# APPLICATIONS OF NOVEL TECHNIQUES FOR APPLIED DAIRY NUTRITION

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## Dedication

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## Introduction

To increase efficiency and precision of dairy cattle nutrition, novel feeding and feed ingredient technologies need to be continually implemented on farm in order to reduce operation **c**osts and meet nutrient demands for greater milk production. To follow the global tendency for intensification, US dairy farms have decreased in number while increasing herd size and productivity (Alvarez et al., 2007). Although dairy operations have traditionally been heavily dependent upon labor, technological advances have moved the industry toward greater reliance on managerial and technological innovations (Khanal et al., 2010). Short (2004) stressed the role of technology in dairy industry evolution, stating it has "changed the way milk is produced," with firm growth and specialization being made possible by technology. Technologies increase the opportunities for moving the dairy industry forward through improving precision of on farm management and helping dairy farmers make more informed decisions. On farm technologies can significantly reduce traditional reliance on intensive labor, whereas increasing output per worker several-fold. Computerized on farm record management systems, automated monitoring of individual animal performance and application of stored information into decision making tools has tremendously increased the capability for dairy herd management. The possibility of measuring, recording, manipulating and evaluating day-to-day animals' activities, production, reproduction, and health status are tools that profoundly improve farm management. Furthermore, it enables farmers to manage the herd as effectively as possible, resulting in more profitable livestock enterprises. For instance,

according to Khanal et al. (2010) the use of on farm computerized feeding system has increased from 21.6% to 28.3% from 2000 to 2005. Computerized feeding systems for cows have become an important tool to control the amount of feed that is delivered to cows. Moreover, it allows farmers to ensure that cows are receiving planed amounts of daily nutrients to sustain milk production. As profit margins for milk production shrink, farmers have become more conscious of the importance of maximizing feeding efficiency. Farmers are trying to feed more accurate rations in an attempt to avoid underfeeding or overfeeding nutrients and reduce feed costs. The importance of minimizing daily variation in the rations has become clear, in order to constantly maintain the balance of nutrients that are offered to cows, for better rumen health, more consistent milk production, resulting in greater profit margins. On farm application of precision feeding is currently in its infancy. Exploratory research is needed to better understand the constraints of on farm applications of precision feeding technology.

Replacement of traditional feedstuffs with by-products has also become a standard practice in the dairy industry to reduce feed costs and increase profitability. Key attributes of successful by-products include consideration of price, consistency, availability, and nutrient profile compared to the traditional ingredients. Use of by-products to replace traditional ingredients in lactating dairy cattle diets has the potential to increase diet digestibility, alter nutrient profile, and decrease feed costs with a net effect of increased profit margins. Considerable attention had been given to the replacement of alternative protein feed sources in

animal diets as an attempt to economically formulate rations. For instance, distillers grains, corn gluten feed, and soy hulls are by-products commonly added to dairy cattle diets.

Alternative or unconventional protein sources will likely play an important role in animal diets in the future to meet the world protein demand by providing nutrients that do not directly compete with human food sources. Aquatic plants such as duckweed are attractive as feedstuffs and contain sufficient amounts of nutrients to be considered as potential alternatives to traditional livestock feed (Linn et. al, 1975). Comparatively, according to Boyd (1968) aquatic plants have higher crude protein and mineral content compared with some conventional forage crops making this alternative nutrient source an intriguing ingredient to replace traditional feedstuffs. More research is needed to understand the effects of feeding aquatic plants to dairy cattle on feed intake, diet digestibility, milk production, and milk components.

Another standard practice to decrease feed costs is the use of sugar by-products as a source of energy in dairy cattle diets. Supplemental sugar can replace some of the dietary starch, resulting in reduced risk for ruminal acidosis while increasing the energy density of the diet. Additionally, supplemental sugar may provide an additional benefit due to increased fiber digestibility and gains in feed efficiency. However, effects of varying sources of sugar on extent fiber digestibility have not yet been clearly defined.

## Chapter 1: Review of Literature

### a) Agriculture usage and history of near infrared reflectance spectroscopy

Near infrared reflectance spectroscopy (NIRS) has become a commonly used and increasingly analytical method in many fields during recent decades due to its characteristics as being fast, cost efficient, non-destructive to samples, and not involving use of hazardous chemicals (Liu et al., 2011). This technology can play a significant role in shaping the future of dairy farming. Near infrared reflectance spectroscopy technology has been used in diverse applications allowing farmers to watch animals closely and being more precise on daily decision. On dairy farms, NIRS has been used to measure somatic cell count (SCC), and so indicate cow's health and milk quality (Tsenkova et al., 2001). Milk SCC indicates the level of infection and resultant inflammation in the mammary gland of dairy cows. Near infrared spectroscopy in the shortwave region has also been successfully used for mastitis detection in quarter milk (Tsenkova et al., 1992). Common economic losses occurring as a result of high SCC, and so mastitis are: reduced milk yield, milk quality, treatment and labor cost, and increased risk of early culling (Tsenkova et al., 2001). Near infrared reflectance spectroscopy technology has allowed producers to detect early inflammation in the mammary gland of dairy cows, and so to provide cows with a rapid treatment that will result on less economic losses. Moreover, NIRS has also been applied to measure the solids composition of milk (Sato et al., 1987; Laporte and Pacuin, 1999; Tsenkova et al., 1999) which can help to monitor cow's health and better

market the milk. Another application of NIR technology is measurements of rumen fluid that can be used as a decision support tool to improve feeding management decisions (Lyons et al., 1993). Measurements of fecal samples using NIRS was applied to predict diet intake and diet quality of domestic animals (Boval et al.,2004; Coleman, 2005; Landau et al., 2006). Improving feed management can reduce the excretion of nitrogen and phosphorous. Adequate manure management practices can improve manure nutrient management and reduces nutrient losses into the environment.

Furthermore, NIRS technology has been used widely and successfully in evaluation of nutrient composition of feedstuffs (Norris et al., 1976; Abrams et al., 1987; Givens et al., 1997). The idea of using NIRS for feed analysis started in the 1950s. The United States Department of Agriculture (USDA) started to explore optical properties of dense, light scattering biological materials (Noms and Butler, 1961). Nom's initial work in NIRS involved transmittance through carbon tetrachloride or methanol extracts or slurries to measure moisture in grains and seeds (Noms and Hart, 1965) or through thin layers of samples, such as meat, for measuring moisture and fat (Ben-Gera and Noms, 1968). In 1976, Norris and co-workers indicated that NIRS had been only used to predict oil, protein and moisture content of grains and oilseeds, and there was a need to apply this technique to forage. Norris and his group successfully used NIRS to measure properties of forages and developed equations for predicting protein, neutral detergent fiber, acid detergent fiber, lignin, in vitro dry matter disappearance, in vitro digestibility with correlations highly linear for each chemical component

(Norris et al., 1976). This work was the fundamental to the establishment of spectro-computer system for forage research at the USDA's Regional Pasture Research Laboratory in collaboration with Pennsylvania State University. Under the supervision of J. Shenk a reflectance monochromator and software to collect store and process NIR data were designed (Givens et al., 1997).

The NIRS region of the electromagnetic field is defined by the American Society of Testing and Materials (ASTM) with an electromagnetic spectrum with wavelength range of 780 - 2526 nm corresponding to wave number range 12820 - 3959 cm -1 (ASTM, 2000). Spectroscopy literally means looking at light and is based on the interaction of electromagnetic radiation within the matter to be analyzed. When a sample is irradiated, light is absorbed selectively according to the specific vibration frequencies of the molecules present and gives rise to a spectrum (Givens et al., 1997). The NIRS bands mainly correspond to the C-H, O-H, and N-H vibrations, which originate from fundamental bands in the midinfrared region (Wu et al., 2008). In general a NIRS analyzer is composed of a light source, a monochromator, a sample holder or a sample presentation interface, and a detector, allowing for transmittance or reflectance measurements (Reich, 2005).

In contrast with traditional chemical analysis, no reagents are required and no wastes are produced on NIRS analysis; moreover, it is a multi-analytical technique: several determinations can be made simultaneously (Givens et al., 1997). Nevertheless, the disadvantages are the need for high precision spectroscopic instruments, dependence on time consuming and laborious

calibration procedures. The use of NIRS requires advanced multivariate calculation techniques that improve linearity and optimize precision of calibrations (Tran et al., 2010). However, once calibrated, the NIR spectrometer is simple to use and operate (Givens et al., 1997).

**b) Application of near infrared reflectance spectroscopy to improve total mixed ration consistency.**

Near infrared reflectance spectroscopy has been used successfully for many years in the determination of major chemical constituents and to estimate digestibility of forage (Sørensen, 2004). Near infrared reflectance spectroscopy has gained wide acceptance within the dairy industry for being a rapid and accurate method to identify composition of feedstuff without any chemical pretreatment that may damage the sample. It has been used widely and successfully in evaluation of forage and feedstuff nutrition (Norris et al., 1976; Abrams et al., 1987; Givens et al., 1997). The feasibility of using NIRS on farm to identify changes in DM content of forages might open the possibility of feeding a more consistent TMR, to replicate the diet formulated by the nutritionists. For instance, changes in DM due to ingredient variability or due to climate can result in changes in final nutrient composition of diets and total amount of DM offered. Near infrared reflectance spectroscopy technology that scans and determines DM changes may be used as a tool to make real-time corrections of DM offered. Precisely rations should be delivered to cows to ensure consistent milk production. Dairy cow's performance relies on short- and medium-term management of daily amounts and quality of forages and feeds given to lactating

herds (Tran et al., 2010). Moreover, underfeeding or overfeeding will affect animal performance, health, and environmental effects of dairy production (NRC, 2001). On a typical dairy farm, the cost of feed can range from 50 – 70% of the total operating cost to produce a kilogram of milk (Bozic et. al., 2012). Therefore, feed cost chiefly determines profit margins. Reducing variation in nutrient composition of diets can lower feed costs, improve animal health, and/or increase milk production (Weiss et. al., 2009).

Routine on farm measurement and correction of ingredient DM on farms presents an opportunity to improve the consistency of TMR preparation. Current on farm strategies to control variation in ingredient DM include hand sampling of forages and submission of samples to a commercial testing laboratory or on farm DM analysis using forage drying equipment. However, method and frequency of on farm DM adjustments is highly variable among farms. According to a study of the largest farms in California, 52.3% of producers evaluate corn silage DM at least once a month, and only 8.3% of dairies determined DM weekly, or more often (Silva Del-Rio, 2010). Differences between formulated TMR and daily TMR consumed by cows are often the reason for lower performance (Chandler, 1990). Therefore, there is a need for a reduction in variability of TMR caused primarily by changes in ingredient DM content on commercial dairy farms.

Determination of DM content in forage and feeds is one of the most frequent and important analyses made because the nutritional composition must be expressed on a DM basis (Windham, 1987). If changes in DM content of ingredients are undetected, and, if as-fed amounts added to the mixer are not

appropriately adjusted, then the diet delivered to the cattle may not be nutritionally balanced (Oetzel et al., 1993). Forages are considered the most variable feed ingredients, whether they are produced on farm or purchased (Mertens, 2006).

New affordable and reliable technology, such as the Intelligent Ration Monitoring System (IRM) (Dinamica Generale,Inc. Montova, Italy) is now available that allows dairy producers to improve the accuracy and precision of TMR preparation. The IRM consists of a NIRS analyzer system mounted in the bucket of the tractor used to add ingredients into a TMR mixer, a scale-head, a wireless link between the tractor and scale head and finally software that integrates the data from the bucket-mounted NIRS with the scale head and calculates the required amount of each ingredient needed to complete the TMR recipe with the current ingredient DM content. Real-time adjustment of DM allows producers to correct for changes in ingredient DM as they occur resulting in a more consistent TMR. Total mixed rations are designed to be consumed as a consistent homogenous mixture to improve diet digestibility compared with consumption of individual feed components by dairy cattle (Coppock et al., 1981). Deviation from the TMR as formulated can result from weighing errors, variation in the DM content of the ingredients, and errors and variation in nutrient sampling and analysis (Buckmaster, 1994). Forages can comprise over 50% of total diet DM fed to lactating cows; therefore it can be presumed that changes in DM content of ingredient are likely to affect final TMR amount fed and nutrient composition. According to Holter (1983) weekly variation in DM content of ensiled

forages within a storage structure can be large, and shift in corn silage DM changed as much as 6 to 7% units in consecutive weeks. Control of nutrient variation should occur during diet formulation (St-Pierre and Harvey, 1986; Tozer, 2000), diet preparation (St Pierre and Weiss, 2006), or at the time feed ingredients are sourced.

**c) Aquatic plant as a feed ingredient for dairy cattle.**

Dairy producers aim to procure ingredients that provide the lowest cost source of nutrients that most closely matches the nutrient requirements of the dairy cow. By-products offer a lower cost alternative to traditional feed ingredients; however, factors such as acceptability, consistency, availability, and quality must be considered before replacing traditional feed ingredients with by-products. Feed cost is the most important cost of animal production systems (Bozic et. al., 2012) and chiefly determines greater margins to the farm. Incorporation of by-products into dairy cow diets has been and will continue to be a common practice as an attempt to reduce feed costs and ensure greater financial returns. A by-product must have the following characteristics to be an attractive alternative to replace traditional feeds: 1) it has to be lower cost than traditional ingredients that it is replacing; 2) it has to have a similar or more valuable nutrient composition than the traditional feed it replaces; 3) it has to be free of anti-nutritional factors such as contaminants that could be harmful to animal health or milk supply. Considerations such as availability of the by-product are also important.

Aquatic plant by-products may be a suitable ingredient to enrich and lower costs of diets. In addition to greater protein values, aquatic plants also have lower lignin and fiber content being an excellent feed ingredient. Duckweeds, also known as floating aquatic macrophytes, are defined as plants that float on water surface usually with submerged roots. Duckweeds can also be classified as higher plants, and they are commonly mistaken for algae. A peculiar characteristic of these aquatic plants is that they are not dependent on soil or water depth (FAO, 2009). They belong to five genera: Lemna, Spirodela, Landoltia, Wolfia and Wolffiella (Journey et al., 1991). These aquatic plants reproduce and grow rapidly doubling their biomass every 2 d making them an intriguing plant for feed and food (Leng et al., 1994). The lipid content of duckweed can vary, being as low as 1.8-2.5 percent in duckweed species grown in nutrient-poor water, and as high as 3-7 percent for duckweed grown in nutrient-rich water (Skillicorn, 1993). Protein concentration of duckweed can reach 35 to 40% when concentration of nitrogen in the water is maintained between 10-30mg/L (Anh and Preston, 1997). Duckweed has high quality protein with a superior amino acid profile than most plant proteins and is similar to animal protein (Rusoff et al.,1980). The nutrient composition value of duckweeds can be compared with that of alfalfa in terms of lysine and arginine. However, duckweeds are also rich in leucine, threonine, valine, isoleucine and phenylalanine (FAO, 2009). Although some species of duckweed measure only 0.3 to 20 millimeters in length (Landolt, 1986), annual DM yield can be 10 to 30 ton/h-1 (Leng et al. 1994). In terms of equivalent protein feed sources, alfalfa can

yield only about 11 metric tons/ha/year, hybrid bermuda grass yields 10 metric tons/ha/year, and endophyte-infected fescue yields 4.5 metric tons/ha/year (Chamblee and Green 1995). Another advantage of duckweeds is that their cell walls have a low lignin content which increases fiber digestibility (Leng et al., 1994). Fiber content of duckweed can be as little as 5 % as these plants do not need to support upright structures (Leng et al., 1994). The entire body of duckweed is composed of non-structural, metabolically active tissue; most photosynthesis is devoted to the production of protein and nucleic acids, making duckweeds very high in nutritional value (FAO, 2009).

