69	9.53	1.44	0.88	0.27	0.36	0.85	0.23
NFC, %	18.00	21.19	20.38	21.10	18.83	21.18	1.38	0.70	0.29	0.87	0.32	0.03

¹ K indicates Keenan mixer, V indicates vertical mixer; 10, 20 and 30 indicate concentration (%) of modified wet distillers grains with solubles.

² Largest standard errors among treatments.

³ Mixer is the *P* value associated with the main effect of mixer.

⁴ C1=linear effect of MWDGS, C2=quadratic effect of MWDGS, C3=linear effect of MWDGS by mixer, C4=quadratic effect of MWDGS by mixer.

Table 3.18. Rumen VFA concentration (mM) and molar proportion (%).

Item	Treatments ¹						SEM ²	Mixer ³	Contrasts ⁴			
	K 10	K 20	K 30	V 10	V 20	V 30			C1	C2	C3	C4
VFA, mM												
Total	102.07	100.20	100.49	104.05	103.02	102.22	3.63	0.47	0.64	0.85	0.97	0.88
Acetate (A)	63.65	60.12	57.81	63.75	61.20	59.29	2.13	0.61	0.02	0.80	0.75	0.94
Propionate (P)	21.78	23.01	25.50	23.15	24.58	25.65	1.59	0.43	0.06	0.87	0.71	0.77
Butyrate	12.89	13.53	13.81	13.33	13.75	13.97	0.64	0.61	0.24	0.81	0.83	0.94
A:P ratio	2.98	2.68	2.38	2.82	2.57	2.35	0.15	0.41	0.001	0.96	0.65	0.95
Isobutyrate	0.82	0.74	0.65	0.83	0.72	0.64	0.03	0.80	<0.0001	0.86	0.66	0.72
Isovalerate	1.34	1.13	0.98	1.28	1.08	0.97	0.14	0.73	0.03	0.77	0.85	0.95
Valerate	1.58	1.66	1.76	1.71	1.69	1.76	0.09	0.46	0.20	0.75	0.50	0.82
Pattern, mol/100 mol												
Acetate	62.56	60.24	57.80	61.46	59.62	58.00	0.96	0.52	0.0002	0.97	0.50	0.92
Propionate	21.24	22.92	25.09	22.21	23.70	25.09	1.08	0.51	0.004	0.92	0.63	0.87
Butyrate	12.51	13.39	13.71	12.62	13.27	13.62	0.36	0.92	0.005	0.49	0.78	0.84
Isobutyrate	0.82	0.74	0.65	0.82	0.71	0.63	0.03	0.43	<0.0001	0.84	0.63	0.72
Isovalerate	1.33	1.14	0.99	1.25	1.08	0.96	0.14	0.65	0.04	0.85	0.87	0.99
Valerate	1.54	1.64	1.75	1.63	1.62	1.71	0.06	0.79	0.01	0.60	0.26	0.58

¹ K indicates Keenan mixer, V indicates vertical mixer; 10, 20 and 30 indicate concentration (%) of modified wet distillers grains with solubles.

² Largest standard errors among treatments.

³ Mixer is the *P* value associated with the main effect of mixer.

⁴ C1=linear effect of MWDGS, C2=quadratic effect of MWDGS, C3=linear effect of MWDGS by mixer, C4=quadratic effect of MWDGS by mixer.

Table 3.19. Apparent digestibility (percentage of intake) of nutrient fractions in the total tract.

Item	Treatments ¹						SEM ²	Mixer ³	Contrasts ⁴			
	K10	K20	K30	V10	V20	V30			C1	C2	C3	C4
Dry matter	64.8	66.0	68.6	66.0	66.3	66.9	2.0	0.95	0.20	0.76	0.42	0.85
NDF	46.7	48.0	53.2	46.6	48.2	51.0	3.9	0.79	0.09	0.63	0.73	0.79
CP	65.1	65.5	67.8	67.0	66.1	65.8	2.9	0.95	0.70	0.68	0.33	0.82
Ash	45.8	50.4	53.7	48.4	50.4	49.1	3.6	0.78	0.16	0.64	0.25	0.84
OM	66.4	67.3	69.8	67.5	67.6	68.3	2.0	0.98	0.24	0.70	0.45	0.88
Crude fat	78.4	81.1	85.0	76.1	81.3	86.1	2.7	0.82	0.0003	0.58	0.03	0.47
NFC	85.7	87.3	88.1	87.6	87.2	87.1	1.3	0.74	0.39	0.91	0.18	0.76

¹ K indicates Keenan mixer, V indicates vertical mixer; 10, 20 and 30 indicate concentration (%) of modified wet distillers grains with solubles.

² Largest standard errors among treatments.

³ Mixer is the *P* value associated with the main effect of mixer.

⁴ C1=linear effect of MWDGS, C2=quadratic effect of MWDGS, C3=linear effect of MWDGS by mixer, C4=quadratic effect of MWDGS by mixer.

Table 3.20 Fractional turnover (% / h) of nutrient fractions in the rumen.

Item	Treatments ¹						SEM ²	Mixer ³	Contrasts ⁴			
	K10	K20	K30	V10	V20	V30			C1	C2	C3	C4
Liquid ⁵	14.96	16.33	13.89	16.93	16.11	18.52	2.75	0.51	0.87	0.92	0.42	0.21
DM ⁶	6.37	6.14	6.25	7.23	6.85	6.79	0.61	0.40	0.40	0.52	0.63	0.98
NDF ⁶	4.84	4.92	5.19	5.47	5.08	5.01	0.68	0.82	0.88	0.64	0.27	0.90
CP ⁶	16.47	16.49	15.71	16.29	15.44	14.19	1.38	0.57	0.09	0.63	0.41	0.87
Ash ⁶	7.30	6.84	6.40	8.20	7.98	6.67	0.99	0.38	0.15	0.68	0.70	0.67
OM ⁶	8.14	8.12	7.96	8.89	8.31	8.05	0.96	0.79	0.29	0.91	0.49	0.75
Crude fat ⁶	16.89	21.69	25.29	20.78	18.77	23.45	4.85	0.95	0.13	0.63	0.42	0.49
NFC ⁶	20.65	14.51	14.14	16.04	16.42	14.30	2.58	0.77	0.02	0.52	0.16	0.12
> 9.5 mm, kg ⁷	34.23	22.03	32.69	23.49	36.10	35.33	8.19	0.74	0.48	0.70	0.36	0.16
> 1.18 mm, kg ⁷	10.04	9.28	9.42	10.39	10.40	9.03	0.99	0.75	0.11	0.81	0.52	0.27
< 1.18 mm, kg ⁷	6.54	7.31	6.18	6.86	5.36	5.72	0.73	0.32	0.20	0.98	0.49	0.08

¹ K indicates Keenan mixer, V indicates vertical mixer; 10, 20 and 30 indicate concentration (%) of modified wet distillers grains with solubles.

² Largest standard errors among treatments.

³ Mixer is the *P* value associated with the main effect of mixer.

⁴ C1=linear effect of MWDGS, C2=quadratic effect of MWDGS, C3=linear effect of MWDGS by mixer, C4=quadratic effect of MWDGS by mixer.

⁵ Calculated by disappearance of cobalt EDTA marker.

⁶ Fractional turnover rate in rumen (%/h) = 100*(intake of component / ruminal pool of component) / 24.

⁷ Turnover of particle size fractions.

Table 3.21. Pearson correlation coefficients for variables associated with milk fat production (*P* values are presented in parentheses).

Item	Milk, kg	Milk fat, %	Milk fat, kg	Dietary Fat, %	Dietary Fat, kg	peNDF, %	peNDF, kg	pH mean	C18:2 <i>t</i> -10, <i>c</i> -12	C18:1 <i>t</i> -10
Milk, kg	1									
Milk fat, %	-0.03 (0.80)	1								
Milk fat, kg	0.68 (< 0.0001)	0.70 (< 0.0001)	1							
Dietary fat, %	-0.03 (0.79)	-0.24 (0.04)	-0.19 (0.11)	1						
Dietary fat, kg	0.48 (< 0.0001)	-0.17 (0.19)	0.22 (0.09)	0.57 (< 0.0001)	1					
peNDF, %	0.10 (0.40)	0.23 (0.05)	0.26 (0.03)	0.03 (0.82)	0.20 (0.11)	1				
peNDF, kg	0.56 (< 0.0001)	0.12 (0.33)	0.49 (< 0.0001)	- 0.04 (0.73)	0.74 (< 0.0001)	0.54 (< 0.0001)	1			
pH, mean	- 0.01 (0.93)	-0.04 (0.81)	-0.06 (0.73)	-0.10 (0.55)	-0.30 (0.09)	-0.09 (0.58)	-0.32 (0.07)	1		
C18:2 <i>t</i> -10, <i>c</i> -12	0.20 (0.10)	-0.67 (< 0.0001)	-0.35 (0.003)	0.36 (0.002)	0.38 (0.002)	-0.02 (0.89)	0.09 (0.49)	-0.35 (0.04)	1	
C18:1 <i>t</i> -10	0.13 (0.27)	-0.72 (< 0.0001)	-0.43 (0.0003)	0.32 (0.007)	0.35 (0.005)	-0.04 (0.75)	0.09 (0.47)	-0.39 (0.02)	0.89 (< 0.0001)	1

Table 3.22. Summary of stepwise regression analysis to explain milk fat secretion (kilograms).

Step	Variable entered ¹	Multiple R ²	Increase in R ²
1	Milk	0.539	0.539
2	CLA	0.733	0.194
3	DIM	0.804	0.072
4	pH	0.826	0.022

¹ Milk - milk production kg/day; CLA - g/100 g of CLA trans-10, cis-12 milk fatty acid; DIM - days in milk; pH - mean rumen pH.

Table 3.23. Summary of stepwise regression analysis to explain milk fat percentage.

Step	Variable entered ¹	Multiple R ²	Increase in R ²
1	CLA	0.405	0.405
2	DIM	0.607	0.202

¹ CLA, g/100 g of CLA trans-10, cis-12 milk fatty acid; DIM – days in milk.

Table 3.24. Economic data for value of milk, cost of feed, and income over feed cost.

Item	Treatments ¹					
	K10	K20	K30	V10	V20	V30
Value of milk ² , \$	13.28	13.08	12.58	13.69	13.01	12.50
Cost of feed ³ , \$	7.27	6.66	6.65	7.63	7.51	7.13
IOFC ⁴ , \$	6.01	6.42	5.93	6.06	5.49	5.37

^a K indicates Keenan mixer, V indicates Vertical mixer; 10, 20 and 30 indicate concentration (%) of modified wet distillers grains with solubles.

^b Calculated with value of milk fat at \$4.96/kg, protein \$5.51/kg, and lactose \$0.44/kg.

^c Calculated with average prices for 2011: alfalfa silage = \$0.15/kg DM; corn silage = \$0.19/kg DM; straw = \$0.11/kg DM; cottonseed = \$0.33/kg DM; alfalfa hay = \$0.18/kg DM; soy hulls = \$0.22/kg DM; soybean meal = \$0.41/kg DM; MWDGS = \$0.21/kg DM; blood meal = \$1.32/kg DM; ground shelled corn = \$0.39/kg DM; minerals and vitamins = \$1.98/kg DM.