Linn et al. (1975) reported value of 18.8% of ADF or lignocellulose contents for Lemna minor compared with alfalfa which had 25.1%. Lignin in the cell wall has been shown to have a detrimental effect on the digestibility of cell wall (Van Soest, 1966). Therefore, cell walls of duckweeds may be more digestible than alfalfa. Huque et al. (1996) examined duckweed as a source of nitrogen and minerals for ruminant animals. Using in situ techniques, these workers were able to demonstrate potential DM disappearance of duckweed in the rumen was 85% (Spirodela), 72% (Lemna) and 93% (Wolfia). Protein of duckweed was highly soluble in the rumen at 24% (Spirodela), 42% (Lemna) and 18% (Wolfia) and overall 80, 87 and 94% respectively of the protein was apparently degraded in the rumen. Additionally, a duckweed (33% diet DM) and corn silage diet produced higher growth rates in Holstein heifers than a diet based on corn silage, concentrate and grass (Rusoff et al., 1978; Rusoff, 1980).

Alternative or unconventional protein sources such as that found in duckweed may be important in the future to meet the world protein demand by providing nutrients in animal diets. This plant may offer a viable alternative to crop production where land is scarce, poor quality or where precipitation is inadequate or variable. Despite the potentially beneficial attributes of duckweed on ruminant animal performance, feeding aquatic plants has received limited attention. Questions still remain regarding the effects of Lemna (duckweed) biomass on diet acceptability to high producing dairy cows, milk yield and milk components.

**d) Sugar by-products supplementation and in vitro digestibility**

Neutral detergent fiber (NDF) fraction plays an important role in rumen health. It stimulates the appropriate motility of the rumen to promote rumination, secretion of saliva to regulate ruminal pH, and development of the ruminal mat that optimizes the fermentation processes (Tafaj et al., 2004; Zebeli et al., 2012). The NRC (2001) recommends a minimum of 25% to 28% dietary NDF, 75% of which should be supplied by forages. However, at very high levels of NDF in the ration, intake and animal performance are also reduced (Mertens et. al., 2009). Neutral detergent fiber has a negative relationship with dry matter digestibility (DMD) which is related to the energy in the feed that is available to the animal and so linked to animal performance (Mertens et. al., 2009). Techniques to increase fiber digestibility and thus animal performance are desirable. Rumen microbial population ability to degrade fiber can be affected by type of forage,

agronomic management, maturity at harvest, and fermentation or processing methods (Galloway et al., 1991). Factors such as hybrid selection, maturity at harvest, starch content and length of fermentation prior to feeding impact corn silage digestibility and subsequent milk yield. For instance, Johnson et al. (1999) reported greater dry matter intake (DMI) and increasing milk production ranging from 0.2 to 2.0 kg/d when cows were fed mechanically processed corn silage vs. non processed corn silage.

Chemical composition of forages can vary greatly, and the sugar content is affected by forage variety, stage of growth at harvest, and method of preservation during storage (Humphreys 1989; Berthiaume et al. 2010). High-sugar forages are expected to increase microbial protein production in the rumen and animal productivity (Penner and Oba, 2009).Therefore, dairy nutritionists and dairy producers are constantly seeking strategies to both increase the digestibility of corn silage and also reduce its variability in digestibility. For instance, Rumin8, a sugar supplement marketed by Milk Specialties Global, Eden Praire, MN, is an accepted source of sugar in the Northeastern US, and increased use of Rumin8 in this region may be due to the forage availability (considerably larger amount of alfalfa and less corn silage). Alfalfa is low in soluble carbohydrates and therefore supplemental sugar may be beneficial.

Oba and Allen (1999) suggested that each unit-increase in neutral detergent fiber digestibility (NDFD) was associated with 0.17 kg increase in DMI and a 0.25 kg increase in 4% fat-corrected milk. Furthermore, these authors also suggested that digestibility of NDF should be measured more routinely to assess

forage quality effects on animal performance. However, DMD and NDFD of forages are not constant and feed additives that increase DMD, NDFD, and therefore DMI are needed to increase cow's performance and increase feed efficiency.

Sugar supplementation has been identified as a method of increasing DMI through multiple mechanisms including: increased diet palatability, increased rumen pH through modification of rumen fermentation, and reduced selective consumption of diet particles (Litherland et al, 2013). However, dietary situations influence the optimum feeding rate of between 2.5 and 5% supplemental sugar (Broderick and Radloff, 2004; Firkins et al., 2008). Varying the source and rate of digestibility of dietary carbohydrates may influence the rumen microbial response. Sugars are highly water soluble carbohydrates supplying a rapid source of energy to rumen microbes which may alter the rumen microbial ecology to increase fiber digestion (Chamberlain et al., 1993). Compared to starch and structural carbohydrates, microbes spend less energy to reduce sugars to smaller units (Golder et al., 2012) which means that rumen microbes can utilize sugars at faster rates, and use this energy to grow more rapidly and increase their capability to degrade more fiber. In addition, sugars can also be converted to volatile fatty acids (VFAs) that can be absorbed through the rumen in a short period of time and then be used as energy source by the host animal (Nafikov and Beitz, 2007). For instance, approximately 70% of sugar in cane molasses is sucrose. Sucrose is largely fermented in the rumen and increases molar yields of acetate and butyrate (Broderick and Radloff, 2004; Hall and

Weimer, 2007; Oelker et al., 2009). Butryate is absorbed through the rumen epithelium and is either oxidized for fuel or stored in the liver.

Sniffen et al. (1992), and Lean et al. (2005) demonstrated that fermentation increased in vitro microbial efficiency to a greater extent with additional dietary sugar. Furthermore, Vallimont et al. (2004) observed a quadratic increase in NDFD when sucrose replaced corn starch in continuous culture, and Ribeiro et al. (2005) showed that bacterial OM production in continuous culture increased linearly from 12.3 to 14.4 g/d as the concentration of sucrose increased from 0 to 8%.

Increasing the rate of carbohydrate fermentation can be beneficial resulting in more effective capture of rumen degradable protein (RDP) and increase the supply of metabolizable protein (MP) (Broderick and Radloff, 2004). Moreover, Stokes et al. (1991) showed some in vitro evidences of enhanced net yield of ruminal microbial protein from the fermentation of sugars.

*In vitro* and *in vivo* research has demonstrated that adding sugar in mixed diets can increase the digestibility of corn silage and subsequent milk yield; however, the mechanism for this effect remains unclear. Moreover, not all sugars are equal and better understanding the nutritional applications of different types of commercial available sugars and their implications on fiber digestion is crucial to manipulate dairy cows' diets and achieve higher feed efficiency.

*In vitro* digestibility trials are commonly performed using Daisy$^{II}$ incubator (Ankom Technology, Fairport,NY). The rotating jar-*in vitro* system is a fast, simple, and inexpensive *in vitro* rumen fermentation technique to measure DMD

and NDFD (Spanghero et al., 2010). Also, this *in vitro* technique has a high degree of accuracy compared with in situ data (Spanghero et al., 2010), and it is used to measure feedstuff digestibility and has potential to serve as a screening tool to evaluate ruminant feeds and feed additives.

**Chapter 2: Effects of ingredient dry matter adjustment using near infrared reflectance and precision feeding software on lactating cow performance on a commercial dairy farm.**

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## Interpretative Summary

The objectives of this study were to determine the effects of method and frequency (daily versus weekly) of wet ingredient dry matter (DM) adjustment on reducing variation of nutrient composition of the TMR, DM consistency of the TMR, milk production and milk components. Two pens averaging 250 Holstein cows per pen on a commercial dairy farm were used in a crossover design with 9 week periods. Milk and milk components production from cows (n = 104) were analyzed to determine the effects of dietary treatments which consisted of 1) once weekly DM amount adjustment of wet ingredients (corn silage, alfalfa silage, and high moisture corn) dried for 12 h in a 100°C oven (Control) or 2) real time (during ingredient addition prior to feeding correction DM of wet ingredients (corn silage, alfalfa silage, and high moisture corn) using a bucket-mounted near infrared reflectance spectroscopy (NIRS) analyzer system – precision dry matter measurements (PDM). We hypothesized that real time DM correction of wet ingredients using PDM would reduce variation of TMR compared with Control. Pens were balanced by milk yield (52.9 and 51.8 ± 1.4 kg) and DIM (110.5 and

111.8 ± 1.3d) for Control and PDM pens respectively. Analysis of weekly TMR samples indicated that method of measuring ingredient DM were similar between treatments. Correlation analysis indicated strong correlation between Control and PDM with r-value of 0.70, 0.79 and 0.66 for corn silage, alfalfa silage and high moisture corn respectively. Variation in ingredient DM was smaller than expected and there were no detectable differences in the nutrient composition of the TMR's. Yield of 3.5% fat corrected milk was similar among treatments and averaged 53.4 and 53.9 ± 1.4 kg/d for Control and PDM, respectively. In conclusion, there were no significant differences in cow performance between ingredient DM adjustment techniques. Cows fed PDM diets performed as well as those fed using traditional DM adjustment techniques. In summary, PDM was comparable with the industry gold standard of weekly sampling, diet DM correction and highly skilled feed personnel. Dairy farms struggling with variability in forage DM or changes in ingredient DM due to precipitation or inexperienced feed personnel may benefit the most from automated precision feeding systems.

## Introduction

Variation in the nutrient content of lactating dairy cows fed diets as a TMR is receiving increased attention because of its effects on cow performance, farm profitability, and efforts to reduce the impact of intensive livestock production on the environment (Fadel et al., 2006; Kohn, 2006; St-Pierre and Weiss, 2006). New affordable and reliable technology, such as the Intelligent Ration Monitoring System (IRM or PDM) (Dinamica Generale,Inc. Montova, Italy) is now available

that allows dairy producers to improve the accuracy and precision of TMR preparation. Routine measurement and correction of ingredient DM during feed mixing presents an opportunity to improve the consistency of TMR preparation. Current on farm strategies to control variation in ingredient DM include hand sampling of forages and submission of samples to a commercial testing laboratory or on farm DM analysis. Frequency of on farm DM adjustments, however, is highly variable among farms. According to a study of the largest farms in California, 52.3% of producers evaluate corn silage DM at least once a month, and only 8.3% of dairies determined DM weekly, or more often (Silva Del-Rio, 2010). There is a need for a reduction in variability of TMR caused primarily by changes in ingredient DM content on commercial dairy farms. Determination of DM content in forages and feed is one of the most frequent and important analyses made because the nutritional quality requires that major constituents be expressed on a DM basis (Windham, 1987). If changes in DM content of ingredients are undetected, and, if as-fed amounts added to the mixer are not appropriately adjusted, then the diet delivered to the cattle may not be nutritionally balanced (Oetzel et al., 1992). Forages are considered the most variable feed ingredients in dairy cattle diets, whether they are produced on farm or purchased (Mertens, 2006). New technology must be implemented to continue to improve the accuracy and precision of feed mixing and delivery.

The IRM or PDM consists of a NIRS analyzer system mounted in the bucket of the tractor used to add ingredients into a TMR mixer, a scale-head, a wireless link between the tractor and scale head, and software that integrates the

data from the bucket-mounted NIRS with the scale head. The NIRS calculates the required amount of each ingredient needed to complete the TMR recipe with the current ingredient DM content. Real-time adjustment of DM allows producers to correct for changes in ingredient DM as they occur resulting in a more consistent TMR. The effects of on farm use of IRM have not yet been published.

Total mixed rations are designed to be consumed as a consistent homogenous mixture to improve diet digestibility compared with consumption of individual feed components by dairy cattle (Coppock et al., 1981). Deviation from the TMR as formulated can result from weighing errors, variation in the DM content of the ingredients, and errors and variation in nutrient sampling and analysis (Buckmaster and Muller , 1994). Forages can comprise over 50% of total diet DM fed to lactating cows; therefore it can be presumed that changes in DM content of ingredient are likely to affect final TMR amount fed and nutrient composition. According to Holter (1983) weekly variation in DM content of ensiled forages within a storage structure can be large, and shift in DM in corn silage changed as much as 6 to 7% units in consecutive weeks. Control of nutrient variation can occur during diet formulation (St-Pierre and Harvey, 1986; Tozer, 2000), diet preparation (St Pierre and Weiss, 2006), or at the time feed ingredients are sourced. A 7-week study involving 3 California dairies showed differences among all formulated and supplied TMR nutrients, concluding that feeding management impacts nutrient delivery to the cow (Rossow et al., 2011). Moreover, differences between formulated TMR and daily TMR consumed by cows are often the reason for lower performance (Chandler, 1990). The

objectives of this study were to determine the effects of method and frequency (daily versus weekly) of wet ingredient DM adjustment amount on reducing variation of nutrient composition of the TMR, DM consistency of the TMR, milk production and milk components. We hypothesized that NIRS will result in more consistent TMR DM and more precise ingredient amounts compared with weekly adjustment for changes in ingredient DM.

## Materials and Methods

### *Housing and Animals*

This study was performed on a commercial dairy farm in South-East Minnesota from July 28$^{th}$ through December 1$^{st}$ of 2010. Five hundred-five dairy cows were arranged in two pens on a commercial dairy farm. Pens were located in a curtain-sided four row free-stall barn with 4.3 meter sidewalls in one barn running East to West with one pen on each side. Each pen had 198 sand bedded free stalls and 198 headlocks. Stocking density of stalls and headlocks ranged between 128 and 130% throughout the study. Prior to the start of the study cows in both pens were balanced by milk yield (52.3kg/d) and days in milk (DIM) (111.1 d). For both pens, cows were moved in and out of pens based on DIM and reproductive status. Throughout the study, however, 104 cows remained in their respective pens study allowing us to evaluate treatment effects applied at the pen level on individual cow milk and milk component production for this subset of cows.

### *Treatments*

Cows were fed 2 treatments in a crossover design with 9 week periods. Dietary treatments consisted of 1) once weekly correction DM of wet ingredients (corn silage, alfalfa silage, and high moisture corn) dried for 12 h in a 100°C oven **(Control)** or 2) real time during mixing and prior feeding correction DM of wet ingredients (corn silage, alfalfa silage, and high moisture corn) using a the bucket-mounted NIRS (Figure 1) analyzer system **(PDM).** Diets had the same ingredient composition and varied only by method and frequency of DM adjustment, weekly versus daily. All ingredients in the diet were hand sampled weekly at 6:30 am during feeding time. The owner of the farm initially established the maximum DM correction limit to 5% from the beginning of the study until October 22nd at which point this correction limit was increased to 10%. For example, if wet ingredients had a daily change of over a 5% or 10% on DM, NIRS system was programmed to adjust only a maximum of 5% or 10% of the DM.

### *Diet, Feed Sampling and Analysis*

The diet was formulated by the producer using on farm ingredients to meet nutrient requirements of high producing lactating dairy cows (NRC, 2001) (Table 1) Ingredient addition to the mixer occurred in the following order: vitamin/mineral mix, Energy Booster 100 (Milk Specialties Global, Eden Prarie, MN), corn gluten, soybean meal, ground corn, protein mix, tallow, alfalfa silage,

high moisture corn and corn silage. Dietary ingredients were mixed for approximately 10 minutes in a vertical mixer wagon (Supreme twin auger 1200T, Supreme International, Alberta, Canada). The amount of feed offered to each pen was adjusted by the farms feed technician daily to ensure approximately 2% feed refusals daily. Corn silage and alfalfa silage were stored in covered piles with openings facing East while the high moisture corn was stored in a covered bunker with the opening facing North. All other ingredients were stored in a commodity shed. Samples of ingredients, TMR and TMR refusals were frozen and submitted to Dairyland Laboratories Inc. (Arcadia,WI) for wet chemistry analysis. Organic matter concentration was calculated as the difference between the DM content and as is content. Ash content was determined using AOAC 942.05. Crude protein was determined using AOAC 990.03. Amylase NDF was determined using methods described by VanSoest et al. (1991). Acid detergent fiber was determined using AOAC 973.18. Lignin was determined by washing ADF residue with 72% sulfuric acid followed by ashing. Ether extract was determined by AOAC 920.39. Sugar was determined by measuring total ethanol soluble carbohydrates. Starch was determined using an enzymatic method described by Knudsen (1997). The two pens were fed in the same order throughout the study. Prior to the first feeding, faces of corn silage and alfalfa silage piles were removed with a tractor mounted rake and high moisture corn face was removed with a rotary knife. After removal from the face, each wet ingredient was mixed with the tractor loader to produce a homogenous pile. The first TMR batch was prepared at 0630 am and the second batch was prepared at

1130 am. The feeding management software DTMTM (Dinamica Generale, Inc., Montova, Italy) recorded all feeding activities, storing daily records of actual ingredients profile of each batch of TMR that was mixed, and total amount of TMR fed. Feed refusals were collected at 0600 am daily for each pen and weighed in the feed mixing wagon.