^d Income over feed costs.

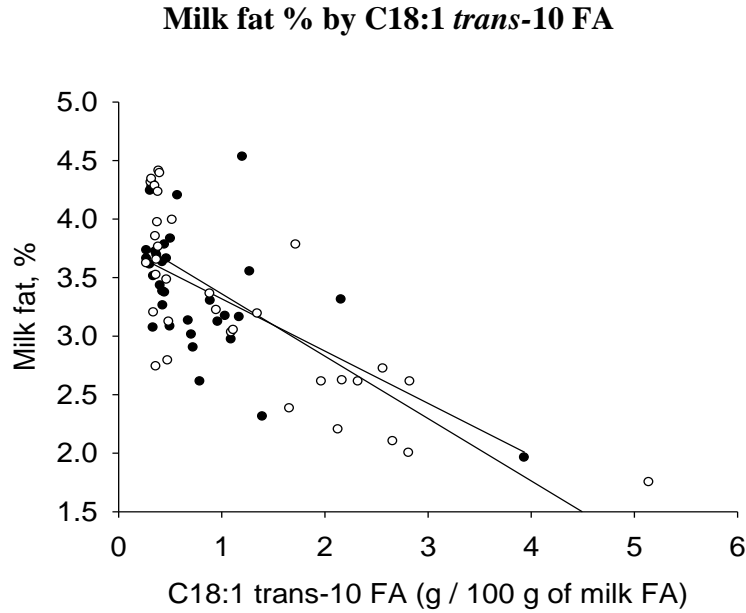


Figure 3.1. Simple regression of milk fat percentage by C18:1 *trans*-10 FA for 24 Holstein cows fed three concentrations of MWDGS mixed with Keenan (KMF; ●) or Vertical auger mixer (VA; ○). Regression for cows fed Keenan diets $y = -0.45x + 3.77$, with a coefficient of determination of 0.32. Regression for cows fed Vertical diets $y = -0.53x + 3.89$, with a coefficient of determination of 0.62.

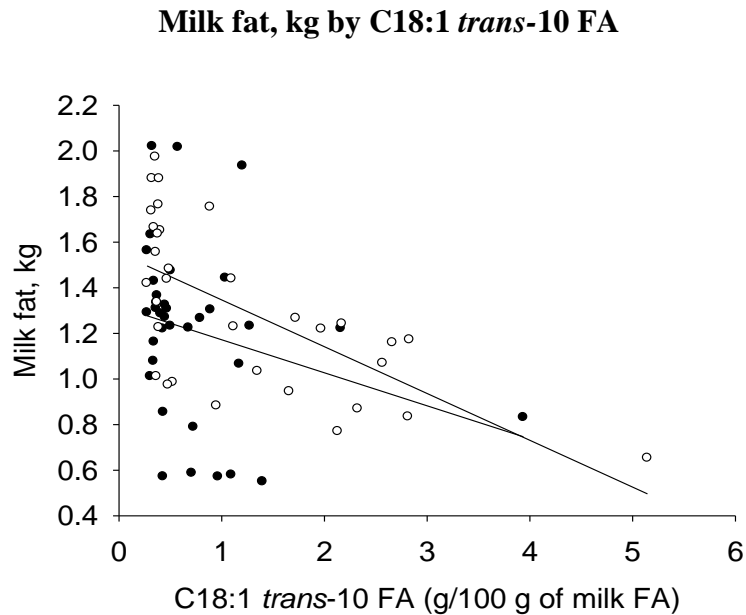


Figure 3.2. Simple regression of milk fat, kg by C18:1 *trans*-10 FA for 24 Holstein cows fed three concentration of MWDGS mixed with Keenan (KMF; ●) or Vertical auger mixer (VA; ○). Regression for cows fed Keenan diets $y = -0.14x + 1.32$, with a coefficient of determination of 0.07. Regression for cows fed Vertical diets $y = -0.21x + 1.55$, with a coefficient of determination of 0.42.

Milk fat % by C18:2 *trans*-10, *cis*-12 FA

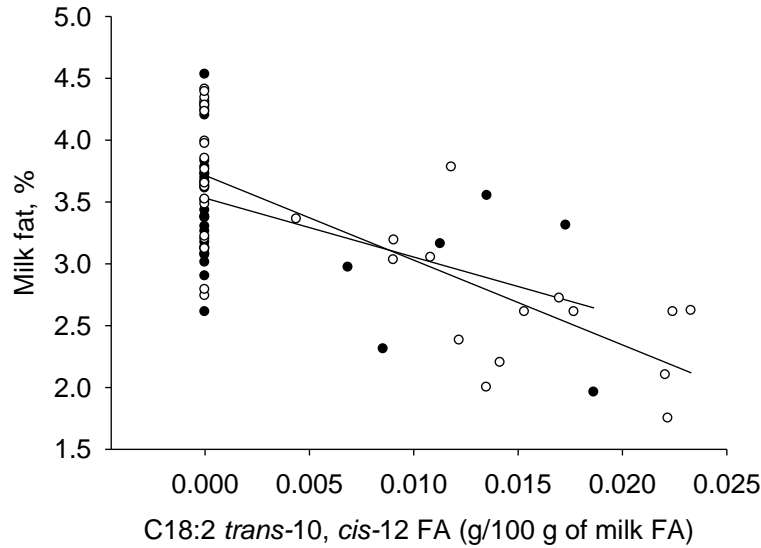


Figure 3.3. Simple regression of milk fat percentage by C18:2 *trans*-10, *cis*-12 FA for 24 Holstein cows fed three concentrations of MWDGS mixed with Keenan (KMF; ●) or Vertical auger mixer (VA; ○). Regression for cows fed Keenan diets $y = -47.69x + 3.53$ with a coefficient of determination of 0.21. Regression for cows fed Vertical diets $y = -68.53x + 3.71$ with a coefficient of determination of 0.58.

Milk fat, kg by C18:2 *trans*-10, *cis*-12 FA

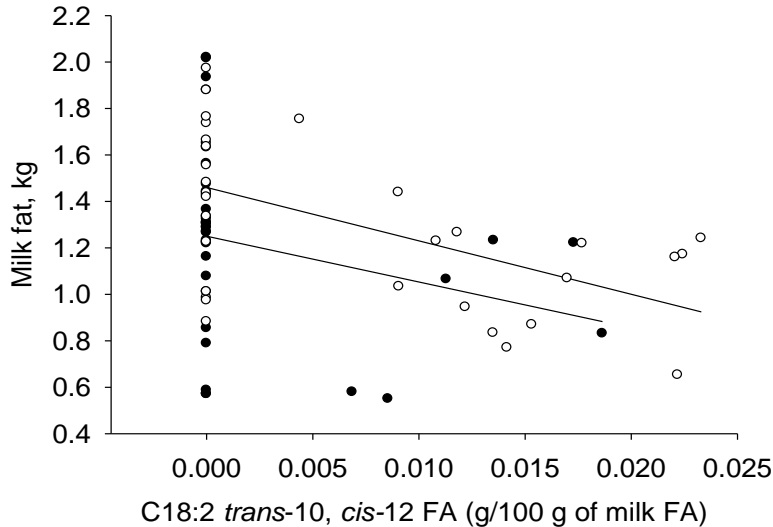


Figure 3.4. Simple regression of milk fat, kg by C18:2 *trans*-10, *cis*-12 FA for 24 Holstein cows fed three concentrations of MWDGS mixed with Keenan (KMF; ●) or Vertical auger mixer (VA; ○). Regression for cows fed Keenan diets $y = -19.73x + 1.25$ with a coefficient of determination of 0.07. Regression for cows fed Vertical diets $y = -23.01x + 1.46$ with a coefficient of variation of 0.30.

Change in milk fat percentage by change in C18:1 *trans*-10 FA

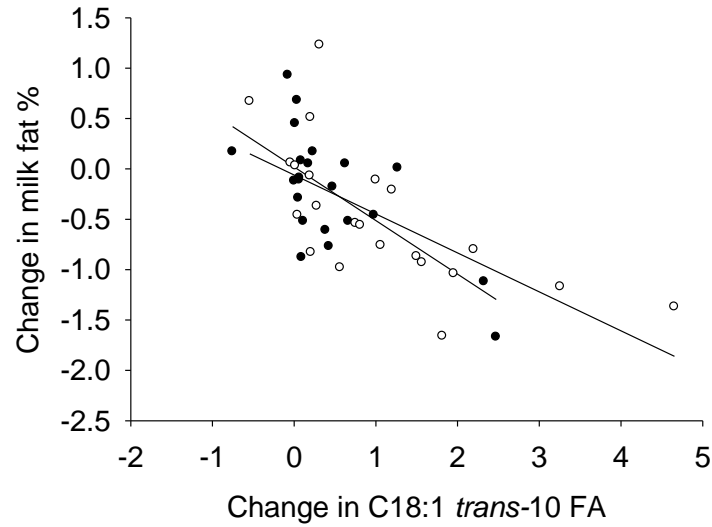


Figure 3.5. Simple regression of change in milk fat percentage by change C18:1 *trans*-10 FA using 10% MWDGS for each cow as a control. The change from 10% to 20% is represented by (●) and the change from 10 to 30% is represented by (○). Regression for the change of 10 to 20% MWDGS $y = 0.02x - 0.53$ with a coefficient of determination of 0.46. Regression for the change of 10 to 30% MWDGS $y = -0.06x - 0.39$ with a coefficient of variation of 0.46.

Change in milk fat kilograms by change in C18:1 *trans*-10 FA

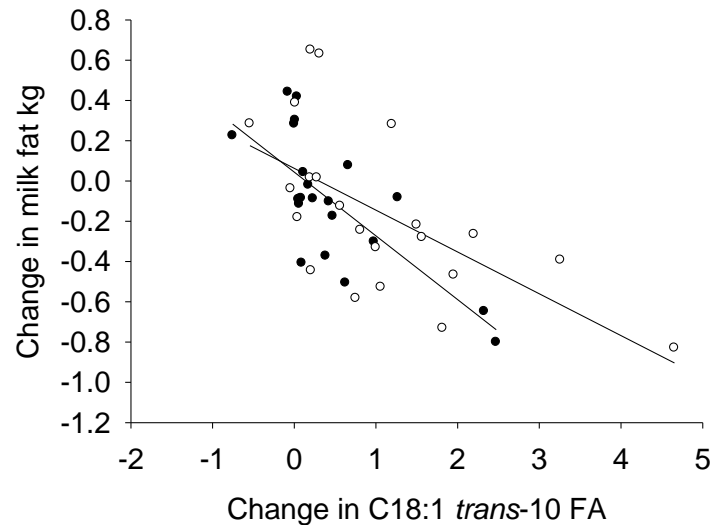


Figure 3.6. Simple regression of change in milk fat kilograms by change C18:1 *trans*-10 FA using 10% MWDGS for each cow as a control. The change from 10% to 20% is represented by (●) and the change from 10 to 30% is represented by (○). Regression for the change of 10 to 20% MWDGS $y = 0.04x - 0.32$ with a coefficient of determination of 0.54. Regression for the change of 10 to 30% MWDGS $y = 0.06x - 0.21$ with a coefficient of variation of 0.38.