### *Body Condition and Fecal Score*

Body condition (BCS) (N = 50) and fecal scores (N = 45) were assigned to a random subset of cows in each pen weekly. BCS was measured on 0.25-unit increments (Ferguson et al., 1994) for a subset of cows weekly. Manure scores were measured using a five point system (Hall, 2005).

### *Precipitation Data*

Total weekly precipitation and average temperature were collected from a local weather station data log at the completion of the trial. The station is located fifteen miles North/West of the dairy farm.

### *Milk Production and Components*

Cows were milked 3 times daily (0700,1300 and 2000h) in a 50 stall rotary parlor equipped with cow identification and calibrated milk meters (Westfalia Surge, Naperville, IL). Milk production for each cow was recorded daily. Milk samples were collected on weeks 2, 4, 6, and 8 of each period from all cows.

Milk samples were preserved (800 Broad Spectrum Microtabs II; D and F Control Systems, Inc. San Ramon, CA), and were analyzed for fat, protein, lactose, urea N, and SCC using mid-infrared procedures (AOAC, 1995) at a commercial laboratory (DHIA, Zumbrota, MN). Milk fat and protein yield (kg/d) were calculated, for the day of sampling based on the product of the milk yield and milk composition on the day of milk sampling.

## Statistical Analysis

All data were analyzed using the Mixed Procedure of SAS (SAS Institute, 2003) as crossover design with cow as the experimental unit for milk production, milk components, BCS and fecal score. Cows (n = 104) which were in the pens for the entire 18 weeks were utilized in statistical analysis. Daily milk production was reduced to weekly means. Model included the effects of treatment, week and treatment x week. The PDIFF statement was used for least squares means separation. All values reported are least squares means and the largest treatment standard error of the mean (SEM). Significance was declared at $P \leq 0.05$, and trends reported if $0.05 < P \leq 0.10$. During week 6 of block 2 there was a technical problem with the PDM treatment and therefore data from this week was treated as missing data for both treatments.

## Results and Discussion

### *Effects of IRM on TMR Consistency*

Dietary fat concentration was higher ($P < 0.05$) for Control vs. PDM TMR; however calculated energy density (NEL) of the two diets were not different (Table 2). All other measured dietary nutrients were similar between treatments (Table 2). TMR DM averaged 46.0 ± 0.6% for Control and PDM. Perhaps weekly sampling of TMR and refusals was insufficient to observe differences in TMR DM content between treatments. The SEM for TMR and feed refusal DM are low for this study. A comparison with similar field trials is difficult as very few studies report diet DM and even fewer studies report the standard error of the mean of the reported DM. DM of TMR refusals was 44.0 and 43.1 ± 0.5% for Control and PDM fed pens and was not different between treatments(Table 3). The lack of differences in the nutrient composition between treatments are not surprising given the consistency in forage quality and the precision of the standard feeding procedures used on this farm. We originally hypothesized that a key benefit of real time corrections for changes in ingredient DM would be consistency of amount of DM offered to ensure that a decrease in ingredient DM would not result in insufficient feed delivery resulting in empty feed bunks. At the outset of this trial, we had intended to keep feed refusals of 2%. Recorded feed refusals did not reach zero for any day. The higher than anticipated amount of feed refusals likely reduced the impact of the precision feeding system. Dry matter content of corn silage, alfalfa silage and high moisture corn is shown in (Table 4). Measured DM for these 3 ingredients did not differ by week within period; however, there were some significant effects due to period indicating that although significant changes in the nutrient composition were not measured

across weeks there were significant changes for some nutrients in forages over the two month period (data not shown). This significant period effect underscores the importance of analyzing forages and making diet adjustments at least every two months in order to maintain feeding precision. Moreover, the lack of a weekly difference in variability of the nutrient composition of the forages indicates the consistency of forage, growth, harvesting and storage on this dairy and indicates that little measurable change in nutrient composition occurs from week to week. Dry matter content of all 3 wet ingredients was positively correlated with both Control and PDM method (Table 5). Results between method of DM content measurement of corn silage samples was significantly correlated ($r = 0.70$; $P < 0.05$). Corn silage DM means were 33.5± 1.7 % and 32.8 ± 1.9 % for Control and PDM respectively. Alfalfa silage sample DM content using both methods were positively correlated ($r = 0.79$; $P < 0.01$). Average DM for alfalfa silage samples were 45.9 ± 5.0 % and 43.9% ± 4.8 for Control and PDM methods respectively. High moisture corn DM samples was positivity correlated ($r = 0.66$) between both methods; however there was only a tendency ($P < 0.10$) for correlation of sample method DM. Results for correlation analysis between Control and PDM indicate that bucket-mounted NIRS system can be used to reliably measure DM content of wet ingredients on farm. These results are similar to other reports of on farm NIRS measured DM content compared to the oven drying method (Akins and Shaver, 2012).

***Effects of IRM on Milk production and Milk components***

We identified 104 cows that remained in their respective pens for the duration of the study and therefore were fed their assigned diets for the full eighteen weeks of the study (Table 6). Milk yield was similar ($P = 0.64$) between treatments and averaged 52.8 and 52.9 ± 0.7 kg/d for Control and PDM. Moreover, yield of 3.5% FCM was similar ($P = 0.40$) between treatments and averaged 53.4 and 53.9 ± 1.4 kg/d for Control and PDM. Boyd and Mertens (2011) reported that abrupt changes in forage DM reduce daily feed intake; however, a change higher than 3% units in forage DM was needed to affect milk yields and components. A significant week effect for milk production variables was measured and likely due to changes in DIM with progressing lactation.

There was a treatment × week interaction for milk protein ($P < 0.05$) as milk protein concentration was higher for Control on week 4 compared with PDM. Additionally, there was also a treatment × week interaction for MUN ($P < 0.05$) as MUN was higher on week 2 for PDM compared with Control. Body condition score and fecal score were not different among treatments. Body condition score for cows on both treatments decreased ($P < 0.05$) throughout the study (Table 7). In addition to adjusting for normal variation in ingredient DM, one of our objectives was to determine if the real-time precision feeding system would be able to adjust for changes in ingredient DM during times of precipitation. We hypothesized that Control would be less sensitive to changes in ingredient DM due to rain events compared with PDM. One significant rain event occurred during week 9 of period 1 (Table 8). During this week, a decrease in milk yield was observed for both treatments and occurred 2 days following the rain event.

The PDM pen received some correction for changes in wet ingredient DM; however, due to the amount of over-feeding that was occurring, cows did not run out of feed on either treatment and therefore no treatment effect on milk yield was observed.

In order to gain a better understanding of the ability of the precision feeding system to account for changes in DM, we selected three days (September 21$^{st}$ – September 23$^{rd}$) during period one in which the greatest precipitation occurred during the study (Table 9). Feed DM measured by the PDM for both daily feedings are reported in Table 10. These DM values are an average DM of each bucket of each ingredient measured by the precision feeding system at the time of TMR preparation. Ingredient DM percentage increased for each ingredient from the 0630 am feeding to the 1130 am feeding. An example of the ability of the bucket-mounted NIRS system to increase precision DM measurements during feeding time was observed on September 23$^{th}$, 2010 in which 11 cm of precipitation was recorded in the area (Table 10). Records from the PDM pen cows showed that the NIRS recorded a decrease in corn silage DM from 31.6% (on September 22$^{th}$) to an average reading (4-5 bucket loads) of 27.0% (on September 22$^{th}$) (Table 10). Both Control and PDM cows received less corn silage DM than formulated, however the PDM cows received 0.4 kg more corn silage/cow/day than Control. We hypothesize that the initial 1-2 cm of the face of the corn silage was moistened by the precipitation. The precision feeding system was able to correct for the change in DM, however, so PDM cows received more corn silage DM as planned. In addition, NIRS

recorded a decrease in high moisture corn DM of 66% (on September 22$^{th}$) to an average reading (4-5 bucket loads) of 36.5% (on September 22$^{th}$) (Table 10). We hypothesized that high moisture corn was highly affected by the rain event compared with the other ingredients due to its silo face facing North in addition to the nearly flat slope across the horizon of the bunker vs. the steeper slope on horizon of the corn silage and alfalfa silage bunkers. High moisture corn was more exposed to the rain than the other ingredients which caused the greatest change in DM content.

**Conclusions**

There were no significant differences in cow performance between ingredient DM adjustment techniques. The Control and PDM cows maintained nearly identical milk production. The feeding routine on this dairy is exceptional as well and is evidenced by the high yield and efficient performance. Treatment differences might be greater on farms managed with less precision or during alternate times of the year with greater relative climatic change. Future studies should not restrict the correction limits for the PDM system as the limit to the correction allowable biases the treatments against the PDM system and reduces potential for measurable animal response. This trial was able to demonstrate that the bucket-mounted near infrared reflectance spectroscopy analyzer system is able to scan forages, wirelessly make corrections for ingredient deviations from the recipe, and maintain feed intake and milk production in comparison with the industry standard of weekly sampling and diet DM correction for changes in

ingredient moisture content. Furthermore, future research should evaluate the effects of precision feeding in early lactation cows where cows are potentially more sensitive to changes in TMR DM. Additionally, the effects of precision feeding on reducing the impact of dairying on the environment should also be explored.

## Chapter 3: Effects of supplementing Lemna Meal vs. Alfalfa Meal on nutrient intake, milk yield, milk components and milk fatty acid composition by lactating dairy cows.

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### Interpretative Summary

Parabel Select Lemna Meal (LM), a product from duckweed, is a novel plant feed that has potential to provide nutrients in animal diets. The objectives of this study were to determine the effects of LM on; nutrient intake milk yield and dairy efficiency. Our hypothesis was that cows fed LM would have similar DMI, milk yield and feed efficiency compared with a more traditional feedstuff such as Alfalfa Meal (AM). Thirty-six (n = 18) multiparous Holstein-crossbred dairy cows were assigned to one of two dietary treatments: 1) TMR with AM (16.2% CP; 1.6 Mcal/kg of NEL, 34.7% NDF) (AMp) or 2) TMR with LM pellets (16.2% CP; 1.6 Mcal/kg of NEL, 33.4% NDF) (LMp). Pellets of AM and LM were made by mixing equal amounts (25% DM basis) of AM or LM with wheat middlings, corn distillers grains with solubles, soy hulls, cane molasses, and soybean oil. Pellets of AM and LM replaced 22% of the DM in the TMR. Prior to the start of the study, treatments were balanced by milk yield (48.6 ± 1.4 kg/d), days in milk (79.3 ± 7.8 d), breed, and body weight (614 ± 16.6 kg). Cows were housed in tie stalls, fed once daily, and milked twice daily. A 7 d dietary adaptation period was used prior to the start of data collection. Data were analyzed using the MIXED models

procedure of SAS in a crossover design with 21 d periods. DMI was similar between treatments and averaged 25.6 and 24.6 ± 0.7 kg/d for AMp and LMp. Yields of milk and 3.5% FCM were similar between treatments and averaged 41.6 and 41.2 ± 1.1 kg/d and 38.5 and 38.7 ± 0.9 kg/d for AMp and LMp respectively. Feed efficiency was not different between treatments and averaged 1.54 and 1.61 ± 0.1 for AMp and LMp respectively. Milk fat (3.1 and 3.2 ± 0.1%) and protein (3.0 and 2.9 ± 0.1 %) concentration were not different between AMp and LMp respectively. Milk fatty acid analysis was conducted on a subset of cows from each treatment (n = 9/treatment). Milk fat yield of butyrate from LMp was greater (40.5 vs. 49.6 ± 2.5 g/d) than that from AMp. Proportion of mono-unsaturated fat tended to be higher in LMp vs. AMp (0.8 vs. 0.7 % ± 0.01). Diet did not affect proportion of omega-3 fatty acids, saturated fat or trans-fatty acids in milk. This study confirmed that following pelleting, dried LM at 5.5 % of diet DM resulted in similar performance compared AM. The fatty acid profile of LM presents an intriguing opportunity to alter lipid composition of milk although the mechanism for this effect is not yet known.

## Introduction

Duckweed, also known as floating aquatic macrophytes, is defined as plants that float on the water surface. A peculiar characteristic of these aquatic plants is they are not dependent on soil or water depth (FAO, 2009). Duckweeds can also be classified as higher plants, and they are commonly mistaken for algae. They belong to five genera: Lemna, Spirodela, Landoltia, Wolfia and

Wolffiella (Journey et al., 1991). These aquatic plants reproduce and grow rapidly doubling their biomass every 2 d making them an intriguing plant for feed and food (Leng et al., 1994). Protein concentration of duckweed can reach 35 to 40 % when concentration of nitrogen in the water is maintained between 10-30 mg/L (Anh and Preston, 1997). Although some species of duckweed measure only 0.3 to 20 mm in length (Landolt,1986), annual DM yield can be 10 to 30 ton/h-1 (Leng et al. 1994). In term of equivalent protein feed source, alfalfa can yield about 11 metric tons/ha/year, hybrid bermuda grass yields 10 metric tons/ha/year, and endophyte-infected fescue yields 4.5 metric tons/ha/year (Chamblee and Green, 1995). Another advantage of duckweed is their cell walls have a low lignin content which increases potential fiber digestibility and make duckweed an intriguing feed ingredient (Leng et al., 1994). Linn et al. (1975) reported fiber analysis of Lemna minor containing 18.8% ADF compared with alfalfa which had 25.1% ADF. Lignin in the cell wall has been shown to have a detrimental effect on the digestibility of plant cell walls in livestock (Van Soest, 1966). Therefore, cell walls of duckweeds may be more digestible than alfalfa.

Alternative or unconventional protein sources such as duckweed may be important in the future to meet the world protein demand by providing nutrients in animal diets. This plant may offer a viable alternative to crop production where land is scarce, poor quality or where precipitation is inadequate or variable. Huque et al. (1996) examined duckweed as a source of nitrogen and minerals for ruminant animals.

Using in situ techniques, these workers were able to demonstrate potential DM disappearance of duckweed in the rumen was 85% (Spirodela), 72% (Lemna) and 93% (Wolfia) (Huque et al., 1996). Protein of duckweed was highly soluble in the rumen at 24% (Spirodela), 42% (Lemna) and 18% (Wolfia) and overall 80, 87 and 94% respectively of the protein was apparently degraded in the rumen (Huque et al., 1996). Additionally, a duckweed (33% diet DM) and corn silage diet produced higher growth rates in Holstein heifers than a corn silage, concentrate, and grass diet (Rusoff et al., 1978, 1980). Despite the potentially beneficial attributes of duckweed, ruminant animal performance alteration due to feeding aquatic plants has received limited attention. Questions still remain regarding the effects of Lemna biomass on diet acceptability by high producing dairy cows, milk yield, and milk components. The objectives of this study were to determine the effects of Parabel Select Lemna Meal (Parabel Inc., Melbourne, FL) on nutrient intake, milk and milk component yield and feed efficiency. We hypothesized cows fed Lemna Meal pellets would have similar nutrient intake, milk yield, and dairy efficiency compared with Alfalfa Meal pellets.

## Materials and Methods

### *Housing*

This experiment was conducted from March 2011 to May 2011 at the University of Minnesota Dairy Teaching and Research Center (St Paul, MN). The Institutional Animal Care and Use Committee of the University of Minnesota

approved the experimental protocol. Cows were housed in individual tie stalls with rubber filled mattresses and bedded with sawdust in a mechanically ventilated barn. Cows were milked twice daily (0100 and 1300 h) and fed once daily (1000 h).

### *Experimental Design and Dietary treatments*

Thirty-six multiparous Holstein-crossbred dairy cows were assigned to one of two dietary treatments: 1) TMR with AM (16.2% CP; 1.6 Mcal/kg of NEL, 34.7% NDF) (AMp) or 2) TMR with LM pellets (16.2% CP; 1.6 Mcal/kg of NEL, 33.4% NDF) (LMp). At the beginning of the study, treatments were balanced by milk yield (48.6 ± 1.4 kg/d), days in milk (79.3 ± 7.8 d), body weight (614 ± 16.6 kg), and body condition score (BCS) (2.6 ± 0.1). Days in milk, milk yield, body weight, and BCS were all similar between treatments at the beginning of the study. A 7 d adaptation period at the beginning of each period was used to allow cows to adjust to the diets.

### *Ingredients, Pellet and Diet Composition*

Chemical compositions of AM and LM prior to pelleting are shown in Table 11. Pellets of AM or LM replaced 22% of the DM in the TMR (Table 12). Pelleting AM and LM were necessary to facilitate consistency of TMR mixing and delivery. Both AM and LM were blended with other ingredients (Table 12) to produce a durable pellet containing 25% AM or LM by a commercial pelleting mill (Form-a-

Feed Stewart, MN). Due to the challenges with heat produced during pelleting both AM and LM (fine particle size) a maximum of 25% of each test ingredient (Table 12) was incorporated into the pellets. Pellet size was 0.84 cm. Pellets were stored in an indoor commodity bay and were fed within 60 d of manufacturing. Pellets of AM and LM replaced 21.9% of the DM in each total mixed ration (TMR) (Table 13). Diets were formulated using the Cornell-Penn-Miner System (CPM Dairy, version 3.0.8; Cornell University, Ithaca, NY; University of Pennsylvania, Kennett Square, PA; and William H. Miner Agricultural Research Institute, Chazy, NY) to supply adequate energy and metabolizable protein for a 650 kg cow producing 40 kg of milk with a fat concentration of 3.5%. Diets were fed at an ad libitum rate and were formulated to be isocaloric, isonitrogenous, and provide an equal amount of forage NDF. Individual ingredient DM was adjusted weekly by drying in a 100°C oven for 24 hours and adjusting ingredient amount fed. Individual ingredients were sampled weekly, frozen at -20°C and composited by period on a wet weight basis. Samples were then analyzed at Dairyland Laboratories, St Cloud, MN using wet chemistry methods. Averages of the nutrient composition of individual ingredients were used in the CPM dairy model to calculate the nutrient composition of the diets. Organic matter (OM) concentration of feed was calculated as the difference between DM content and ash content. Ash content was determined using AOAC 942.05. Crude protein (CP) was determined using AOAC 990.03. Heat-stable, alpha-amylase-treated and sodium sulfite NDF (%aNDF) was determined using an ANKOM 200 fiber analyzer (Ankom Technology, Macedon, NY) based on

procedures described by VanSoest et al. (1991). Acid detergent fiber (ADF) was determined using AOAC 973.18. Lignin was determined using AOAC 973.18. ADF insoluble crude protein (AD-ICP) was determined by AOAC 973.18 and Ether extract (EE) was determined by AOAC 920.39. Concentrations of minerals in feed were determined by AOAC 985.01. Total sugar (expressed as invert, TSI) was determined by AOAC 968.28. Starch was determined using an enzymatic method described by Knudsen (1997).

Feed offered and feed refused were measured daily and recorded electronically. Individual cow DMI was measured daily and was calculated by correcting as-fed offered minus refusals and multiplied by weekly diet DM for each treatment. Body weight was measured and BCS was assigned on 0.25-unit increments (Ferguson et al., 1994) for each cow at the beginning and ending of each 21-d period.

### *Milk Yield and Composition*

Cows were milked twice daily and weekly samples were obtained from consecutive a.m. and p.m. milking's. Milk samples were preserved (800 Broad Spectrum Microtabs II; D and F Control Systems, Inc. San Ramon, CA). Milk samples were analyzed for fat, protein, lactose, urea nitrogen, and SCC using mid-infrared procedures (AOAC, 972.16) at a commercial laboratory (DHIA, Zumbrota, MN). Milk fatty acid analysis was conducted via gas chromatography (AOAC Official Method 996.06) (Rtech laboratories, St Paul, MN).

## Statistical Analysis

Data were analyzed using MIXED models procedure of SAS (Littell et al., 1996) with a crossover design with 21 d periods. Cows were allowed a 7 d adaptation period prior to measurements during each period. Data measured over time were subjected to ANOVA by using the REPEATED statement in the MIXED procedure of SAS (Littell et al., 1996). Model contained the effects of treatment, time and treatment × time. For each variable analyzed, cow nested within treatment was subjected to 3 covariance structures: compound symmetry, auto-regressive order 1 and unstructured covariance. The covariance structure that resulted in the Akaike information criterion closest to zero was used (Littell et al., 1996). Least squares means for treatment effects were separated by using the PDIFF statement when the overall F-test was $P < 0.05$. Trends are indicated when $P < 0.10$.

## Results and Discussion

As we hypothesized, DMI was not different ($P = 0.28$) between treatments and averaged 25.6 and 24.6 ± 0.7 kg/d for AMp and LMp respectively (Table 14). Dry matter intake expressed as a percentage of body weight (BW) was not different between treatments and averaged 4.2 and 4.0 ± 0.1 % of BW for AMp and LMp (Table 14). Due to the 1.0 kg/d lower DMI of LMp vs. AMp it is plausible that 5.5% of the diet as Lemna Meal may be approaching the upper limit threshold for inclusion rates within the system employed in this study. It is

unclear what factors may constrain DMI at higher Lemna Meal inclusion amounts but we hypothesize that adaptation to this novel feed ingredient by adding increasing amounts into the diet over time might result in higher DMI. There was a treatment × week interaction for DMI ($P < 0.05$). Cows fed AMp had the highest intakes on week 2 compared with week 1 and 3. Cows fed LMp had higher intake on week 1 and 2 compared with week 3. Even though there were statistical differences across weeks within treatments, it can be said that differences were not biologically significant. Differences represented on average 1.0 kg/d change in DMI. Body weight change was not different between treatments; however, AMp cows gained 14.1 kg while LMp cows lost 14.3 kg. Body condition score change was not different between treatments. Yields of milk and 3.5% FCM were similar between treatments averaging 41.6 and 41.2 ± 1.1 kg/d and 38.5 and 38.7 ± 0.9 kg/d for AMp and LMp, respectively (Table 14). Due to similarities in DMI and 3.5% FCM yield, feed efficiency was not different among treatments and averaged 1.54 and 1.61 ± 0.01 for AMp and LMp, respectively (Table 14). Milk fat concentration and yield were not different between treatments. Milk fat concentration was lower than anticipated. Insufficient fiber or fiber that lacks a coarse texture may result in low ruminal pH, decreased microbial efficiency, or depressed milk fat percentage (Mooney and Allen, 1997). On the other hand, physical effective fiber in lactating cow diets increases total chewing time, resulting in increased saliva secretion due to greater mastication, and thus a greater saliva flow into the rumen (Krause et al., 2002). Reduced physical fiber structure and particle size which occurs during pelleting of ingredients results in

reduced time spent ruminating, fast rate of digestion in the reticulo-rumen (Meyer et. al., 1959). Saliva is continually produced by cattle and is integral in providing buffer and liquid within the rumen (Bowman, et al., 2003). Rumination behavior was not monitored in this study nor was rumen pH measured to test these hypotheses. Due to low fat yield, the fat:protein ratio was low; however, fat:protein was similar between treatments (Table 14). There was a week and treatment × week interaction ($P < 0.05$) for protein yield which followed the same trend for DMI. Concentrations of lactose, other solids, non fat milk solids, milk urea nitrogen (MUN), and somatic cell count (SCC) were not different between treatments. Milk urea nitrogen was higher for both treatments than anticipated and is likely due to low dietary starch (22.0% of DM) to efficiently capture soluble nitrogen in the rumen. However, Huque et al. (1996) reported that duckweed proteins were highly degraded in the rumen of cattle (Table 14).

We conducted milk fatty acid (FA) analysis on a random subset of cows from each treatment (n = 18) (Table 15). Milk fat yield of butyrate (C4:0) from LMp was greater ($P < 0.05$) than from AMp. Grummer (1991) suggested that almost all C4:0 to C14:0 FA and approximately one-half C16:0 FA in milk fat is derived from de novo FA synthesis in the mammary gland. However, the ability to modified milk fat is greatly dependent on the efficiency by which FA are transferred from diet to the smooth endoplasmic reticulum of mammary secretory cells where FA esterification takes place. In this present study, higher yields of C4:0 for LM fed cows may indicate that LMp has higher transfer efficiency of FA into milk compared with AMp. Butyrate has been shown to have anti-proliferative,

apoptotic and differentiating effects on colon cancer cells (Harrison et. al., 1999). Typical FA profile of bovine milk fat is 70% saturated FA, 25% monounsaturated FA (MUFA), and 5% polyunsaturated FA (PUFA) (Van Eenennaam and Medrano, 2008). Additionally, there has been recent interest in supplementing salts of C4:0 in the milk replacer of nursery calves as a means of increasing reticulo-rumen and intestinal development and health. Butyrate supplementation in milk replacer resulted in higher reticulo-rumen weight and greater rumen papillae length and width when compared with control fed calves (Gorka et al., 2009). Infusion of butyric acid into the rumen stimulated rumen papillae growth in newborn calves (Tamate et al., 1962; Mentschel et.al., 2001). Yield of the remainder of fatty acids in milk were not different between treatments. Proportion of mono-unsaturated fat tended (P = 0.07) to be higher in LMp vs. AMp (Table 16). Work by Boeckaert et.al (2008) showed that algae supplementation altered the milk fatty acid composition considerably. Moreover, algae supplementation affected the rumen biohydrogenation of C18:2 n-6 and C18:3 n-3 as reflected by the changes in milk fatty acid composition. Polyunsaturated fatty acids in milk were similar between treatments. Additionally, there were no differences between treatments for proportions of omega-3 FA, saturated FA or trans-FA (Table 16).

Cows on this study were fed LM for a short period of time (3 weeks), and the effects of feeding LM for longer period of time remain unknown. Moreover, LM was delivered as pellets and it is unknown if processing and storage, such as ensiling alters acceptability of LM. Physical processing such as pelleting is known to decrease rumination, increase rate of passage from rumen and

increase rate of intake (Meyer et. al.,1959). Furthermore, due to the amount of LM fed, intake of LM fed was limited to 1.4 kg/d in this study, and greater amounts of LM might results in larger changes in milk FA composition. Maximum feeding rates of LM in dairy cow diets should to be investigated in order to evaluate the impact of this novel ingredient on the cow performance.

## Conclusions

In this experiment, diets containing 1.3 kg or 5.5% of diet DM as AM or LM resulted in similar DMI and milk yield. The results from this study confirmed that following pelleting, 1.3 kg/d or 5.5% of diet DM of LM is an acceptable feed ingredient for inclusion into the diet of high producing lactating dairy cows. Future research should evaluate the feeding value of fresh LM in dairy cattle diets and evaluate if higher proportions of traditional dietary ingredients can be replaced by LM. Additionally, the effects of long-term feeding of LM on cow performance warrants further exploration, and FA profile of this novel ingredient presents an intriguing opportunity to alter the lipid composition of milk and meat for added human health benefits. Protection of these FA from alteration by ruminal microbes will likely be necessary to increase transfer of LM FA into ruminant products.

## Chapter 4: Effects of alternate sources of sugar by-products on rumen in vitro dry matter and neutral detergent fiber digestibility in corn silage varying in quality.

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### Interpretative Summary

Varying the source and rate of digestibility of dietary carbohydrates may influence the rumen microbial response. Sugars are highly water soluble carbohydrates supplying a rapid source of energy to rumen microbes resulting in an increase in fiber digestion. However, not all sugars are equal and better understating nutritional application of different type of commercial available sugar and their implications on fiber digestion is crucial to manipulate dairy cows' diets and achieve higher feed efficiency. Cow response to supplemental sugar may be forage dependent, and factors such as starch and protein concentration, potential NDFD, and rate of digestion may impact rumen fermentation and therefore cow's response to sugars. The objectives of this study were: Experiment I: to determine the effects of 4.15% solution (weight DM basis) of RUMIN8 (RUM) using a single dose (RUM1) or three pulses doses (RUM3) and no sugar supplementation (CON1) on IVDMD and IVNDFD of corn silages (CS) varying in potential NDFD (Conventional vs. BMR), time elapse of ensiling (< 1 month vs. > 3 months), dry matter content (< 35% vs. > 35%), and starch content (30% vs. 40%). We hypothesized that pulse doses of Rumin8 would better mimic feeding conditions

and increase CS IVDMD and IVNDFD and these effects would be dependent upon the quality of corn silage; Experiment II: to determine the effects of three pulse dose of 6.54% solution (weight DM basis) of RUM, lactose (LAC), sucrose (SUC), molasses (MOL), or whey permeate (WHP) on CS. We hypothesized that RUM would increase IVDMD and IVNDFD compared with other commercially available sugar supplementation products. In both experiments in vitro incubation was performed using Daisy II incubator, (Ankom Technology, Fairport,NY) rotating jar-in vitro system. Length of incubation was 30 hours. Amounts of 1.3 g (4.15% solution) and 2.1g (6.54% solution) of sugar were calculated to represent in vivo diet inclusions of 0.7% and 1.0 % of diet DM /cow/day. Results from the first experiment suggested that either single dose or three pulses doses of RUM did not add any benefit to in vitro fermentation and IVDMD and IVNDFD of CS compare to Control. This data suggests that effects of sugar on digestibility may be only in the beginning when sugar digesting bacteria are still prolific. Results for second experiment suggest LAC and SUC increased in vitro fermentation with significantly greater IVDMD and IVNDFD of CS. This work provides insight of different type of sugars for future in vivo research.

**Introduction**

Techniques to increase fiber digestibility and increase feed efficiency need to be continually implemented on farm in order to increase animal performance. Dairy nutritionists and dairy producers are seeking strategies to both increase the digestibility of corn silage and also reduce variability in digestibility. Varying the

source and rate of digestibility of dietary carbohydrates may influence the rumen microbial response. Sugars are highly water soluble carbohydrates supplying a rapid source of energy to rumen microbes which may alter the rumen microbial ecology to increase fiber digestion (Chamberlain et al., 1993). However, rumen microbial population ability to degrade fiber is variable and can be affected by type of forage, agronomic management, maturity at harvest, and fermentation or processing methods (Galloway et al., 1991). Factors such as hybrid selection, maturity at harvest, starch content and length of fermentation prior to feeding impact corn silage digestibility and subsequent milk yield. For instance, Johnson et al. (1999) reported greater DMI and increased milk production ranging from 0.2 to 2.0 kg/d when cows were fed mechanically processed corn silage vs. corn silage without processing.

*In vitro* and *in vivo* research has demonstrated that adding sugar in mixed diets can improve the digestibility of corn silage and subsequent milk yield; however, the mechanism for this effect remains unclear. Not all sugars are equal and better understanding the nutritional applications of different types of commercial available sugars and their implications on fiber digestion may offer insight into factors benefiting rumen fermentation.

The objectives of this study were: Experiment I: to determine the effects of 4.15% solution (weight DM basis) of RUMIN8 using a single dose or three pulses doses and no sugar supplementation on IVDMD (in vitro dry matter digestibility) and IVNDFD (in vitro neutral detergent fiber) of corn silages varying in potential NDFD (Conventional vs. BMR), time elapse of ensiling (< 1 month vs. > 3

months), dry matter content (< 35% vs. > 35%), and starch content (30% vs. 40%). We hypothesized that pulse doses of Rumin8 would better mimic feeding conditions and increase CS IVDMD and IVNFD and these effects would be dependent upon the quality of corn silage; Experiment II: to determine the effects of three pulse dose of 6.54% solution (weight DM basis) of five different sugars RUM, lactose, sucrose, molasses, or whey permeate on corn silage. We hypothesized that RUM would increase IVDMD and IVNDFD compared with other commercially available sugar supplementation products.

## Materials and Methods

### *Supplemental Sugar*

The experiment took place at the University of Minnesota laboratory located at the Department of Minnesota in St.Paul, MN. Five sources of supplemental sugars were used in two in vitro experiments: Rumin8 (RUM); Lactose (LAC); Sucrose (SUC); Molasses (MOL) and Whey Permeate (WHP).

Rumin8 is a commercial blend of 80% highly soluble lactose sugars commonly used in North Eastern dairies in the United States to enrich dairy cow's diets and enhance fiber digestibility. Recommended feeding rate of this product is a minimum of 0.23 kg per cow per day.