Change in milk fat percentage by change in C18:2 *trans*-10, *cis*-12 FA

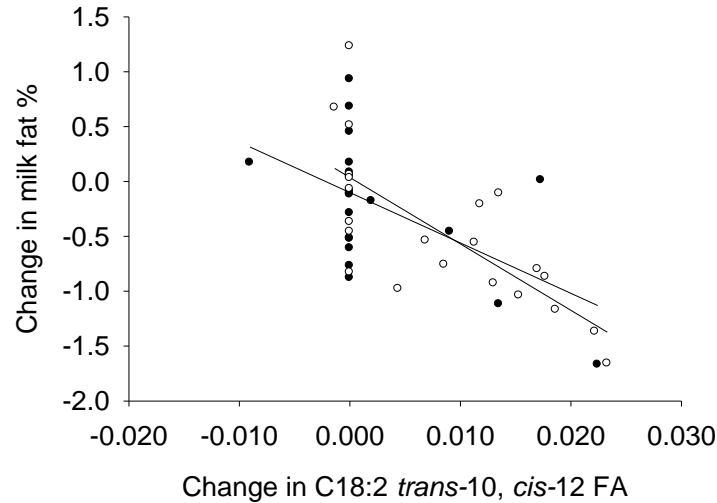


Figure 3.7. Simple regression of change in milk fat percentage by change C18:2 *trans*-10, *cis*-12 FA using 10% MWDGS for each cow as a control. The change from 10% to 20% is represented by (●) and the change from 10 to 30% is represented by (○). Regression for the change of 10 to 20% MWDGS $y = -0.10x - 45.91$ with a coefficient of determination of 0.29. Regression for the change of 10 to 30% MWDGS $y = -0.04x - 60.45$ with a coefficient of variation of 0.53.

Change in milk fat kilograms by change in C18:2 *trans*-10, *cis*-12 FA

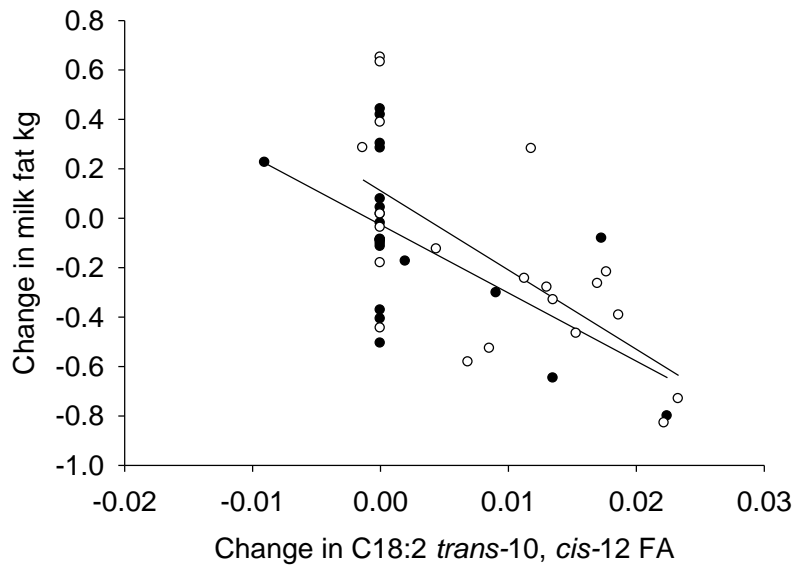


Figure 3.8. Simple regression of change in milk fat kilograms by change C18:2 *trans*-10, *cis*-12 FA using 10% MWDGS for each cow as a control. The change from 10% to 20% is represented by (●) and the change from 10 to 30% is represented by (○). Regression for the change of 10 to 20% MWDGS $y = -0.03x - 27.59$ with a coefficient of determination of 0.35. Regression for the change of 10 to 30% MWDGS $y = 0.11x - 32.08$ with a coefficient of variation of 0.44.

Keenan and Vertical Mixer Wagon 10% MWDGS

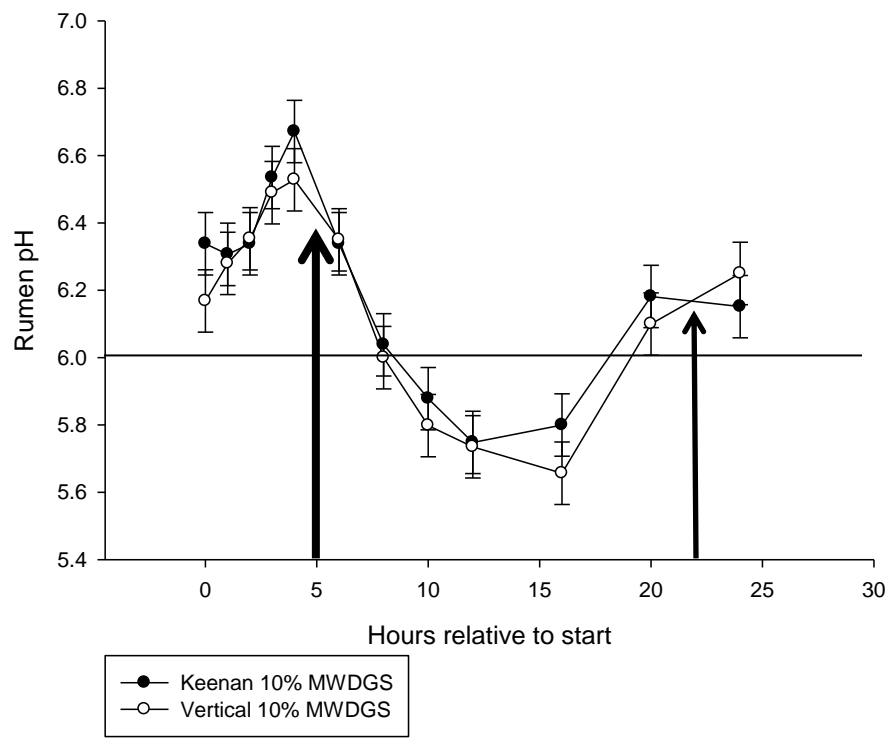


Figure 3.9. Rumen pH for lactating cows fed diets containing 10% MWDGS mixed with Keenan (KMF; ●) or with vertical augur (VA; ○). Rumen pH under 6.0 by mixer and the heavy arrows represent feeding times.

Keenan and Vertical Mixers 20% MWDGS

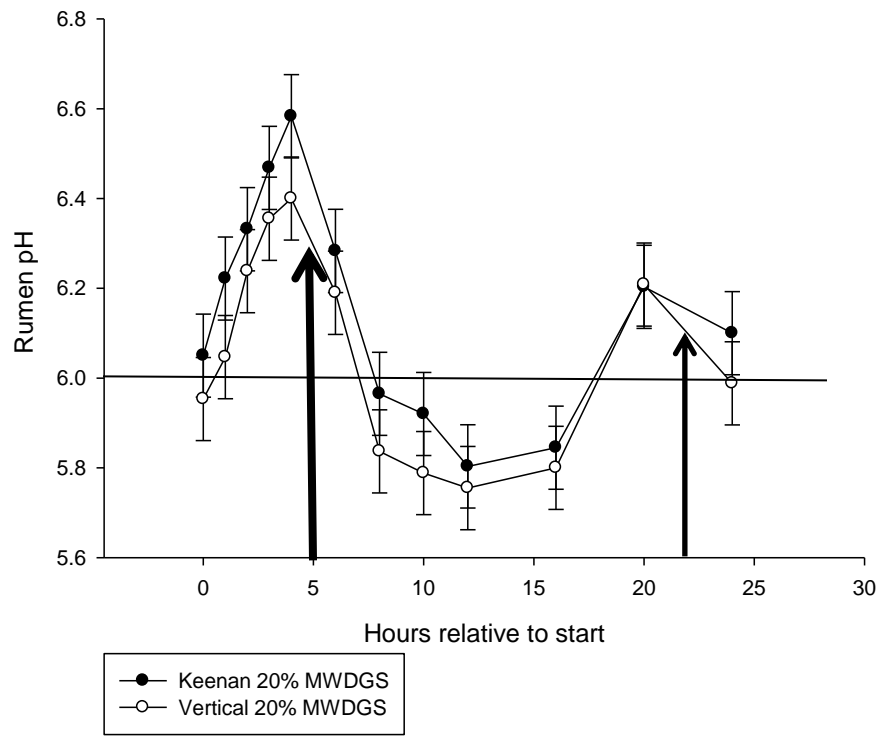


Figure 3.10. Rumen pH for lactating cows fed diets containing 20% MWDGS mixed with Keenan (KMF; ●) or with vertical auger (VA; ○). Rumen pH under 6.0 by mixer and the heavy arrows represent feeding times.

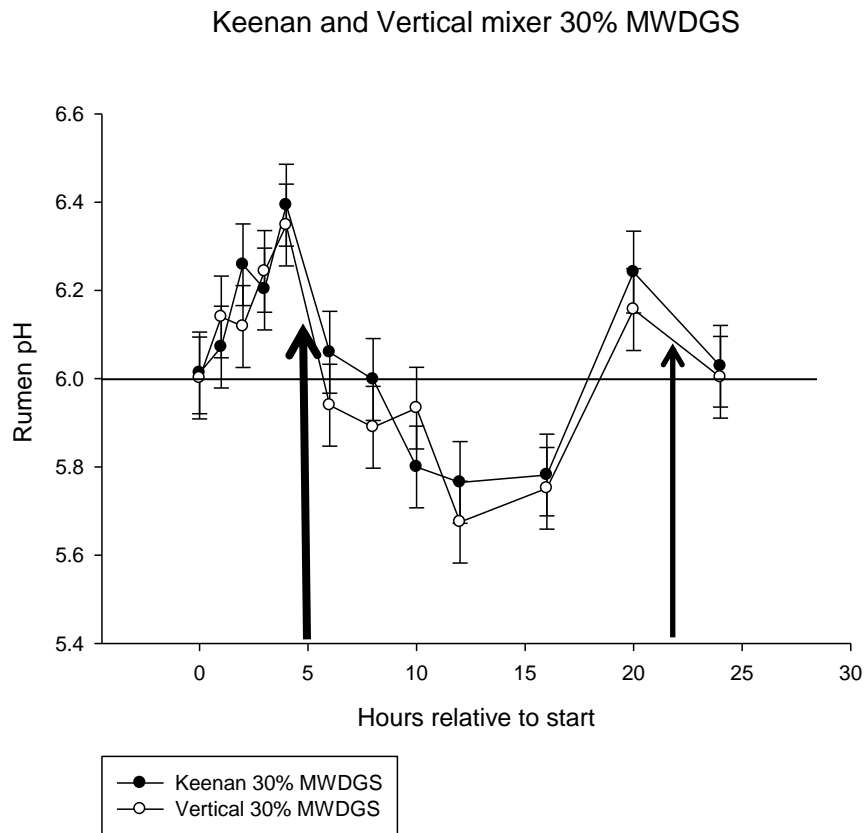


Figure 3.11. Rumen pH for lactating cows fed diets containing 30% MWDGS mixed with Keenan (KMF; ●) or with vertical auger (VA; ○). Rumen pH under 6.0 by mixer and the heavy arrows represent feeding times.

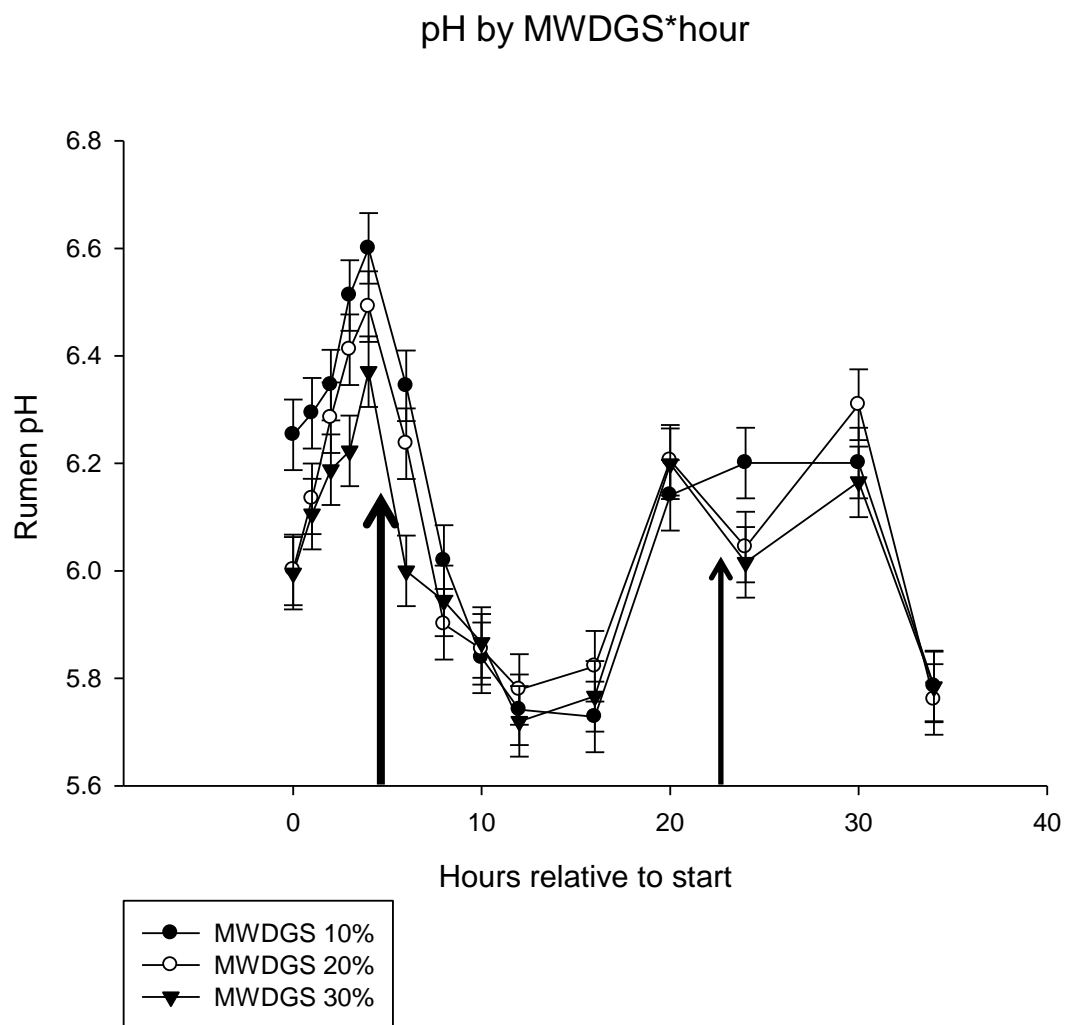


Figure 3.12. Rumen pH of 12 lactating Holsteins fed MWDGS at three concentrations (10% MWDGS; ●), (20% MWDGS; ○), and (30% MWDGS; ▼) for 14 time points taken over 34 hours. Heavy arrows represent feeding times. Notable effects in the model included the hour*MWDGS ($P = 0.01$) and hour ($P < 0.0001$).

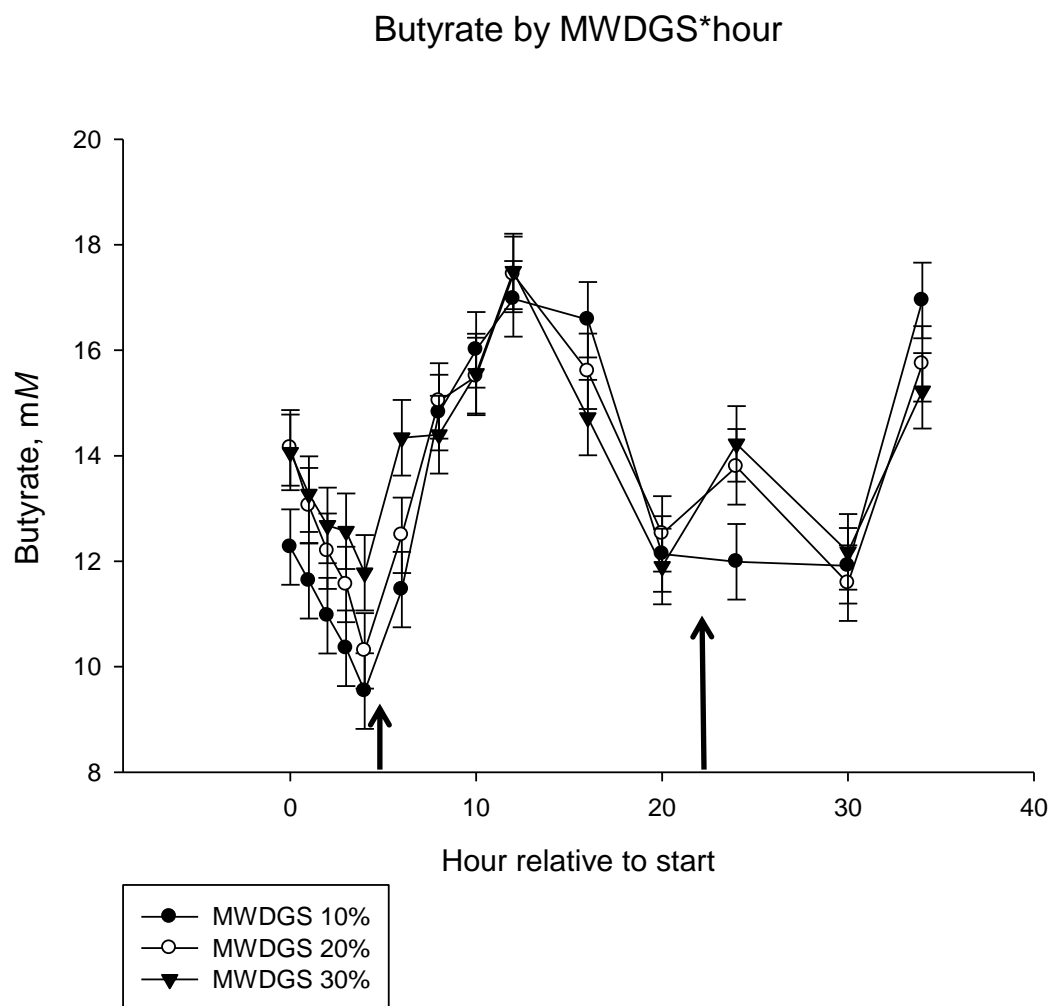


Figure 3.13. Rumen butyrate (mM) of 12 lactating Holsteins fed MWDGS at three concentrations (10% MWDGS; ●), (20% MWDGS; ○), and (30% MWDGS; ▼) for 14 time points taken over 34 hours. Heavy arrows indicate feeding times. Notable effects in the model included the hour*MWDGS ($P = 0.03$) and hour ($P < 0.0001$).

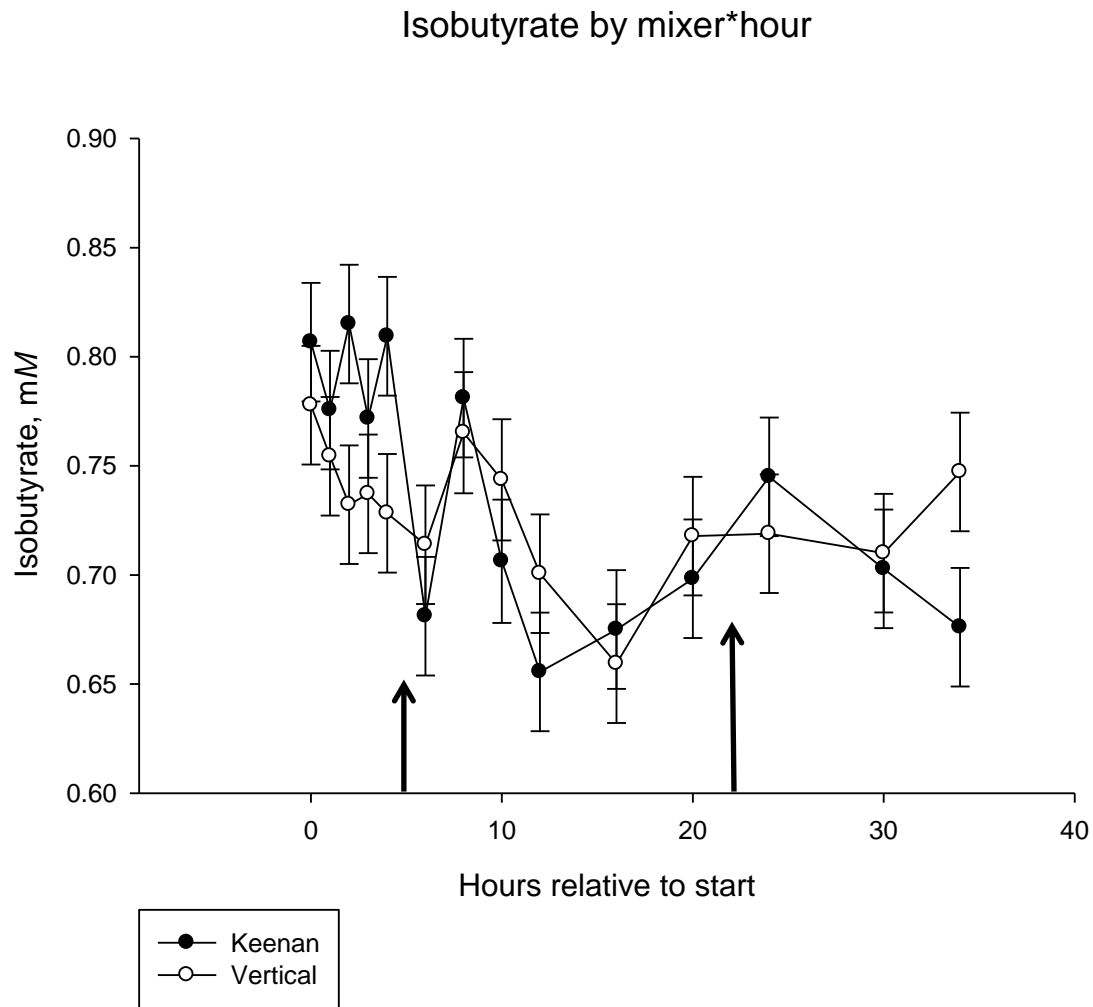


Figure 3.14. Rumen isobutyrate (mM) of 12 lactating Holsteins fed MWGDS at three concentrations mixed with two mixer wagons Keenan (KMF; ●) and vertical auger (VA; ○) for 14 time points taken over 34 hours. Heavy arrows represent feeding times. Notable effects in the model included the hour*mixer ($P = 0.02$), hour ($P < 0.0001$), and concentrations of MWGDS ($P < 0.0001$).