Lactose is the major carbohydrate (approximately 70% of DM) found in whey, a liquid by-product of cheese manufacturing. Lactose is produced from deproteinized whey that is evaporated, crystallized and dried.

Sucrose is a disaccharide derived from fructose and glucose. It is commonly added into dairy cow's diets to improved diet palatability and decrease sorting. Sucrose and cane molasses supplementation to lactating cows increased DMI (Broderick et al., 2004; Broderick et al., 2008; Penner and Oba, 2009).

Molasses is mostly sucrose (glucose + fructose). Molasses is largely fermented in the rumen and increases molar yield of acetate and butyrate (Broderick and Radloff, 2004; Hall and Weimer, 2007; Oelker et al., 2009). Sugars in molasses based liquid feeds are fermented rapidly in the rumen (Weisbjerg et al. 1998). Neutral detergent fiber digestibility was increased by molasses supplementation and peaked at the dietary sugar concentration of 7.2 or 7.4% (Broderick and Radloff, 2004).

Whey permeate is a by-product of the cheese industry obtained by the removal of protein from the whey. The dry matter of whey consists mainly of lactose (glucose + galactose), about 73%, which is fermented easily and may be an excellent source of soluble carbohydrate (energy) for ruminal microbes. Whey permeate also contains 3- 8% soluble protein, as well as major minerals such as Ca, P, and K. Liquid whey is an attractive ingredient for lactating dairy cattle because of its desirable price, chemical composition and physical properties (DeFrain et. al., 2004). Research has suggested that feeding whey has similar effects on rumen fermentation as feeding lactose, regardless of whey type; butyrate concentration increased in lactating dairy cows (Huber et al. 1967). Moreover, whey provides a moderate amount of rumen degradable protein

(RDP) and is a good source of lactose, which is extensively used by ruminal microbes (Firkins et al.,2008).

### *Corn Silage Samples*

Samples of corn silage were either collected from commercial dairies in Minnesota or were available from previous in house research. The selected corn silage samples included corn silages varying in: 1) potential NDFD (Brown midrib (BMR) vs. conventional). Brown midrib corn hybrids have greater NDFD, and they have increased in popularity; however, effects of sugar supplementation on this type of corn compared with conventional corn still remain; 2) time elapse of ensiling (< 1 month vs. > 6 months). Low ensile CS have a lower starch digestibility compared to long ensile. It may affect the fibrolytic bacterial population; 3) dry matter content (<35% vs. >35%). Greater dry matter content is an indicative of excessive maturity and therefore lower digestibility; and 4) starch content (30% vs. 40%). Corn silage with greater starch content have negatives effects on rumen pH, and fibrolytic bacterial population.

Corn silages were dried in a forced air oven for 48 h at 55°C and ground through a 1-mm screen of a Wiley mill (Arthur H. Thomas, Philadelphia, PA) and 0.5g of sample was weighted in duplicate into Ankom F57 bags (ANKOM Technology Corp., Fairport, NY) and sealed prior to analysis for IVDMD and IVNDFD. The pore size of the bags was 57 microns. Chemical composition of the eight corn silages are shown on Table 17. The two donor cow diets were all

alfalfa hay and corn silage-based TMR, chemical composition is shown in Table 18.

### *Daisy$^{II}$ incubator System*

DaisyII incubator, (Ankom Technology, Fairport,NY) rotating jar-*in vitro* system is an inexpensive technique to measure ruminal feedstuff digestibility (Spanghero et al., 2010). This technique has potential to serve as a screening tool to evaluate ruminant feeds and feed additives. Measurement of IVDMD has been used extensively to analyze feeds because of a high degree of correlation to in vivo digestibility (Marten et al., 1980). DaisyII incubator system contains four 4-L digestion jars, which slowly rotate in a digestion chamber that is maintained at 39.5°C. Samples to be analyzed are heat sealed into F57 bags (Ankom Technology, Fairport, NY) and inserted into the digestion jars. Each digestion jar holds 25 bags, and 100 bags can be analyzed simultaneously.

### *General procedures of in vitro fermentation on experiment I and experiment II.*

DaisyII incubator digestion jars were filled with prewarmed ($39^0$C) 1600ml of McDougall's buffer solution: NaHCO3, $Na_2HPO_4$, KCl, NaCl, $MgSO_4$, $CaCl_2$. Rumen fluid was collected 30 minutes before incubation from two fistulated cows housed in individual tie stall at the Dairy Research and Teaching Center located in St. Paul, MN. Rumen fluid was filtered through 8 layers of cheesecloth and then 400mL were poured into each digestion jar with the 1600ml of McDougall's

buffer solution, with the corn silage samples bags in place, making the total of two litters per rotating jar. Empty Ankom filter bag (containing glass wool) were included in each jar, for each in vitro rumen digestion experiment, to correct for NDF contributed by rumen fluid inoculum. During the period in which bags were being placed into jars, McDougall's buffer solution and rumen fluid into digestion jars, a constantly amount of $CO_2$ gas was purged into the system in order to remove the $O_2$ and keep an anaerobic environment.

After 30 h of incubation, sample bags for IVDMD were removed from the digestion jars, rinsed (six times with cool water), and dried in a forced-air oven for 48 hours at 55oC. In vitro DMD was calculated using equation [1] after determining the weight of the undigested residue. Same steps were done for bags for in vitro neutral detergent fiber digestibility (IVNDFD), except that bags were placed in a freezer (-20°C) after being rinsed for further NDF analysis. Ankom fiber bag digestion system (Ankom Technology Corp., Fairport,NY) was used for detergent analysis and heat-stable α-amylase (Ankom Technology Corp., Fairport,NY) was included in analysis of NDF analysis. In vitro NDFD was calculated using equation [2].

**Equation [1]**

$$IVDMD(\%)\left(\frac{Initial\ DM(g) - Undigested\ Residue(g)}{Initial\ DM(g)}\right)\mathbf{X\ 100}$$

**Equation [2]**

$$IVNDFD(\%)\left(1-\left(\frac{100-IVDMD}{NDF}\right)\right)\mathrm{X}\ 100$$

The 30 h incubation time was selected to describe the digestion potential of NDF in high producing lactating dairy cows. Oba and Allen (2005) stated that: 1) retention time of indigestible NDF in cows at maintenance is likely less than 48 hours, and 2) grinding forages greatly increases their rate of digestion so the incubation time must be lowered to compensate.

***Specific procedures for experiment I***

During experiment I, three rotating jars were used which represent the three treatments: no sugar (Control), single dose of Rumin8 (RUM1) and three pulses doses of Rumin8 (RUM3). This experiment analyzed only the effects of RUMIN8 using either one dose or three pulse doses or no sugar supplementation (Control) on seven different corn silages. For Control incubations, deionized water without sugar was injected into the jar. Amount of sugar to make the sugar solution was calculated based work done by Martin et al.(2000). Three grams of sugar was added to 1000 mL of rumen fluid. Since on this present study the amount of 400 mL of rumen fluid was used, 1.3 g was calculated to reach same concentrations described on Martin et al.(2000) Therefore, for RUM1 treatment, a solution of Rumin8 (1.3g of Rumin8 diluted on 30mL of deionized water or 4.15% of sugar solution) was prepared and injected into the digestion jar. For RUM3, three equal amounts of the 4.15% Rumin8 solution were injected into rotating jar

at time points 0, 10 and 20 hours after placed bags into jar (10 mL/time point). Our goal was to simulate the use of Rumin8 in three feedings in a 30 h period. Injections occurred through septa installed in the lids of each jar. The septa was designed to minimize the amount of oxygen entering the system during the dosing as well as minimizing temperature change occurring in the jar during the addition of test compounds.

***Specific procedures for experiment II***

In experiment II, our experimental design of in vitro incubations compared the effects of three pulses doses of no sugar (Control), RUMIN8 (RUM), lactose (LAC), sucrose (SUC), molasses (MOL), whey permeate (WHP), in five different corn silages. Six rotating jars were used on this experiment, one jar for each treatment. After rumen fluid and buffer were placed into the digestion jar, 2.1 g of DM of each sugar supplement were mixed into 30 mL of distilled water (6.54% of sugar solution). Pulse doses of 10 mL of each product were injected into its respective jar at 0, 10 and 20 hr. Injections occurred through septa installed in the lids of each jar as it is described on experiment I. Amount of sugar supplementation added into the rotating jars was based on the amount of sugar fed to dairy cows during normal conditions. Sugar supplementation added to typical dairy cattle diets results in 0.230 kg per head per day (recommendation use product for RUMIN8). Each rotating jar has a volume of 400mL of rumen fluid and buffer which brings the value of 2.1 g of DM of sugar was added into each jar in order to mimic the normal feed conditions of sugar supplementation. Sugar

supplementation for this experiment was calculated based on rumen fluid of cows suggested by Church (1988).

## Statistical Analyses

For both experiments data was subjected to ANOVA using the MIXED procedure of SAS (Littell et al., 1996). Sample bags containing corn silage samples were used as the experimental unit. Both experiments were analyzed as a completely randomized design with a factorial arrangement of treatments. Least squares means for corn silage and sugar supplementation effects were separated by use of the PDIFF statement when the overall F-test was $P < 0.05$. Trends are indicated when $P < 0.10$.

## Results and Discussion

### *Experiment I*

Effects of dosing frequency on corn silage in vitro dry matter and neutral detergent fiber digestibility.

Although IVDMD after 30 h fermentation (Figure 3) was similar ($P = 0.37$) across treatments, RUM1 had numerically greater DM disappearance of 79.5% compared with both Control (78.6%) and RUM3 (77.7%). Numerically greater IVDMD agreed with lowest %aNDF (Figure 4) after 30 h incubation. In vitro NDFD after 30 h fermentation was similar ($P = 0.48$) among treatment (Figure 5); however, numerically greater for Control than treated with RUM1. Values

averaged 39.4, 42.3 and 37.6 ± 2.76% for Control, RUM1 and RUM3, respectively.

Perhaps, microorganisms treated with RUM1 which received one single sugar dose were able to utilize the energy available for sugar supplement, grow and multiply faster compared with RUM3 which received sugar supplement in smaller and more frequent doses during the 30 h fermentation. Moreover, maybe 30 h incubation time was too long for pulse sugar dose to have any benefit. Changes *in vitro* fermentation due to supplemental sugar may occur quickly in the rumen at the beginning of the fermentation. It is difficult to tell the effects on dosage timing since we did not measure early points of the fermentation on this study.

***Interactions between sugar dosing frequency and type of corn silage on in vitro dry matter digestibility and neutral detergent fiber digestibility***

There were significant interactions ($P$ = 0.05) between dosing frequency of sugar and CS type on IVDMD after 30 h fermentation (Figure 6). In vitro DMD was lower for low starch corn silage samples treated with Rumin8 for either single or pulse dose. However, pulse dose had the lowest IVDMD (69.3%). High Starch CS sample IVDMD results were similar between CON and RUM1 treatments; however, RUM3 treated samples had the lowest IVDMD (77.4%) compared with Control and RUM1 (84.6 and 84.7± 1.2% respectively). Short ensile CS had similar IVDMD among treatments. Pulse sugar dose increased IVDMD by 6.2% and 5.0% compared with CON and RUM1 respectively (Figure 7). Low dry matter CS samples treated with RUM3 had significant higher IVDMD

(83.8%) compared with Control (76.0%) and RUM1 (76.9%). High DM CS samples treated RUM3 had the lowest IVDMD compared with high DM CS samples treated with Control and RUM1 samples. One of the hypotheses for this experiment was that BMR samples would have a higher in vitro DMD and NDFD. Brown midrib corn is less lignified than normal corn silages and has more digestible fiber (Jung and Allen, 1995).

Not surprisingly, independently of frequency sugar dosing high starch, short ensile, long ensile CS and BMR samples had similar IVDMD and IVNDFD (Figure 7 and Figure 8). It may have resulted from their higher starch content (42.5, 40.4 and 43.5 ± 1.6%) and the lower NDF (30.1, 33.6, 30.2 ± 2.0%) for high starch, short ensile and long ensile CS respectively (Table 17) compared with the other samples. Results for BMR sample may be due to low lignified cell walls compared to conventional CS. On the other hand, low starch CS samples had the lowest IVDMD compared with all other silages which probably due to higher initial %aNDF (Table 17).

One the main objectives of experiment I was to investigate how *in vitro* DMD and NDFD of corn silage is affected by either single or pulse dose of sugar interact with corn silages with different chemical composition affect. Variation among the results occurred in all three treatments. However, the differences within and across feed and treatments have shown that fiber digestibility is not only dependent on the type and frequency of sugar dosing; it is dependent on the chemical composition of corn silages. This evidence provides insight into the complexity of interactions among sugar sources and amounts and corn silage

and the variety of production responses that occur on farm. Further research with greater sample replication is needed to tease out the effects of these complex interactions. Martin et al. (2000) found that most carbohydrates associated with soluble sugars were utilized by microorganism between 6 and 8 h during in vitro digestibility assay. This data suggests that effects of sugar on rumen microorganism fermentation occur soon after feeding when sugar digesters are prolific.

We conclude that under conditions of this experiment, frequency of sugar supplementation does not affect in vitro fiber digestibility. Moreover, nutrient composition of CS can influence *IV*DMD and IVNFD, with higher starch and lower NDF of CS having the greatest response to sugar supplementation.

## *Experiment II*

### *Effects of different sources of sugar on corn silage in vitro dry matter digestibility and neutral detergent fiber digestibility*

There were significant differences among sugar supplements on *IV*DMD and *IV*NFD of CS with lactose and sucrose sugars being similar between each other, and yielding the highest positive responses compared with the other sugars (Figure 9, Figure 10 and Figure 11). *In vitro* DMD averaged 79.2 and 78.8 ± 0.7% for lactose and sucrose, respectively which resulted in an average of 4% increase compared with the other sugars. Results of *IV*NDFD suggested that lactose and sucrose also improved fiber digestibility by 12.7 and 10.8%, respectively compared with other sugars (Figure 11). After the experiment was

completed, analysis of the supplemental sugars revealed differences in sugar concentration among the test products. This data demonstrates that all sugars are not created equal and differences in cow performance due to sugar supplementation are likely due to both source and amount of specific sugars fed (Table 19).

Broderick et al.(2008) reported that DMI increased linearly as sucrose supplementation increased to 7.5% of dietary DM. Similarly, Penner and Oba (2009) reported that feeding sucrose at 4.7% of dietary DM increased DMI in early lactating cows. In sheep fed grass silage, feeding xylose and fructose increased the molar proportion of propionate in rumen fluid, while feeding sucrose and lactose increased the molar proportion of butyrate (Chamberlain et al. 1993). Sucrose is expected to ferment completely in the rumen due to the rapid hydrolysis and subsequent fermentation of monosaccharides, but a minor portion (i.e.,5%) of lactose may escape rumen fermentation and reach the duodenum, and be available for enzymatic digestion (Weisbjerg et al. 1998). Moreover, feeding lactose in place of ground corn increased butyrate, but decreased propionate concentration in the rumen fluid of lactating dairy cows (DeFrain et al. 2004).

### *Interactions between supplemental sugar and type of corn silage on in vitro dry matter digestibility and neutral detergent fiber digestibility.*

Sugar supplementation combined with some CS characteristics resulted in a significant DMD and NDFD increase. For BMR corn silage, *IV*DMD and

*IV*NDFD increased ($P < 0.001$) when treated with WHP followed by SUC supplementation compared with the other sugar supplements. For conventional CS, in vitro DMD and NDFD increased ($P < 0.001$) supplements with LAC (2.6% and 7.4%) and SUC (3.4% and 5.7%) compared with the same CS treated with other sugar sources. High DM corn silage had increased *in vitro* DMD and NDFD ($P < 0.001$) for samples treated with LAC (1.8% and 6.84%), SUC (3.78% and 12.0%). Short ensile CS sample IVDMD and IVNDFD were significantly increased by lactose (5.0% and 16%) and sucrose (5.0% and 15%) sugar supplementation compared with the other sources of sugars. Increases in *IV*NDFD up to 16% for short ensile CS samples are excited. Short ensiled corn silage are thought to have a lower digestibility compared to long ensile CS, as silage material has not been exposed to acids and microbial activity from the fermentation process. Due to lower nutrient digestibility of short ensiled CS, milk production is typically reduced, however, supplemental sugar may at least in part increase of digest are usually lower, and sugar supplementation might help providing rapidly available energy for microbial growth. Fiber digestibility of long ensiled CS samples varied from 17.5 to 59.7%. However, *IV*NDFD was significantly increased by LAC and SUC and averaged 59.8% and 46.7%, respectively. Moreover, long ensile CS treated with LAC and SUC had the highest *IV*DMD and *IV*NDFD among all CS treated with sugars. Chemical composition (Table 17) of long ensile CS samples might have contributed to this outcome. Samples for this CS had high starch values (43.5%) and lower aNDF% (30.2%) compared to all other CS samples. Research has shown that silages

fermented for long period of time had a higher nutrient availability in the rumen. For instance, starch degradability is higher for long ensile CS compared to short ensile CS. Starch degradability seems to be higher for long ensile CS proving more energy for support high milk yields.