CHAPTER 4

EFFECTS OF MIXER TYPE ON UTILIZATION OF DIETS WITH MODIFIED WET DISTILLERS GRAINS WITH SOLUBLES FOR LACTATING JERSEY COWS

INTRODUCTION

Jersey cows have been shown to have different feeding behavior than Holsteins, both in that they eat smaller, more frequent meals (Senn et al., 1995), and that Jerseys spend a longer time ruminating each unit of fiber than other breeds (Welch et al., 1970). There are no specific recommendations in the NRC (2001) for feeding Jersey cattle because of lack of research on differences between Holsteins and Jerseys (Aikman et al., 2008). Differences in physiology between breeds may influence the amount of MWDGS that can be included in Jersey diets. In addition, mixer wagons may produce a diet that differs in physical presentation. Physical presentation of diets influences rumination, saliva production, and milk fat production (Shaver, 1990; Bal et al., 2000).

Lactating Jersey cattle producing milk high in protein and fat were used to determine if differences existed between the same diets mixed with different mixer wagons. The hypothesis of this study was that the Keenan mixer wagon would allow for 10% inclusion of MWDGS without the negative effect of lowered milk fat production. The objective of this study was to compare feed intake, milk production, milk composition and feed conversion efficiency (FCE) for Jersey dairy cows fed diets containing 10% MWDGS mixed either in Keenan or vertical augur mixers.

MATERIALS AND METHODS

Experimental Design and Management of Cows

All procedures were conducted under protocols approved by the University of Illinois Laboratory Animal Care Advisory Committee. Sixteen lactating Jersey cows were enrolled in the study. One cow did not complete the study due to health problems not associated with dietary treatments (cancer).

Cows were divided into two groups, one being fed with the Keenan Klassic 140 paddle-type mixer with knives (K; Keenan Systems, Borris, Ireland) and the other with the Kuhn-Knight VSL-142 vertical auger mixer (V; Kuhn North America, Inc., Brodhead, WI). Cows were blocked and enrolled in a switchback design being fed 10% MWDGS with periods that lasted 49 d. The diet was formulated to meet National Research Council (NRC, 2001) requirements for lactating cows. Both diets were mixed once a day and fed as a TMR in the morning, before 0900 h. Cows were housed in tie stalls throughout the experiment and were milked three times daily (0430, 1230, and 2030 h).

Data Collection, Sampling Procedures and Analytical Methods

Intake of each cow was measured daily during the trial. Samples of feed ingredients were obtained weekly and analyzed for DM content (AOAC, 1984). Weekly samples of individual ingredients were frozen at -20° C, composited by period of a concurrent trial (Chapter 3) and analyzed for contents of DM, CP, NDF, ADC, Ca, P, Mg and K (Dairy One, Ithaca, NY). Orts were given a score of 1 to 4 for visual moisture content daily, 1- same as TMR offered, 4- completely saturated with water. Orts were collected during weeks 4 and 7 of each period for DM content (AOAC, 1984).

Body weight and body condition scores (Wildman et al., 1982) were determined for each cow weekly. Three individuals assigned body condition scores independently and unaware of treatment throughout the experiment.

Four weeks prior to the trial starting, milk samples were taken for 6 consecutive milkings and analyzed for fat, protein, lactose, urea N, and somatic cell count (Dairy Lab Services, Dubuque, IA); these measurements were used for pre-trial covariate values. Milk weights were recorded daily and samples were obtained from 3 consecutive milkings during wk 4 and 7 of each period. Milkings were composited in proportion to milk yield at each sampling and were analyzed for fat, protein, lactose, urea N, and somatic cell count (Dairy Lab Services, Dubuque, IA).

Calculations and Estimates

Dry matter of orts were estimated by averaging the DM for all samples that were collected for each score and assigning that value to all corresponding DM for orts. Feed conversion efficiency was calculated as energy-corrected milk divided by DMI.

Statistical Analysis

Cows were blocked by parity, days in milk, and production (Table 4.1). Within each block half of the cows were assigned to each mixer and fed a diet containing 10% MWDGS. Milk production, milk composition, dry matter intake and feed conversion efficiency were analyzed as switchback design for two treatments using the MIXED procedures of SAS (Version 9.2, SAS Institute Inc., Cary, NC). The fixed model effect was mixer. The random model effect was cow within period. Data for milk, fat, and protein were adjusted by analysis of covariance

using the respective pretrial measurement. The linear model for this experiment was written as follows:

$$y_{ijkl} = \mu + M_i + P_j + C(P)_{jk} + \varepsilon_{ijk}$$

where y_{ijkl} represents observation_{ijkl}; μ represents the overall mean; M_i represents the fixed effect of the i th mixer; P_j represents the random effect of j th period; $C(P)_{jk}$ represents the random effect of the l th cow nested within the j th period. The residual term ε_{ijklmn} was assumed to be normally, independently, and identically distributed with variance σ_e^2 .

RESULTS

Milk production (Table 4.2) was similar for diets mixed with the Keenan and vertical mixer, averaging 23.5 kg/d and 23.7 kg/d, respectively ($P = 0.92$). Milk fat percentage and yield was similar between mixer wagons ($P = 0.75$ and $P = 0.51$, respectively). There were no differences in milk protein yield and percentage between treatments ($P = 0.72$ and $P = 0.84$, respectively). Therefore, energy-corrected milk (ECM) did not differ between treatments ($P = 0.92$). These data indicate that, at the inclusion rate of 10% for MWDGS, there were no detectable differences between the two mixer wagons used. Increasing concentrations of MWDGS may be needed to evaluate if differences exist between mixer wagons at higher levels of MWDGS.

DISCUSSION

Inclusion rates of 10% MWDGS represent total dietary fat concentrations of less than 5%, which is viewed as an acceptable level of fat for lactating dairy diets; however, because of the increased milk fat secretion from Jersey cattle there may be different critical levels of dietary fat for Jersey cattle to avoid milk fat depression. Both mixer wagons provided a diet with

physical characteristics that did not lead to MFD. This lack of difference is consistent with Holstein cows fed the same diet containing 10% MWDGS (Chapter 3). Future research with increasing amounts of MWDGS is needed with Jersey cattle to determine the optimal concentration of inclusion of MWDGS.

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Table 4.1. Characteristics of the 16 lactating Jersey cows used.

Item	Mean		Range		SD	
	Group 1 ¹	Group 2 ²	Group 1 ¹	Group 2 ²	Group 1 ¹	Group 2 ²
Parity	2.6	2.7	1 to 9	1 to 5	2.4	1.5
BW ³ , kg	455	455	375 to 594	375 to 548	65	51
BCS ⁴	2.93	2.90	2.22 to 3.83	2.23 to 3.47	0.44	0.41
DIM ⁵	112.5	113.25	9 to 197	1 to 188	67.7	68.3

¹ Treatments were diets mixed by Keenan followed by vertical mixer and back to Keenan.

² Treatments were diets mixed by vertical mixer followed by Keenan and back to vertical mixer.

³ Body weight taken as an average of each period.

⁴ Body condition score taken as an average of each period.

⁵ Days in milk at the beginning of the trial.

⁶ Days carried calf at the beginning of the trial.

Table 4.2 DMI, milk production, milk components, and feed conversion efficiency of Jersey cattle.

	Keenan ¹	Vertical ²	SEM ³	Mixer ⁴
DMI, kg	18.9	19.2	0.6	0.80
Milk, kg	23.5	23.7	1.3	0.92
ECM ⁵ , kg	26.4	26.2	1.5	0.92
Milk fat, %	4.90	4.84	0.15	0.75
Milk fat, kg	1.17	1.13	0.04	0.51
Milk protein, %	3.54	3.58	0.08	0.72
Milk protein, kg	0.83	0.84	0.05	0.84
FCE ⁶	1.52	1.51	0.08	0.92

¹ Diet containing 10% MWDGS mixed with Keenan mixer.

² Diet containing 10% MWDGS mixed with Kuhn-Knight vertical mixer.

³ Standard error of largest term.

⁴ *P* value associated with the fixed effect of mixer.

⁵ Energy corrected milk = [(12.82 × kg milk fat) + (7.13 × kg milk protein) + (0.323 × kg milk)] (Tyrell and Reid, 1965)

⁶ Feed conversion efficiency calculated as ECM/DMI.

CHAPTER 5

EFFECTS OF MIXER TYPE ON UTILIZATION OF DIETS WITH 17% MODIFIED WET DISTILLERS GRAINS WITH SOLUBLES FOR LACTATING HOLSTEIN COWS

INTRODUCTION

Research conducted on distillers products has recommended that inclusion of up to 20% of diet DM can be achieved for lactating dairy cows (Nichols et al., 1998; Anderson et al., 2006). Feeding more than this amount has the potential for over-feeding protein and phosphorus. Although research has included up to 20% distillers without the negative effects of milk fat depression, the industry recommendations for DGS still remain at 5 to 10% of the diet DM. Reports from the field at higher levels of inclusion indicate lowered milk fat production.

Several studies indicate that replacing corn silage with alfalfa based silages reduces the risk of milk fat depression when supplementing with a fat source (Onetti et al., 2002; Krause and Combs, 2003). However, the yield, availability, and price of corn silage make it a more appealing option than alfalfa silage to Midwestern dairy producers. There is a need to reduce milk fat depression while still utilizing the less expensive and nutritionally dense feedstuff MWDGS. Use of Keenan MechFiber TMR mixers has been reported to lead to more consistent feed mixtures and therefore a more consistent rumen environment. Potentially, using Keenan MechFiber would allow for greater inclusion of MWDGS and still allow for the high rates of corn silage that is typical in the Corn Belt.

MATERIALS AND METHODS

Experimental Design and Management of Cows

All procedures were conducted under protocols approved by the University of Illinois Laboratory Animal Care Advisory Committee. Sixty-five lactating Holstein cows were enrolled in the study. Four cows were sold before the completion of the trial.

Cows were divided into two groups, one being fed with the Keenan Klassic 140 paddle-type mixer with knives (K; Keenan Systems, Borris, Ireland) and the other with the Kuhn-Knight VSL-142 vertical auger mixer (V; Kuhn North America, Inc., Brodhead, WI). Cows were blocked by production, lactation number, and DIM then enrolled in a crossover design being fed 17% MWDGS for periods that lasted 70 d. The diet was formulated to meet National Research Council (NRC, 2001) requirements for lactating cows. Three diets of 10, 20, and 30% MWDGS were mixed with each mixer and 50%, 30%, and 20% of the total diet from each amount of MWDGS was blended to achieve a final diet of 17% MWDGS. The diets were delivered starting at 0700 h and the last batch was delivered by 1100 h. The order of diet delivery was 10, 20 and 30% MWDGS with up to 2 h between loads being delivered. Cows were housed in lots with sand-bedded stalls throughout the experiment and were milked three times daily (0500, 1300, and 2100 h).

Data Collection, Sampling Procedures, and Analytical Methods

Four weeks prior to the trial beginning, milk samples were taken from 6 consecutive milkings and analyzed for fat, protein, lactose, urea N, and somatic cell count (Dairy Lab Services, Dubuque, IA). Milk weights were recorded daily and samples were obtained from 3 consecutive milkings during wk 5 and 10 of each period. Milkings were composited in

proportion to milk yield at each sampling and were analyzed for fat, protein, lactose, urea N, and somatic cell count (Dairy Lab Services, Dubuque, IA).

Maximum, minimum, and average ambient temperature was recorded for the duration of the trial (National Weather Service, Silver Springs, MD). Temperatures were averaged by week throughout the trial.

Statistical Analysis

Cows were blocked by parity, days in milk, and production. Within each block half of the cows were assigned to each mixer type and fed a diet containing 17% MWDGS. Milk production and milk composition were analyzed using a crossover design for two treatments using the MIXED procedures of SAS (Version 9.2, SAS Institute Inc., Cary, NC). Fixed model effects were mixer, period, week, and their interactions. Random model effect was cow within period. Data for milk, fat, and protein were adjusted by analysis of covariance using the respective pretrial measurement as well as being adjusted for days carried calf. The linear model for this experiment is written as follows:

$$y_{ijklm} = \mu + M_i + P_j + W_k + MP_{ij} + MW_{ik} + MPW_{ijk} + C(P)_{lj} + \varepsilon_{ijklm}$$

where y_{ijklm} represents observation $ijklm$; μ represents the overall mean; M_i represents the fixed effect of the i th mixer; P_j represents the fixed effect of j th period; W_k represents the fixed effect of the k th week; MP_{ij} represents the interaction between mixer and period; MW_{ik} represents the interaction between mixer and week; MPW_{ijk} represents the interaction between mixer, period, and week; $C(P)_{lj}$ represents the random effect of the l th cow nested within the j th period. The residual term ε_{ijklm} was assumed to be normally, independently, and identically distributed with variance = σ_e^2 .

RESULTS AND DISCUSSION

The mixer effect on milk production (Table 5.1) between the two lots was not significantly different ($P = 0.41$). However, the period ($P = 0.001$) and week ($P < 0.0001$) effects were significant with milk production decreasing as DIM increased as expected. This decrease would be explained by the normal lactation curve because as DIM increase post-peak we would expect decreased milk production. The period \times mixer effect was significant, which is illustrated in Figure 5.1.

Milk fat percentage decreased dramatically during period 1 and was maintained during period 2. The vertical mixer had a larger decrease in milk fat than the Keenan during period 1. Once the low milk fat was reached during wk 10 of the trial, neither of the mixers was able to recover milk fat. The mixer effect was not significant ($P = 0.27$); whereas, the period and week effects were both significant ($P = 0.01$ and $P < 0.0001$, respectively). The three-way interaction among mixer, period, and week was significant at $P = 0.003$ (Figure 5.2).

Milk protein percentage remained constant for the cows in lot 1 while there was more variation in the cows in lot 3. The mixer and period effects were not significant for milk protein percent ($P = 0.97$ and $P = 0.59$, respectively). The effect of week was significant ($P = 0.004$) and the interaction among mixer, period, and week was significant ($P = 0.001$; Figure 5.3).

The reduction in milk fat as 17% MWDGS was included in the diet agrees with the findings of Leonardi et al. (2005) who reported that milk fat was decreased when the diet contained 15% DGS. However, Anderson et al. (2006) and Kleinschmit et al. (2006) both fed 20% DGS without decreasing milk fat for mid-lactation cows. We are unaware of any research trials with DGS that reported milk fat secretion being affected by time. Mpapho et al. (2006) fed 15% DGS throughout the entire lactation while cows maintained milk fat of 4.07%. Other

research trials supplementing fatty acids indicated that after as little as 5 days there was decreased milk fat secretion (Giesy et al., 2002). In longer-term studies supplementing CLA, the reduction in milk fat persisted as long as the CLA supplementation continued (Perfield et al., 2002); therefore, because the same level of distillers was fed for the entire trial, we would expect the same amount of CLA reaching the mammary gland and continuing to inhibit milk production. Our results were impacted by time and are not consistent with infusion or dietary fatty acid trials. Heat stress often leads to or aggravates MFD; in our study the lowest milk fat contents were reached as summer temperatures increased (Figure 5.4). Heat stress conditions also may have made it more difficult for any potential differences due to mixer to be displayed.

CONCLUSIONS

Milk production decreased most likely because of the increase in DIM as the trial progressed following a normal lactation curve. Milk fat production was reduced by week 10, which is longer than is typical for higher fat diets that cause a reduction in milk fat. Although there were no mixer effects, there were significant interactions with time and mixer. The Keenan mixer wagon performed better in the first period and there was little change in milk fat after wk 10 of the trial; both mixers were able to maintain the level of milk fat. In addition to heat stress effects, another possible explanation for the differences in periods is that the carryover effects of milk fat depression may be longer than we were able to measure. Changes in the rumen microbial population should be monitored in the future to determine the extent of changes in the rumen.

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Table 5.1. Main effects of milk production and milk composition of 65 Holstein cows fed 17% MWDGS.

Item	Lot 1				Lot 3					<i>P</i> -values ²		
Period	P1	P1	P2	P2	P1	P1	P2	P2				
Week	W5	W10	W5	W10	W5	W10	W5	W10				
Mixer	Keenan	Keenan	Vertical	Vertical	Vertical	Vertical	Keenan	Keenan	SEM ¹	Mixer	Period	Week
Milk, kg	37.8	34.9	34.0	33.9	37.6	37.3	34.0	31.4	1.08	0.41*	0.001	<0.0001
ECM ³ , kg	35.4	31.0	30.5	27.9	35.7	32.0	31.4	28.7	0.98	0.90	<0.0001	<0.0001
Fat, %	3.75	3.44	3.49	3.37	3.91	3.14	3.15	3.07	0.15	0.27‡	0.01	<0.0001
Fat, kg	1.38	1.14	1.13	1.02	1.44	1.13	1.12	1.00	0.06	0.57‡	<0.0001	<0.0001
Protein, %	3.13	3.16	3.16	3.16	3.07	3.20	3.15	3.15	0.03	0.97‡	0.59	0.004
Protein, kg	1.17	1.09	1.06	0.98	1.14	1.18	1.14	1.06	0.03	0.36*•‡	0.001	0.0004

¹ Largest standard errors among treatments.

² Mixer is the *P* value associated with the main effect of mixer; Period is the *P* value associated with the main effect of period of the trial; Week is the *P* value associated with week of the trial.

³ Energy corrected milk = [(12.82 × kg milk fat) + (7.13 × kg of milk protein) + (0.323 × kg milk)] (Tyrrell and Reid, 1965).

* Mixer x period was significant (*P* < 0.05).

• Mixer x week was significant (*P* < 0.05).

‡ Mixer x period x week was significant (*P* < 0.05).

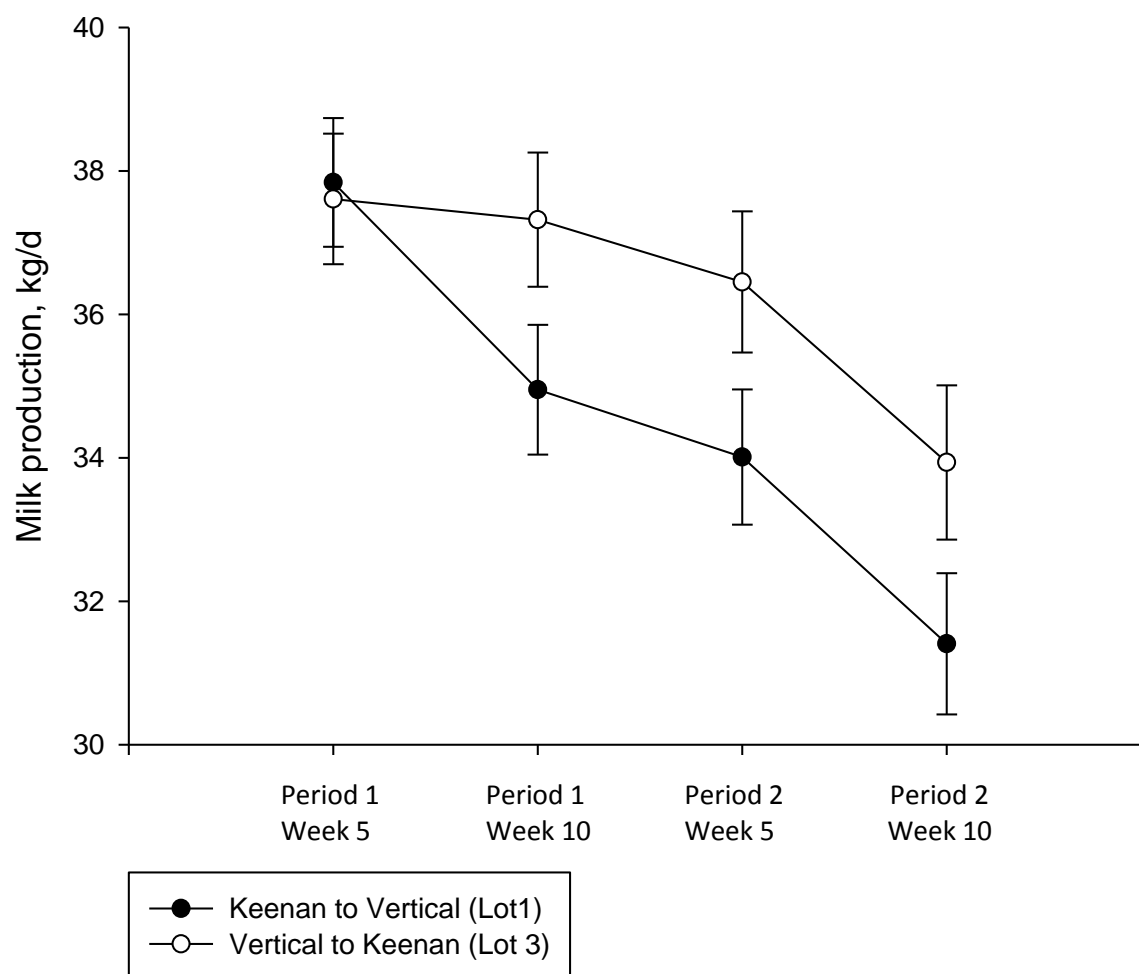


Figure 5.1. Milk production by period and sequence for lot cows fed 17% MWDGS. Notable effects in the model included the period ($P = 0.001$), week ($P < 0.0001$), and period \times mixer ($P = 0.04$).