Lactose and sucrose supplementation effects might have increased the effects of energy availability for rumen microbes resulting on greater *IV*DMD and IVNDFD compared to other CS. Chemical composition of CS show a broad range of NDF and starch ranging from 30.0 to 46.0% and 17.7 and 43.5%, respectively. It was expected to find treatment differences among results for *IV*DMD and *IV*NDFD of these CS.

Results from experiment II suggested that all CS responded positively to supplementation of lactose and sucrose. Lower CP and supplemental sugar may have produced a lower ammonia pool in-vitro, which aided in fiber digestion. Supplemental sugar replaced that normally found in silage for those CS with lower NDF and ADF, which resulted in a greater potential for a response in fiber digestion.

**Conclusions**

Pulse dosing sugar supplementation over 30 hr incubation did not increase in vitro DMD of NDFD. Perhaps the effects are only in the early phase of the incubation when sugar digests are still prolific. Sugar tends to slow starch digestion and less lactate is produced by sugar fermentation than starch fermentation. Response to sugar is silage dependent, and results suggested that

CS with low sugar/high starch/ low crude protein had the greatest response to sugar supplementation. However, lactose and sucrose appear to have the greatest benefit. Pulse dosing feeding should better mimic feeding condition and better stimulate rumen fermentation; however, in vitro rumen fermentations on this study obtained the same results compared to single sugar dosing.

## Conclusions and Implications

The bucket-mounted near infrared reflectance spectroscopy analyzer system is able to scan forages, wirelessly make corrections for ingredient deviations from the recipe. This reliable technology enables farmers to control variations during TMR preparation in real time whereas increases the degree of precision feeding on-farm. However, variation on ingredients DM needs to present on farm in order to make worthwhile the investment of such technology.

Lemna meal was demonstrated to be an adequate ingredient to supply protein to dairy cows diets. Cows fed Lemna meal had similar performance than cows fed Alfalfa Meal. However, changes in milk FA profile found on cows fed 5% of Lemna Meal are intriguing. The effects of long-term feeding of Lemna meal on cow performance needs to further explored. Furthermore, future research should focus on evaluating the limits amounts of inclusions of this novel ingredient in dairy diets.

Sugar supplementation represents an opportunity to increase fiber digestibility of corn silages, especially for new corn silage with short fermentation time and for silages with high potential NEFD. Results from 30 h in vitro incubation have shown that DMD and NDFD are silage dependent. Moreover, lactose and sucrose appear to have the greatest benefit on those parameters.

Pulse dosing feeding should better mimic feeding condition and better stimulate rumen fermentation; however, *in vitro* rumen fermentations over 30 h do not increase in vitro DMD and NDFD compared to single sugar dosing. Future

*in vitro* research should evaluate the effects of supplemental sugar on early stage of *in vitro* fermentation and its impact on fiber digestion.

**Table 1.**Ingredient composition of TMR diet fed to multiparous lactating Holstein cows fed as Control[1] or PDM[2].

| Ingredient | % diet DM |
|---|---|
| Corn silage | 36.6 |
| Alfalfa silage | 22.5 |
| High moisture corn | 17.9 |
| Lactation protein mix[3] | 6.9 |
| Soybean meal, 48% | 6.4 |
| Corn gluten feed pellets | 5.2 |
| Finely ground corn | 2.1 |
| Energy Booster 100[4] | 1.4 |
| Tallow | 0.8 |
| Vitamin/mineral mix | 0.1 |

[1] Once weekly correction DM of wet ingredients (corn silage, alfalfa silage, and high moisture corn) dried for 12 h in a 100°C oven.
[2] Real time (during ingredient addition prior to feeding) correction DM of wet ingredients (corn silage, alfalfa silage, and high moisture corn) using a bucket-mounted near infrared reflectance spectroscopy (NIRS) analyzer system.
[3]Lactation Protein mix contained corn distillers grains with solubles, 48% soybean meal, soy expellers and canola meal.
[4]Milk Specialties Corporation, Carpentersville, IL.

**Table 2.** Chemical composition of TMR diets fed to multiparous lactating Holstein cows fed as Control[1] or PDM[2].

| Variable | Treatment | | SEM | *P*-Value | | |
|---|---|---|---|---|---|---|
| | Control[1] (n=18) | PDM[2] (n=18) | | Trt | Week | Trt × Week |
| DM, % | 46.0 | 46.0 | 0.6 | 0.8 | 1.0 | 1.0 |
| CP, % | 17.9 | 17.7 | 0.3 | 0.6 | 0.4 | 1.0 |
| ADF, % | 20.8 | 20.6 | 0.4 | 0.7 | 0.3 | 1.0 |
| NDF, % | 29.0 | 28.9 | 0.5 | 0.9 | 0.3 | 1.0 |
| $NE_L$, Mcal/kg | 1.6 | 1.6 | 0.01 | 0.8 | 0.2 | 0.8 |
| Starch, % | 27.5 | 28.0 | 0.6 | 0.5 | 0.6 | 1.0 |
| Sugar, % | 2.5 | 2.5 | 0.2 | 0.9 | 0.07 | 0.7 |
| Fat, % | 4.7 | 4.4 | 0.1 | <0.05 | <0.05 | 0.2 |
| Ash, % | 8.0 | 8.1 | 0.2 | 0.7 | 0.3 | 0.9 |
| DCAD, mEq/kg | 184.0 | 187.0 | 7.5 | 0.8 | 0.4 | 1.0 |
| Ca, % | 0.9 | 0.9 | <0.1 | 0.8 | 0.2 | 0.9 |
| P, % | 0.4 | 0.4 | <0.1 | 0.7 | 0.05 | 0.8 |
| Mg, % | 0.3 | 0.4 | <0.1 | 0.2 | <0.05 | 0.4 |
| K, % | 1.6 | 1.6 | <0.1 | 0.9 | 0.3 | 1.0 |
| S, % | 0.2 | 0.2 | <0.1 | 0.8 | 0.7 | 0.4 |
| Na, % | 0.5 | 0.5 | <0.1 | 0.4 | 0.3 | 1.0 |
| Cl, % | 0.6 | 0.6 | <0.1 | 0.3 | 0.6 | 1.0 |

[1] Once weekly correction DM of wet ingredients (corn silage, alfalfa silage, and high moisture corn) dried for 12 h in a 100°C oven. [2] Real time (during ingredient addition prior to feeding) correction DM of wet ingredients.

**Table 3.** Feed refusal chemical composition of TMR fed to multiparous lactating Holstein cows fed as Control[1] or PDM[2].

| Variable | Treatment | | SEM | *P*-value | | |
|---|---|---|---|---|---|---|
| | Control (n=18) | PDM (n=18) | | Trt | Week | Trt × week |
| DM, % | 44.0 | 43.1 | 0.5 | 0.22 | 0.49 | 0.88 |
| Starch, % | 24.7 | 25.0 | 0.4 | 0.61 | < 0.05 | 0.86 |
| CP, % | 16.9 | 16.9 | 0.5 | 0.98 | 0.27 | 0.93 |
| ADF, % | 23.3 | 23.7 | 0.4 | 0.57 | < 0.05 | 0.81 |
| NDF, % | 32.5 | 32.5 | 0.5 | 0.99 | < 0.05 | 0.83 |

[1] Once weekly correction DM of wet ingredients (corn silage, alfalfa silage, and high moisture corn) dried for 12 h in a 100°C oven.
[2] Real time (during ingredient addition prior to feeding) correction DM of wet ingredients (corn silage, alfalfa silage, and high moisture corn) using a bucket-mounted near infrared reflectance spectroscopy (NIRS) analyzer system.

**Table 4.** Comparison of ingredient DM measurement used on total mixed rations diets fed to multiparous lactating Holstein cows fed as Control[1] or PDM[2].

| Variable | Treatment | | SEM | *P-value* | | |
|---|---|---|---|---|---|---|
| | Control[1] (n=18) | PDM[2] (n=18) | | Trt | Week | Trt × Week |
| Corn silage | 33.5 | 32.7 | 0.9 | 0.23 | 0.57 | 0.91 |
| Alfalfa silage | 45.4 | 42.6 | 4.5 | 0.25 | 0.57 | 0.93 |
| High moisture corn | 69.2 | 69.7 | 4.1 | 0.72 | 0.63 | 0.93 |

[1] Once weekly correction DM of wet ingredients (corn silage, alfalfa silage, and high moisture corn) dried for 12 h in a 100°C oven.
[2] Real time (during ingredient addition prior to feeding) correction DM using a bucket-mounted near infrared reflectance spectroscopy (NIRS) analyzer system.

**Table 5**. Pearson correlations of ingredients dry matter determinations used on total mixed rations diets fed to multiparous lactating Holstein cows fed as Control[1] or PDM[2].

| Ingredients | Treatment | | | | r |
|---|---|---|---|---|---|
| | Control (n = 18) | | PDM (n = 18) | | |
| | Mean | SE | Mean | SE | |
| Corn silage | 33.5 | 1.7 | 32.8 | 1.9 | 0.70* |
| Alfalfa silage | 45.9 | 5.0 | 43.9 | 4.8 | 0.79** |
| High moisture corn | 69.5 | 3.5 | 70.0 | 3.8 | 0.66*** |

[1] Once weekly correction DM of wet ingredients (corn silage, alfalfa silage, and high moisture corn) dried for 12 h in a 100°C oven.
[2] Real time (during ingredient addition prior to feeding) correction DM using a bucket-mounted near infrared reflectance spectroscopy (NIRS) analyzer system.
* $P < 0.05$; ** $P < 0.01$;*** $P < 0.10$.

**Table 6.** Individual cow (n = 104) milk and milk components yield from multiparous lactating Holstein cows fed as Control[1] or PDM[2].

| | Treatment | | | *P*-Value | | |
|---|---|---|---|---|---|---|
| Variable | Control | PDM | SEM | Trt | Week | Trt × week |
| Milk, kg/d | 52.8 | 52.9 | 0.7 | 0.64 | < 0.01 | 0.25 |
| 3.5% FCM[3], kg/d | 53.4 | 53.9 | 1.4 | 0.40 | 0.30 | 0.17 |
| Fat, % | 3.5 | 3.6 | 0.1 | 0.13 | < 0.01 | 0.27 |
| Fat, kg/d | 1.9 | 1.9 | 0.1 | 0.23 | < 0.01 | 0.19 |
| Protein, % | 2.9 | 2.9 | 0.1 | 0.57 | < 0.01 | < 0.01 |
| Protein, kg/d | 1.6 | 1.6 | 0.02 | 0.49 | < 0.01 | 0.25 |
| Fat:protein | 1.2 | 1.2 | 0.05 | 0.14 | < 0.01 | 0.21 |
| Urea N, mg/dL | 14.8 | 14.9 | 0.4 | 0.77 | < 0.01 | < 0.01 |
| SCC, × 1000 | 83.7 | 95.8 | 50.0 | 0.56 | 0.94 | 0.36 |

[1] Once weekly correction DM of wet ingredients (corn silage, alfalfa silage, and high moisture corn) dried for 12 h in a 100°C oven.
[2] Real time (during ingredient addition prior to feeding) correction DM of wet ingredients (corn silage, alfalfa silage, and high moisture corn) using a bucket-mounted near infrared reflectance spectroscopy (NIRS) analyzer system.
[3]3.5%Fat-corrected milk (kg) = 0.4324 × (milk yield) + 16.2162 × (fat yield).

**Table 7.** Body condition score (BCS) (n = 50) and fecal score (n = 45) for multiparous Holstein cows fed as Control[1] or PDM[2].

| | Treatment | | | *P*-Value | | |
|---|---|---|---|---|---|---|
| Variable | Control | PDM | SEM | Trt | Week | Trt × Week |
| BCS, 5-point scale[3] | 2.8 | 2.8 | <0.1 | 0.51 | 0.01 | 0.64 |
| fecal score, 5-point scale[4] | 2.7 | 2.7 | <0.1 | 0.87 | 0.16 | 0.94 |

[1] Once weekly correction DM of wet ingredients (corn silage, alfalfa silage, and high moisture corn) dried for 12 h in a 100°C oven.
[2] Real time (during ingredient addition prior to feeding) correction DM of wet ingredients (corn silage, alfalfa silage, and high moisture corn) using a bucket-mounted near infrared reflectance spectroscopy (NIRS) analyzer system.
[3]Thin = 1.0 and obese = 5.0
[4]loose watery manure = 1.0 and firm dry manure = 5.0

**Table 8.** Total weekly precipitation and average ambient temperature during the study.

| | Week | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 |
| | Period 1 | | | | | | | | |
| Total rain, cm | 2.2 | 1.3 | 5.2 | 0.3 | 1.0 | 1.8 | 6.9 | 4.7 | 11.0 |
| Average temperature, °C | 25.0 | 25.6 | 21.1 | 16.1 | 20.6 | 12.8 | 18.9 | 14.4 | 15.0 |
| | Period 2 | | | | | | | | |
| Total rain, cm | 0 | 0 | 0 | 2.0 | 0 | 0.1 | 5.7 | 0.6 | 0.9 |
| Average temperature, °C | 16.1 | 11.7 | 13.3 | 4.4 | 7.8 | 13.3 | 2.2 | -3.9 | -9.4 |

**Table 9.** Number of cows housed in the Control[1] and PDM[2] pens during the main rain event during the study.

| Date | Control | PDM | Precipitation |
|---|---|---|---|
| | Cows per pen | | cm/d |
| September 21, 2010 | 248.0 | 258.0 | 1.4 |
| September 22, 2010 | 249.0 | 257.0 | 3.2 |
| September 23, 2010 | 249.0 | 258.0 | 11.0 |

[1] Once weekly correction DM of TMR wet ingredients (corn silage, alfalfa silage, and high moisture corn) dried for 12 h in a 100°C oven.
[2] Real time (during ingredient addition prior to feeding) correction DM of TMR wet ingredients (corn silage, alfalfa silage, and high moisture corn) using a bucket-mounted near infrared reflectance spectroscopy (NIRS) analyzer system.

**Table 10.** Dry matter percentage reported by PDM[1] feeding system.

| Ingredient | September 21 | September 22 | September 23 |
|---|---|---|---|
| | DM, % recorded by PDM | | |
| | ---------- 0630 am feeding ---------- | | |
| Alfalfa silage | 34.6 | 43.0 | 40.0 |
| High moisture corn | 61.9 | 66.0 | 36.5 |
| Corn silage | 30.1 | 31.6 | 27.0 |
| | ---------- 1130 am feeding ---------- | | |
| Alfalfa silage | 42.3 | 47.4 | 40.0 |
| High moisture corn | 68.0 | 67.7 | 58.2 |
| Corn silage | 32.4 | 30.8 | 30.5 |

[1] Real time (during ingredient addition prior to feeding) correction DM of TMR wet ingredients (corn silage, alfalfa silage, and high moisture corn) using a bucket-mounted near infrared reflectance spectroscopy (NIRS) analyzer system.