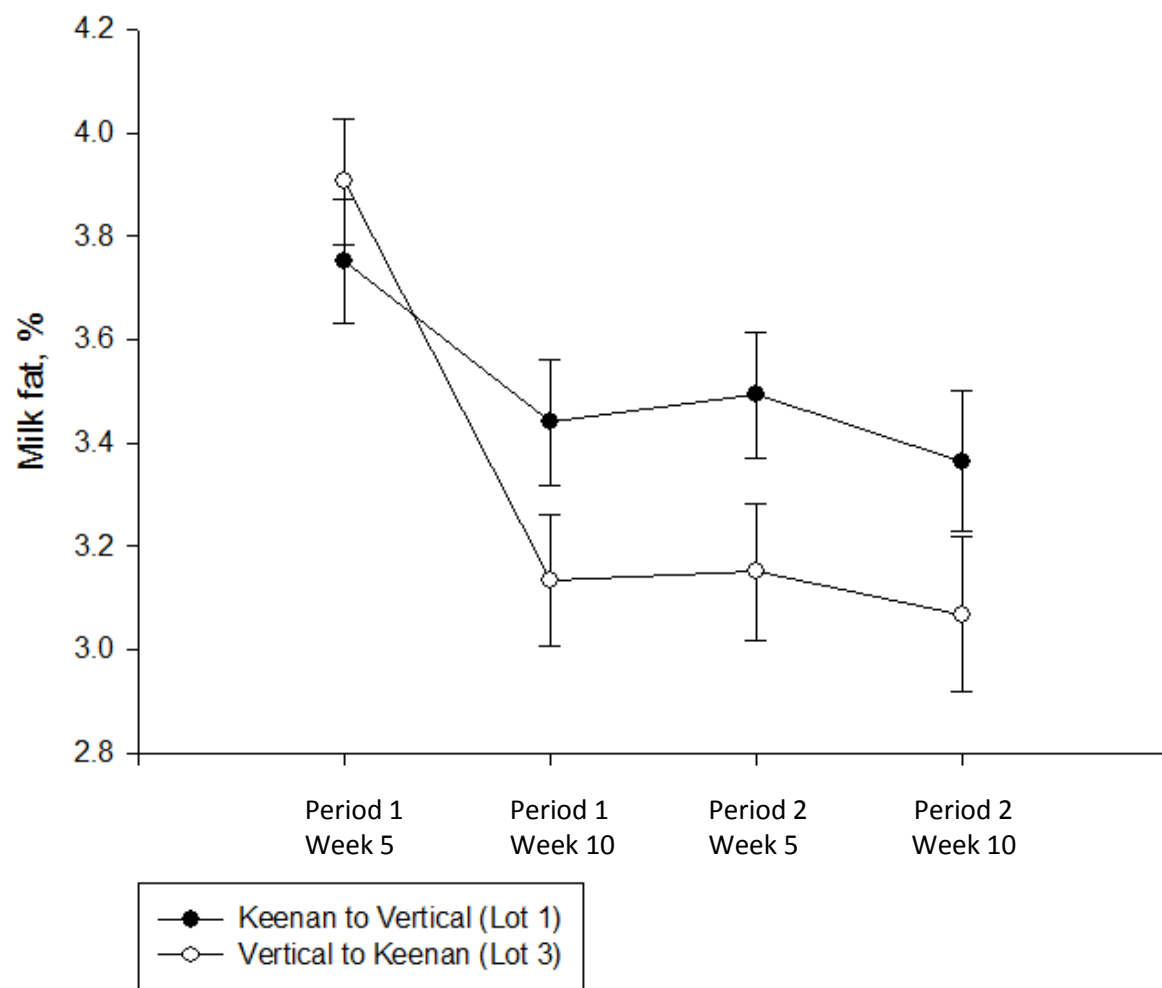


Figure 5.2. Milk fat percentage by period and sequence for lot cows fed 17% MWDGS. Notable effects in the model include the interactions of period \times mixer ($P = 0.08$), week \times mixer ($P = 0.07$) and period \times week \times mixer ($P = 0.003$).

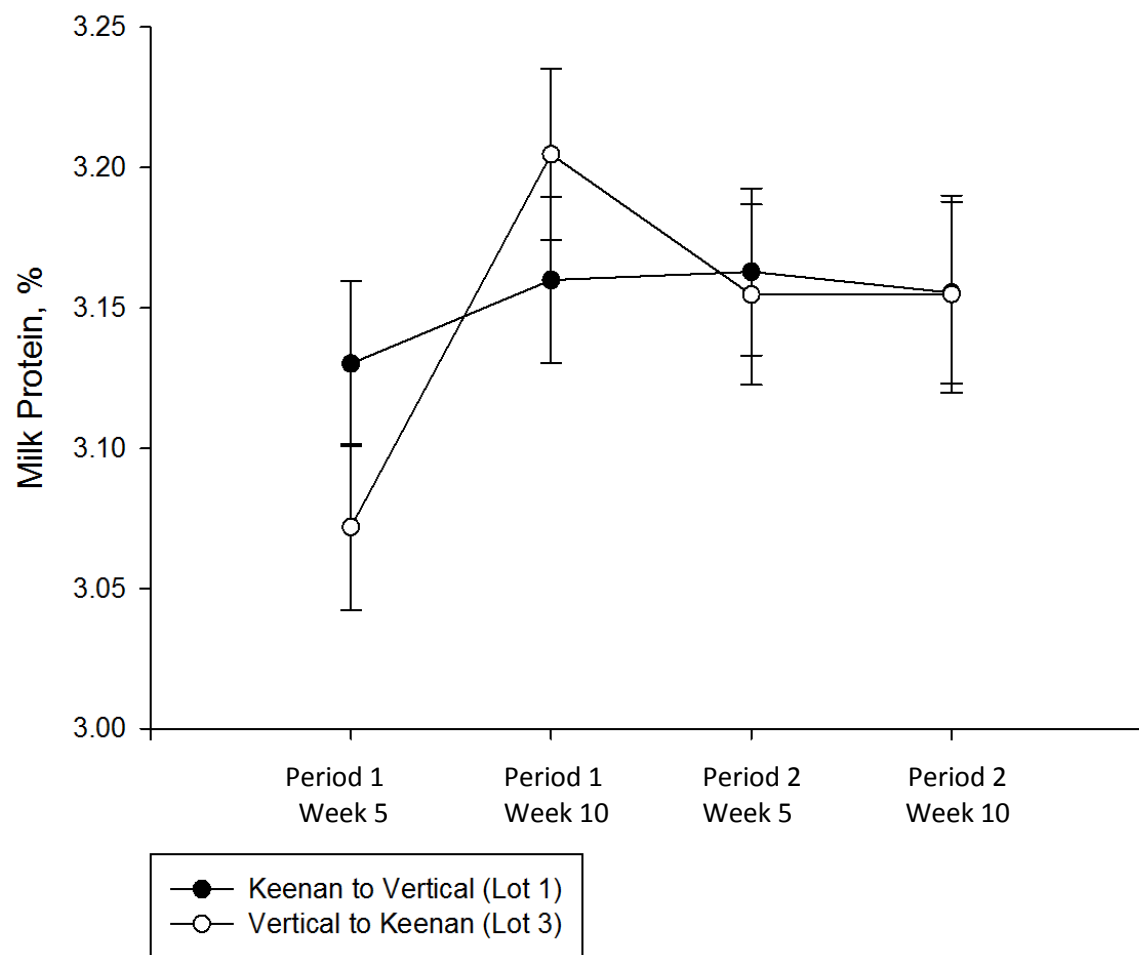


Figure 5.3. Protein percentage by period and sequence for lot cows fed 17% MWDGS. Notable effects in the model include the interactions of week \times mixer ($P = 0.08$) and period \times week \times mixer ($P = 0.001$).

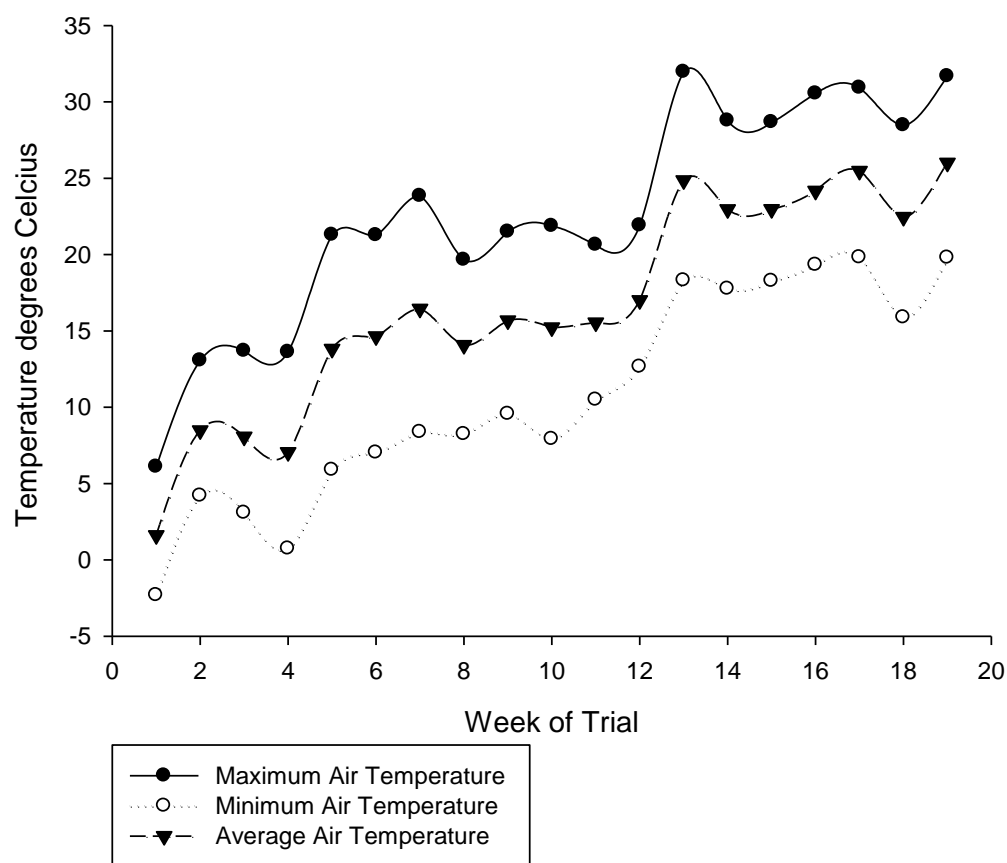


Figure 5.4 Air temperature by week of trial for cows fed 17% MWDGS. Maximum air temperature (●); minimum air temperature (○); average air temperature (▼). (National Weather Service, Silver Spring, MD)

CHAPTER 6

SUMMARY AND CONCLUSIONS

Consistent with our hypothesis, use of the Keenan TMR mixer allowed greater inclusion of MWDGS without causing severe milk fat depression. The differences in milk fat secretion can be partially explained by concentrations of CLA, *trans*-10, *cis*-12, days in milk, and by rumen pH. Physical differences in the presentation of the TMR lead to measurable differences in milk composition and dry matter intake. The rations mixed by the vertical mixer had a larger mean particle size and larger variation in distribution of particles, which allowed for more sorting than the same rations prepared with the Keenan mixer. Cows fed with the Keenan mixer had lower DMI, which could also be related to the ability of cows to sort with the vertical mixer. Lowered DMI contributed to greater FCE for the rations mixed with the Keenan.

The concentration of MWDGS influenced the amount of PUFA in the milk, which is to be expected because there is a correlation between dietary PUFA and milk PUFA. However, the interaction between mixer and concentration of MWDGS for specific *trans* isomers of 18:1 and conjugated linoleic acid (CLA) indicate that there were differences in the rumen environment among treatments. There was no statistical difference in mean rumen pH between mixer wagons or area under pH 6.0 among treatments, in contrast to what we would expect based on the differences in milk fatty acid composition. Concentrations of 30% MWDGS should be fed with caution based on reductions in milk fat, particularly when the vertical mixer is used.