**Table 11.** Chemical composition of Alfalfa Meal (AM) and Parabel Selected Lemna Meal (LM)[1] prior to pelleting.

| | AM | LM |
|---|---|---|
| Nutrient | DM, % | |
| DM, % | 90.3 | 99.4 |
| CP, % | 19.0 | 16.3 |
| ADF, % | 32.3 | 34.0 |
| NDF, % | 38.8 | 48.8 |
| Lignin,% | 7.1 | 8.5 |
| Fat, % | 2.7 | 1.3 |
| Sugar, % | 0.1 | 0.6 |
| Ash, % | 11.0 | 8.5 |
| Ca, % | 1.8 | 2.1 |
| P,% | 0.3 | 0.3 |
| Mg, % | 0.3 | 0.4 |
| K, % | 2.1 | 0.9 |

[1] Parabel ™. Melbourne, FL.

**Table 12.** Alfalfa Meal Pellets (AMp) and Selected Parabel Lemna Meal pellets (LMp)[1] ingredients and nutrient composition.

| | AMp | LMp |
|---|---|---|
| Ingredient | Amount of DM, % | |
| Wheat midds | 61.5 | 57.4 |
| Dehydrated alfalfa | 25.0 | 0.0 |
| Parabel Select Lemna Meal[1] | 0.0 | 25.0 |
| Corn distillers grains with solubles | 5.0 | 9.0 |
| Soy hulls | 5.0 | 4.1 |
| Molasses | 1.7 | 1.7 |
| Soybean oil | 0.5 | 0.5 |
| Calcium carbonate | 0.0 | 0.9 |
| Nutrient | | |
| DM, % | 88.4 | 89.7 |
| CP, % | 18.4 | 18.3 |
| ADF, % | 22.5 | 17.6 |
| aNDF, % | 41.8 | 34.9 |
| Ash, % | 6.3 | 6.5 |
| Lignin, % NDF | 13.2 | 9.6 |
| AD-ICP, % of DM | 1.1 | 1.0 |
| Soluble protein, % CP | 30.7 | 23.1 |
| Starch, % | 15.1 | 15.3 |
| Sugar, % | 6.6 | 4.2 |
| Ca, % | 0.6 | 1.3 |
| P,% | 0.9 | 0.6 |
| Mg, % | 0.5 | 0.3 |
| K, % | 1.2 | 1.1 |
| S, % | 0.2 | 0.2 |
| Na, % | 0.1 | 0.1 |
| Cl,% | 0.3 | 0.3 |

[1] Parabel ™. Melbourne, FL.

**Table 13.** Total mixed ration ingredient composition for lactating dairy cows fed diets containing 22% of Alfalfa Meal Pellets (AMp) or Selected Parabel Lemna Meal pellets (LMp)[1].

| Ingredients | Treatments[2] | |
|---|---|---|
| | AMp | LMp |
| | DM, % | |
| Alfalfa pellet | 21.9 | 0.0 |
| Lemna pellet | 0.0 | 21.9 |
| Corn silage, processed | 31.8 | 31.8 |
| Alfalfa hay, chopped | 14.4 | 14.4 |
| Corn grain, ground | 10.0 | 10.0 |
| Liquid feed[3] | 2.2 | 2.2 |
| Cottonseed, whole with lint | 4.9 | 4.9 |
| Soy bean meal, 48% | 2.5 | 2.5 |
| Lactation protein mix[4] | 12.3 | 12.3 |
| Nutrients | | |
| DM, % | 54.4 | 54.5 |
| Forage, % | 46.0 | 46.0 |
| CP, % | 16.2 | 16.2 |
| $NE_L$, Mcal/kg | 1.6 | 1.6 |
| ADF, % | 24.1 | 23.3 |
| aNDF, % | 34.7 | 33.4 |
| Sugar, % | 5.4 | 5.8 |
| Starch, % | 22.0 | 22.0 |
| Ether extract, % | 4.2 | 4.5 |
| Ash, % | 7.3 | 7.7 |
| Ca, % | 0.8 | 0.9 |
| P,% | 0.5 | 0.5 |
| Mg, % | 0.3 | 0.4 |
| K, % | 1.3 | 1.3 |
| DCAD, Meq/100g | 18.7 | 18.2 |

[1] Parabel ™. Melbourne, FL.

[2]Cows were fed TMR with either alfalfa meal pellets (AM) or lemna meal pellets (LM) to provide 5.5% of diet DM as either AM or LM.

[3]Prepared by Quality Liquid Feeds Dodgeville, WI. Ingredients: cane molasses, condensed whey, calcium carbonate, urea, ammonium sulfate, magnesium sulfate, ammonium polyphosphate, magnesium oxide, vitamin E supplement, zinc sulfate, manganese sulfate, xanthan gum, copper sulfate, sodium selenite, vitamin A supplement, ethylenediamine dihydriodide, ferrous sulfate, cobalt sulfate and vitamin D3 supplement.

[4] Nutrient composition as a percent of DM: 29.8% CP, 19.2% ADF, 34.8% aNDF, 15.7% ash, 7.2% EE, 1.3% Ca, 0.9% P, 0.4% Mg, 2.2% K, 0.39% S, 1.7% Na, 0.8% Cl.

**Table 14.** Least squares means of dry matter intake (DMI), intake as a percentage of body weight, milk yield and composition for cows fed diets containing 22% of Alfalfa Meal Pellets (AMp) or Select Parabel Lemna Meal pellets (LMp)[1].

| | Treatments | | | *P*-Value | | |
|---|---|---|---|---|---|---|
| Variable | AMp | LMp | SEM | Trt | Week | Trt × week |
| DMI, kg/d | 25.6 | 24.6 | 0.7 | 0.28 | <0.05 | <0.05 |
| DMI, % BW | 4.2 | 4.0 | 0.1 | 0.30 | - | - |
| Milk, kg/d | 41.6 | 41.2 | 1.1 | 0.81 | <0.05 | 0.46 |
| 3.5% FCM, kg/d | 38.5 | 38.7 | 0.9 | 0.87 | <0.05 | 0.19 |
| Feed efficiency[2] | 1.5 | 1.6 | <0.1 | 0.39 | <0.05 | 0.51 |
| Fat, % | 3.1 | 3.2 | 0.1 | 0.37 | 0.71 | 0.56 |
| Fat, kg/d | 1.3 | 1.3 | < 0.1 | 0.66 | 0.23 | 0.25 |
| Protein, % | 3.0 | 2.9 | < 0.1 | 0.75 | <0.05 | <0.05 |
| Protein, kg/d | 1.2 | 1.2 | < 0.1 | 0.53 | <0.05 | <0.05 |
| Fat:protein | 1.0 | 1.1 | < 0.1 | 0.26 | 0.15 | 0.20 |
| Lactose,% | 4.8 | 4.8 | < 0.1 | 0.87 | <0.05 | 0.55 |
| Other solids, % | 5.6 | 5.6 | < 0.1 | 0.87 | <0.05 | 0.49 |
| Solids not fat, % | 8.3 | 8.3 | < 0.1 | 0.72 | <0.05 | <0.05 |
| Total solids, % | 11.7 | 11.8 | 0.1 | 0.62 | 0.43 | 0.80 |
| MUN, mg/dL | 16.7 | 17.1 | 0.6 | 0.29 | <0.05 | 0.76 |
| SCC,(×1000 `cells/mL) | 148.5 | 199.9 | 58.0 | 0.53 | 0.80 | 0.23 |

[1] Parabel ™. Melbourne, FL.
[2]Calculated dividing kg of DM consumed by kg of 3.5 FCM milk produced.

**Table 15**. Least squares means for milk fatty acid yield and composition for cows fed diets containing 22% of Alfalfa Meal Pellets (AMp) or Selected Parabel Lemna Meal pellets (LMp)[1]

| Variable | Treatment | | SEM | *P*-value |
|---|---|---|---|---|
| | AMp | LMp | | Treatment |
| | Fatty Acids, g/d | | | |
| C4:0 | 40.5 | 49.6 | 2.5 | < 0.05 |
| C6:0 | 22.3 | 23.3 | 1.5 | 0.7 |
| C8:0 | 10.6 | 10.5 | 0.8 | 0.9 |
| C10:0 | 24.9 | 23.9 | 1.7 | 0.7 |
| C12:0 | 30.1 | 28.8 | 2.1 | 0.7 |
| C13:0 | 2.0 | 2.0 | 0.2 | 0.9 |
| C14:0 | 115.2 | 116.8 | 7.1 | 0.9 |
| C14:1 | 7.4 | 8:6 | 0.7 | 0.3 |
| C15:0 | 13.5 | 15.1 | 0.7 | 0.1 |
| C16:0 | 321.0 | 330.3 | 17.3 | 0.7 |
| C16:1 | 12.3 | 13.9 | 0.9 | 0.2 |
| *trans* -C16:1 | 4.7 | 5.1 | 0.3 | 0.3 |
| C17:0 | 7.9 | 8.6 | 0.4 | 0.2 |
| C 17:1 | 2.0 | 2.6 | 0.2 | 0.1 |
| C18:0 | 136.1 | 150.7 | 10.5 | 0.3 |
| C18:1 | 256.8 | 288.8 | 15.1 | 0.2 |
| *trans* -C18:1 | 56.2 | 56.5 | 4.9 | 1.0 |
| C18:2 | 39.1 | 42.9 | 1.9 | 0.2 |
| *trans* -C18:2 | 9.6 | 11.1 | 0.6 | 0.1 |
| C18:3 | 4.4 | 4.5 | 0.3 | 0.9 |
| C20:0 | 2.0 | 2.3 | 0.2 | 0.4 |
| C20:3 | 1.4 | 1.6 | 0.2 | 0.5 |

[1] Parabel [TM]. Melbourne, FL.

**Table 16.** Least squares means for milk and milk fat components yield, fatty acid yield and composition for a subset (n = 18) cows fed diets containing 22% of Alfalfa Meal Pellets (AMp) or Selected Parabel Lemna Meal pellets (LMp)[1].

| Variable | Treatment | | SEM | *P* - value |
|---|---|---|---|---|
| | AMp | LMp | | |
| Milk yield, kg/day | 41.4 | 41.5 | 2.4 | 0.97 |
| Fat,% | 2.8 | 2.9 | 0.2 | 0.55 |
| Fat, kg/day | 1.1 | 1.2 | < 0.1 | 0.32 |
| *cis*-monounsaturated fat, % | 0.7 | 0.8 | < 0.1 | 0.07 |
| *cis*-polyunsaturated fat, % | 0.01 | 0.01 | < 0.1 | 1.00 |
| Omega-3 fat, % | 1.8 | 1.9 | 0.1 | 0.60 |
| Saturated fat, % in sample | 2.9 | 3.2 | 0.2 | 0.36 |
| Total fat%, (as triglycerides) | 0.2 | 0.2 | < 0.1 | 0.50 |
| *trans*-fat, % | 0.1 | 0.1 | < 0.1 | 0.19 |
| Saturated fat in total fat, % (as Triglycerides) | 0.6 | 0.60 | < 0.1 | 0.50 |

[1] Parabel [TM]. Melbourne, FL.

**Table 17.** Nutrient composition: starch, sugar, crude protein, ADF, NDF and ASH contents of eight corn silages on DM basis used on two incubations experiments.

| Corn Silage Type | DM (%) | | | | | |
|---|---|---|---|---|---|---|
| | Starch | Sugar | CP | ADF | NDF | Ash |
| BMR (2009) | 17.70 | 3.20 | 8.30 | 25.20 | 45.80 | 4.50 |
| Low Starch (2009) - Conventional | 15.80 | 2.60 | 9.70 | 25.10 | 46.60 | 4.50 |
| High Starch (2012) | 42.50 | 2.60 | 8.00 | 18.20 | 30.10 | 4.20 |
| Short ensile (2011)* | 40.40 | 1.90 | 7.60 | 21.50 | 33.60 | 4.50 |
| Long ensile (2012)** | 43.50 | 0.90 | 6.60 | 20.90 | 30.20 | 5.10 |
| 33% DM (2010) | 31.90 | 2.00 | 7.40 | 23.40 | 38.50 | 3.50 |
| 41% DM (2011) | 29.60 | 2.20 | 9.00 | 23.80 | 41.40 | 4.90 |

*Corn silage after one month ensile.
** Corn silage after six months ensile.

**Table 18.** Ingredient and nutrient composition of diets fed to rumen fluid donor cows used on *in vitro* incubation experiments.

| Ingredients | Diet |
|---|---|
| | DM% |
| Alfalfa Hay | 13.70 |
| Corn Silage, processed | 38.30 |
| Corn Gluten | 10.18 |
| Corn grain, ground | 15.73 |
| Liquid feed[1] | 3.59 |
| Lactation protein mix[2] | 18.50 |
| Nutrients | |
| DM, % | 50.8 |
| Forage,% | 47.10 |
| CP, % | 17.20 |
| RUP, CP% | 54.33 |
| RDP, CP% | 45.67 |
| ADF,% | 19.9 |
| NDF,% | 30.82 |
| $NE_L$, Mcal/kg | 1.67 |
| Ether extract, % | 6.50 |
| Sugar, % | 7.07 |
| Starch, % | 23.48 |
| Ether extract, % | 4.10 |
| Ash, % | 7.52 |
| Ca, % | 0.75 |
| P,% | 0.52 |
| Mg, % | 0.31 |
| K, % | 1.79 |

[1]Prepared by Quality Liquid Feeds Dodgeville, WI. Ingredients: cane molasses, condensed whey, calcium carbonate, urea, ammonium sulfate, magnesium sulfate, ammonium polyphosphate, magnesium oxide, vitamin E supplement, zinc sulfate, manganese sulfate, xanthan gum, copper sulfate, sodium selenite, vitamin A supplement, ethylenediamine dihydriodide, ferrous sulfate, cobalt sulfate and vitamin D3 supplement.
[2] Nutrient composition as a percent of DM: 39.4% CP, 16.9% aNDF, 12.7% ash, 5.4% EE, 1.1% Ca, 0.7% P, 0.5% Mg, 3.1% K, 0.5% S, 0.9% Na, 0.5% Cl.

**Table 19.** Sugar profile of Whey Permeate, Rumin8, Lactose, Sucrose, and Molasses used on experiment II**.**

| Samples | Sugar profile (%) | | | | |
|---|---|---|---|---|---|
| | Fructose | Glucose | Lactose | Maltose | Sucrose |
| Whey Permeate | < 0.1 | < 0.1 | 60.8 | < 0.1 | < 0.1 |
| Rumin8 | 0.1 | 0.1 | 56.3 | 0.1 | 0.1 |
| Lactose | < 0.1 | < 0.1 | 99.2 | < 0.1 | < 0.1 |
| Sucrose | < 0.1 | 0.1 | < 0.1 | < 0.1 | 99.2 |
| Molasses | 10.4 | 9.4 | < 0.1 | < 0.1 | 32.4 |

**Figure 1**. Bucket-mounted near infrared reflectance spectroscopy used to scan individual wet ingredients (corn silage, alfalfa silage, and high moisture corn) on real time (during ingredient addition prior to feeding) and adjust ingredient dry matter amount for each bucket load.

**Figure 2**. Barn layout and pen orientation on a commercial dairy farm used on the crossover design with 9 week periods to determine the effects of varying method used to correct for total mixed ration ingredient dry matter.