The 10% MWDGS diet was not high enough to see any differences between mixer wagons as confirmed with the Jersey cattle. The concentration of MWDGS is within the range of acceptable feeding rates in the field and should be tested at higher levels to determine if differences exist between Jerseys and Holsteins and between mixer wagons.

Cows fed the 17% MWDGS diet showed evidence of milk fat depression by week 10 of the trial. These results agree with field reports that milk fat is reduced at concentrations of DGS at 15%. It is possible that the delivery of the MWDGS allowed for cows to eat different diets when they came to the bunk. The first TMR had 10% MWDGS while the last TMR delivered had 30% MWDGS; this variation in the diet could have contributed to the variation in the rumen. Changes in the rumen can lead to alternative biohydrogenation pathways causing milk fat depression. Samples to determine rumen microbial populations might help confirm why changes in milk fat production occurred.

Appendix A. Pre-trial means and unadjusted (raw) means for production and intake variables for 24 lactating Holsteins in Study 1.

Variable	Treatments					
	K10	K20	K30	V10	V20	V30
Pre-trial milk, kg/d	38.58	38.58	38.58	40.57	40.57	40.57
Milk, kg/d	34.92	34.69	34.98	39.16	38.83	41.86
Pre-trial ECM, kg/d	35.34	35.34	35.34	37.86	37.86	37.86
ECM, kg/d	32.12	30.89	31.45	36.03	34.23	34.08
Pre-trial fat, %	3.66	3.66	3.66	3.84	3.84	3.84
Fat, %	3.53	3.43	3.38	3.70	3.33	2.84
Pre-trial protein, %	2.83	2.83	2.83	2.80	2.80	2.80
Protein, %	3.09	3.07	3.00	2.95	2.95	2.86
Pre-trial lactose, %	4.83	4.83	4.83	4.83	4.83	4.83
Lactose, %	4.74	4.62	4.56	4.68	4.69	4.68
Pre-trial fat, kg/d	1.42	1.42	1.42	1.56	1.56	1.56
Fat, kg/d	1.24	1.16	1.22	1.43	1.29	1.18
Pre-trial protein, kg/d	1.09	1.09	1.09	1.13	1.13	1.13
Protein, kg/d	1.08	1.05	1.02	1.14	1.14	1.20
Pre-trial lactose, kg/d	1.87	1.87	1.87	1.96	1.96	1.96
Lactose, kg/d	1.67	1.63	1.60	1.88	1.82	1.96
Pre-trial DMI, kg/d	25.23	25.23	25.23	25.49	25.49	25.49
DMI, kg/d	24.27	23.02	24.77	24.97	25.93	26.12
Pre-trial FCE, kg/kg	1.41	1.41	1.41	1.49	1.49	1.49
FCE, kg/kg	1.39	1.37	1.32	1.43	1.32	1.30

Appendix B. Summary of results from feeding 17% MWDGS to two pens of lactating dairy cattle for 20 wk.

Item	Lot 1				Lot 3			
	Week 5	Week 10	Week 15	Week 20	Week 5	Week 10	Week 15	Week 20
Mixer	Keenan	Keenan	Vertical	Vertical	Vertical	Vertical	Keenan	Keenan
Cows in lot	60	60	60	66	96	94	94	101
No. colored breed	2	1	0	0	8	11	17	18
No. lactation 1	18	18	17	18	44	47	45	45
Lactation no.	2.4±1.4	2.4±1.4	2.4±1.4	2.3±0.9	1.9±1.2	1.9±1.1	1.8±1.0	1.9±1.0
DIM	183±128	191±120	156±92	177±93	169±137	163±131	130±81	146±83
Milk, kg	32.3±11.2	37.2±12.5	40.7±12.3	39.3±9.8	30.7±8.0	38.1±9.3	39.2±9.0	37.6±9.2
Pre-milk, kg	35.8±9.9	36.3±9.2	35.7±9.1	37.1±9.0	34.0±8.8	35.2±9.1	34.3±9.9	33.6±10.7
Fat, %	3.60±1.01	3.45±0.78	3.23±1.10	3.42±1.17	4.00±0.76	3.60±0.71	3.37±0.88	3.35±0.99
Pre-fat, %	3.82±0.76	3.85±0.80	3.89±0.83	4.00±0.82	3.87±0.90	3.79±0.95	4.19±1.03	4.12±1.09
Protein, %	3.10±0.34	3.07±0.34	3.10±0.30	3.08±0.36	3.10±0.38	3.07±0.31	3.16±0.33	3.13±0.30
Pre-protein, %	3.08±0.32	3.03±0.28	3.01±0.29	3.04±0.29	3.09±0.39	3.12±0.39	3.17±0.44	3.24±0.48
DMI, kg	26.2	26.4	27.8	22.4	23.1	23.4	25.7	20.8
FCE, kg/kg	1.24	1.41	1.46	1.76	1.33	1.63	1.52	1.80

Appendix C. Nutrient specifications of formulated diet using NRC.

Item	10% MWDGS	20% MWDGS	30% MWDGS
DM, %	50.99	49.37	47.85
NEL, Mcal/kg	1.68	1.68	1.68
CP, % of DM	17.43	17.47	17.52
NRC Metabolizable protein, g/d	2718	2760	2802
Soluble protein, % of DM	4.93	4.69	4.46
Soluble protein, % of CP	28.28	26.85	25.46
ADF, % of DM	22.09	21.79	21.49
NDF, % of DM	34.40	35.27	36.13
NDF from forage, % of DM	22.76	22.76	22.76
Fat, % of DM	4.16	4.30	4.45
Ash, % of DM	7.32	7.75	8.19
NFC, % of DM	36.69	35.20	33.72
Ca, % of DM	0.73	0.73	0.73
P, % of DM	0.41	0.42	0.43
Mg, % of DM	0.29	0.29	0.29
K, % of DM	1.43	1.40	1.38
S, % of DM	0.23	0.28	0.32
Na, % of DM	0.34	0.42	0.49
Cl, % of DM	0.31	0.33	0.35
Fe, ppm	300	276	253
Zn, ppm	82	82	82
Cu, ppm	16	16	15
Mn, ppm	77	75	74
Co, ppm	0.2	0.2	0.2
I, ppm	0.5	0.4	0.4
Se, ppm	0.38	0.41	0.44
DCAD (mequiv/kg)	279	273	267

Appendix D. Nutrient analysis of 6 (composited by period) samples of MWDGS.

Nutrient	Period					
	1	2	3	4	5	6
Dry Matter, % of as fed	48.4	49	48.6	45.1	47	48.3
Crude Protein, % of DM	27.9	26.3	26.7	27.2	28.3	27.7
Adjusted Crude Protein, % of DM	27.3	24.9	20.3	21.9	23.4	23.2
Soluble Protein, % of CP	21	25	28	22	24	31
ADF, % of DM	11.1	14.2	19.7	18.5	19.2	17.5
NDF, % of DM	29.6	36.5	34.7	34.6	34	34.4
Lignin, % of DM	3.4	2.7	4.8	4.8	5.2	4.8
NFC, % of DM	31.9	28.2	30.8	27.5	26.1	27.5
Crude fat, % of DM	11.3	10.7	9.4	11.9	12.3	11.5
Ash, % of DM	5.28	5.54	5.4	5.66	6.12	5.76
Calcium, % of DM	0.13	0.13	0.1	0.14	0.12	0.13
Phosphorus, % of DM	0.86	0.71	0.68	0.7	0.82	0.81
Magnesium, % of DM	0.30	0.30	0.28	0.28	0.33	0.33
Potassium, % of DM	1.07	1.30	1.05	1.06	1.22	1.20
Sulfur, % of DM	0.73	0.65	0.58	0.60	0.71	0.71
Sodium, % of DM	0.37	0.40	0.35	0.37	0.49	0.42
Iron, ppm of DM	166	144	144	154	176	234
Zinc, ppm of DM	49	46	45	46	51	52
Copper, ppm of DM	6	6	5	5	6	6
Manganese, ppm of DM	15	16	15	15	17	17
Molybdenum, ppm of DM	1.1	1.4	1.2	1	1.2	1.2

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Research Experience:

2009-present Graduate Research Fellow, Ruminant Nutrition
University of Illinois Urbana-Champaign: (Advisor Dr. James Drackley)

- Organized, planned and executed three concurrent research trials with lactating dairy cattle fed modified wet distillers grains with solubles
- Responsible for coordinating farm staff, undergraduate workers and graduate students
- Prepared samples for analysis
- Analyzed data using SAS[®] and presented results to companies that funded research

Teaching Experience:

Fall 2008 Adjunct Professor

- Dairy Herd Management at Morrisville State College
 - Developed curriculum
 - Instructed class
 - Organized guest speakers and labs
 - Facilitated discussion about current trends in the dairy industry

Fall 2006 Teaching Assistant

- Dairy Cattle Principles at Cornell University
 - Instructed labs
 - Graded papers and exams

Spring 2005 Teaching Assistant

- Dairy Cattle Evaluation at S.U.N.Y. Cobleskill
 - Presented lectures
 - Listened to and critiqued oral reasons

Additional Work Experience:

2007 – 2008 Assistant Herdsman

- Strathdale Dairy Farm and Meon Valley Dairy
 - Milked and monitored health of 1000 and 600 cow dairy farms
 - Assisted with calvings and matings
 - Managed youngstock program

Summer 2008 Marketing and Technical Dairy Intern

- Monsanto
 - Developed dairy tours program
 - Assisted with marketing strategy for World Dairy Exposition
 - Executed database cleanup

Summer 2006 Genetic Management Intern

- ABS Global
 - Set up interviews with dairy producers
 - Edited, produced and marketed a promotional video
 - Input data into computer mating program

1999 – 2007 Farm Worker

- Will-Cara Dairy Farm
 - Milked, fed and cared for 100 head of dairy cattle on family's registered Holstein farm
 - Assisted with harvest of crops

Relevant coursework:

- Dairy Science Techniques
- Intro to Dairy Nutrition
- Intro to Dairy Cattle Management
- Dairy Record Management
- Dairy Cattle Management
- Animal Health
- Bovine Hoof Care & Maintenance
- Forages and Seed Crops
- Genetics
- Animal Nutrition
- Dairy Cattle Principles
- Dairy Herd Management
- Junior Dairy Fellows

- Dairy Cattle Nutrition
- Advanced Dairy Nutrition
- Whole Farm Nutrient Management
- Advanced Ruminant Nutrition
- Techniques in Animal Nutrition
- Evidence Based Decision Making on Dairy Farms
- Organic Chemistry
- Applied Statistical Methods
- Biochemistry
- Protein and Energy
- Experimental Design

Professional Organizations:

- American Dairy Science Association

Honors and Awards:

- Third place Graduate Student Oral Competition, ADSA, 2011
- Jonathan Baldwin Turner Fellowship, University of Illinois, 2009-2011
- Cornell Tradition Fellowship, Cornell University, 2005-2007
- American FFA Degree, 2005
- Outstanding Senior in Animal Science, S.U.N.Y. Cobleskill, 2005