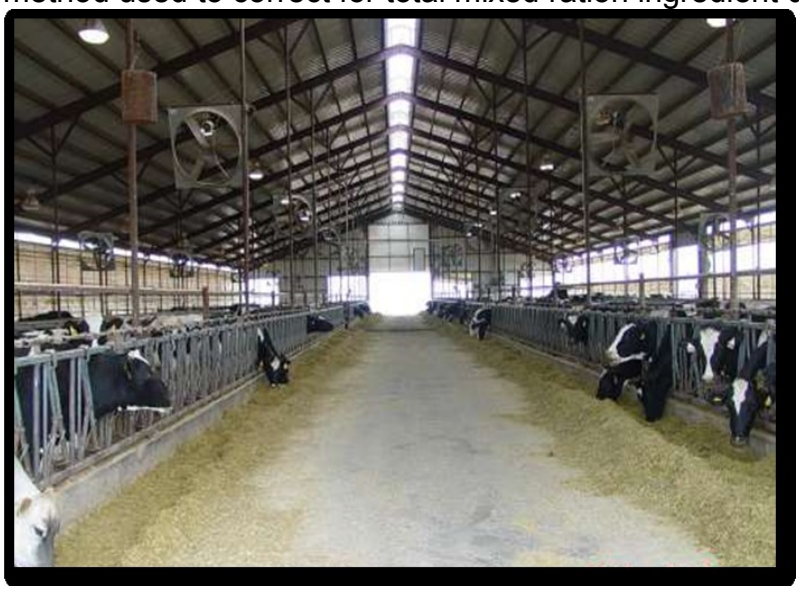
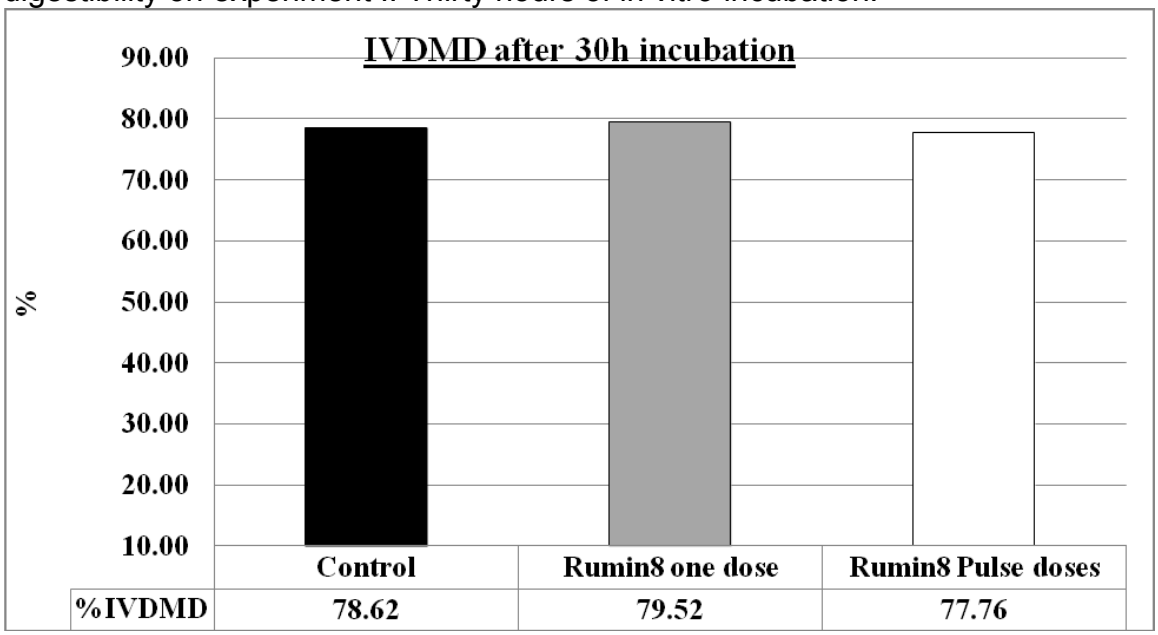
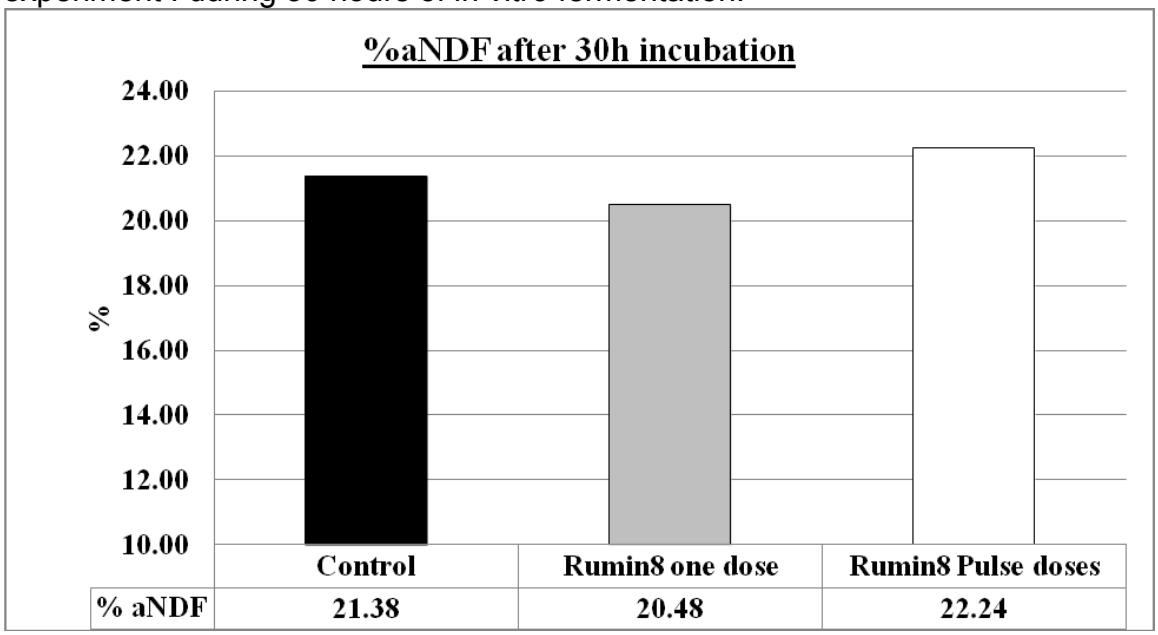


**Figure 3**. Effects of pulse dose frequency of sugar on *in vitro* dry matter digestibility on experiment I. Thirty hours of *in vitro* incubation.



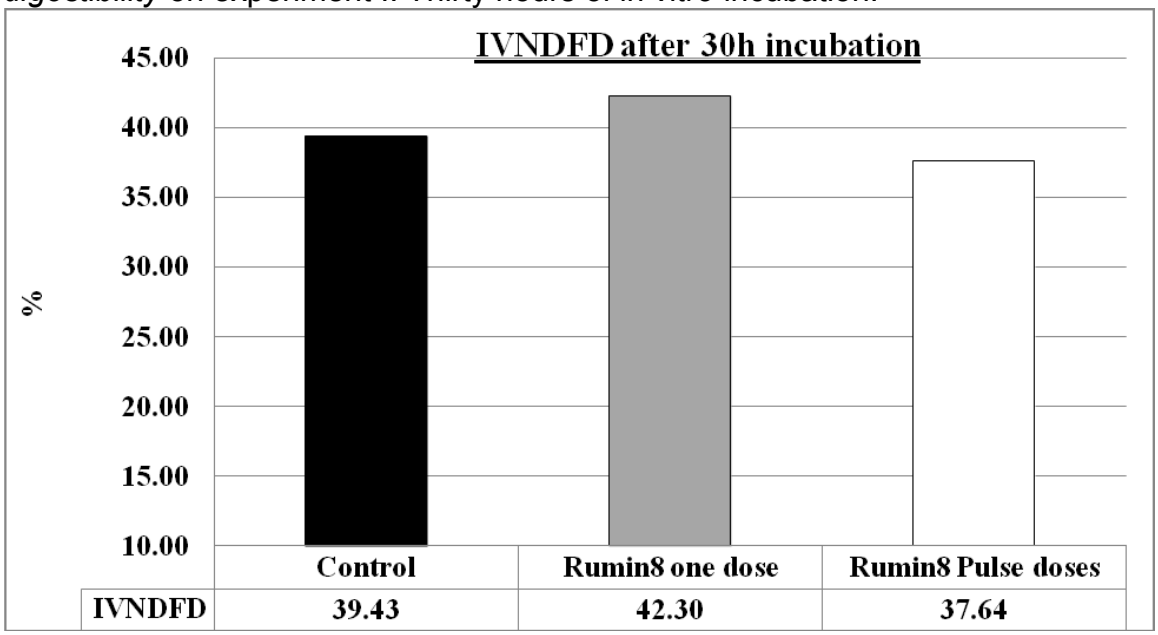

*P-value* = 0.37
SEM = 0.83

**Figure 4.** Effects of pulse dose frequency of sugar on neutral detergent fiber on experiment I during 30 hours of *in vitro* fermentation.



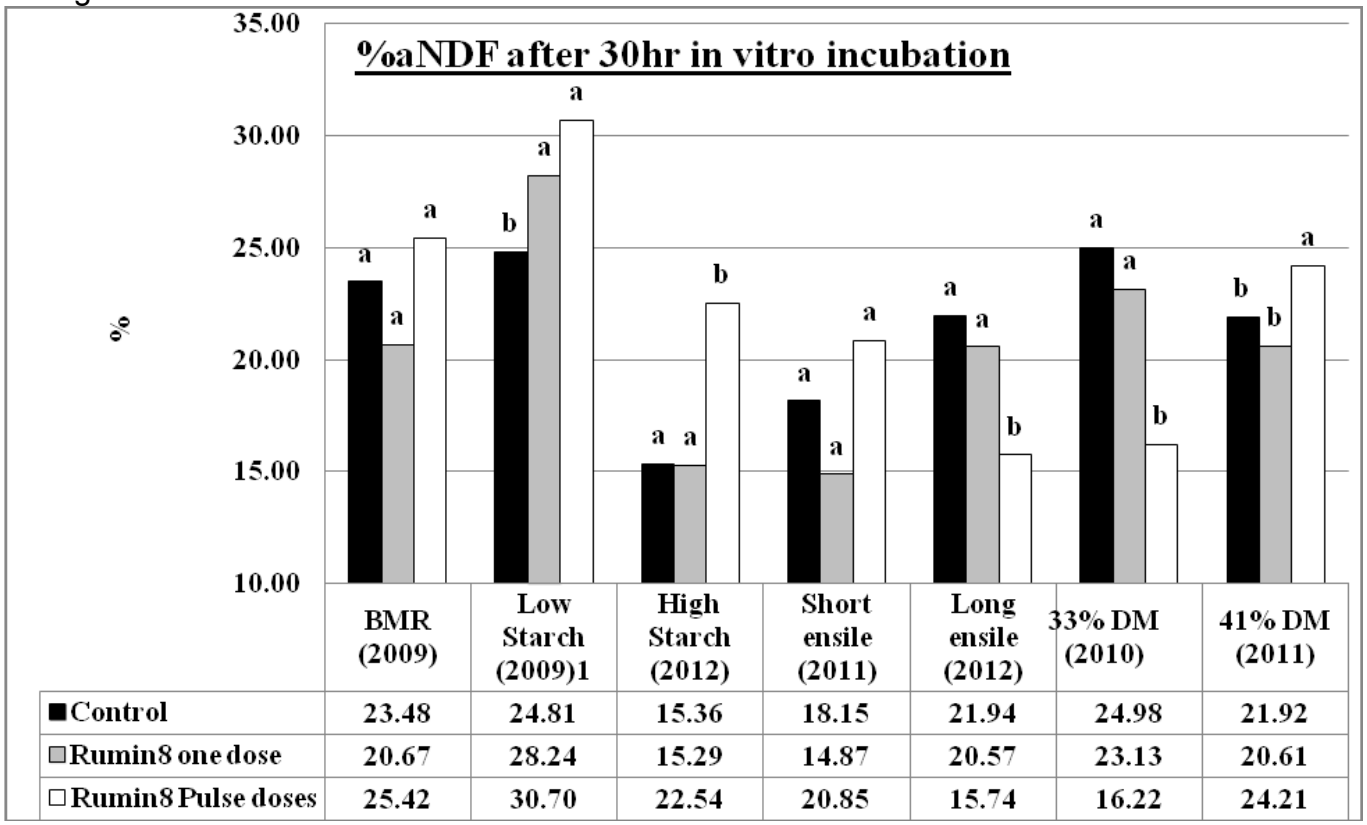

*P-value* = 0.37
SEM = 0.80

**Figure 5**. Effects pulse dose frequency of sugar on *in vitro neutral detergent fiber digestibility* on experiment I. Thirty hours of *in vitro* incubation.




*P-value* = 0.48
SEM = 2.76

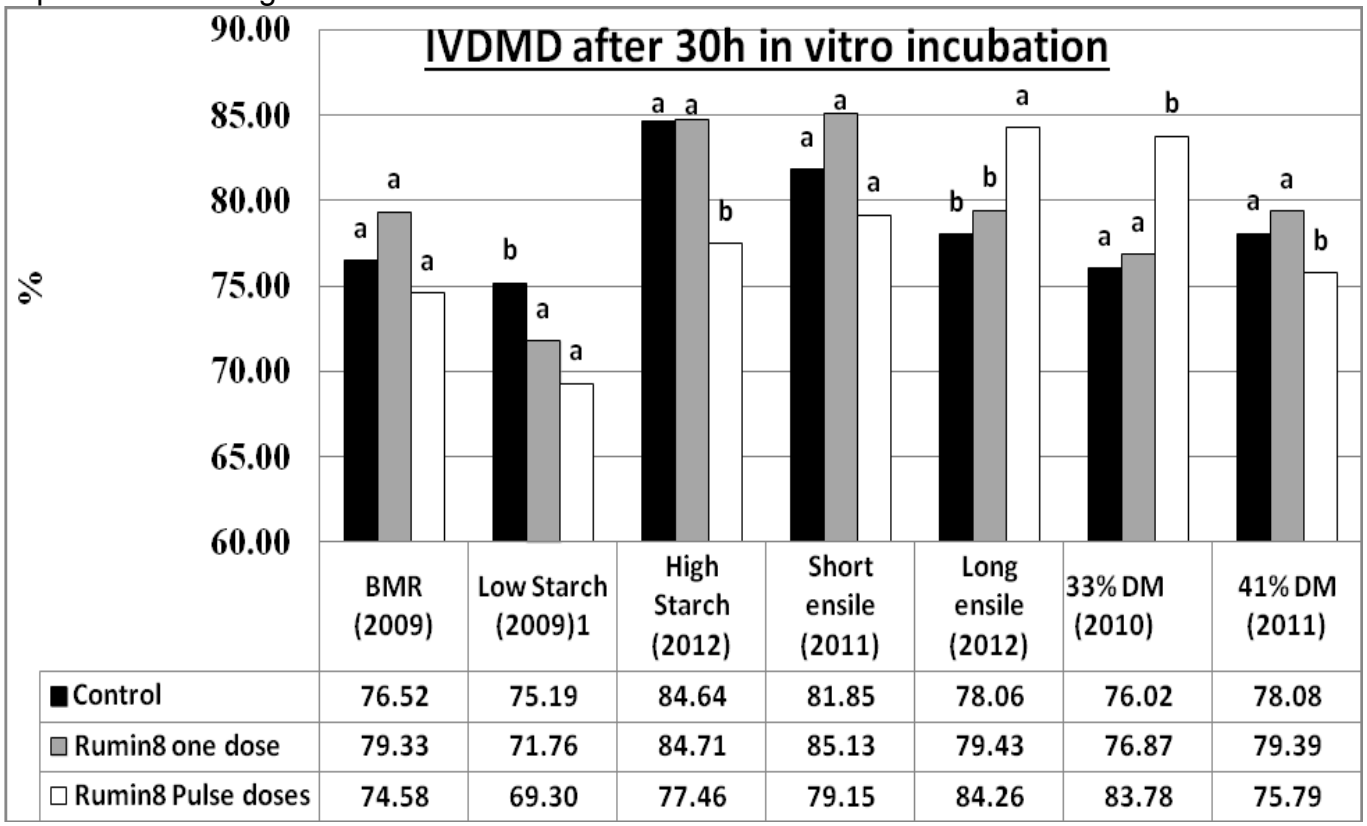
**Figure 6.** Effects of type of silage and pulse dose frequency of sugar on neutral detergent fiber on experiment I during 30 hours of *in vitro* fermentation




| | BMR (2009) | Low Starch (2009)1 | High Starch (2012) | Short ensile (2011) | Long ensile (2012) | 33% DM (2010) | 41% DM (2011) |
|---|---|---|---|---|---|---|---|
| Control | 23.48 | 24.81 | 15.36 | 18.15 | 21.94 | 24.98 | 21.92 |
| Rumin8 one dose | 20.67 | 28.24 | 15.29 | 14.87 | 20.57 | 23.13 | 20.61 |
| Rumin8 Pulse doses | 25.42 | 30.70 | 22.54 | 20.85 | 15.74 | 16.22 | 24.21 |

*P-value* = 0.05
SEM = 2.19

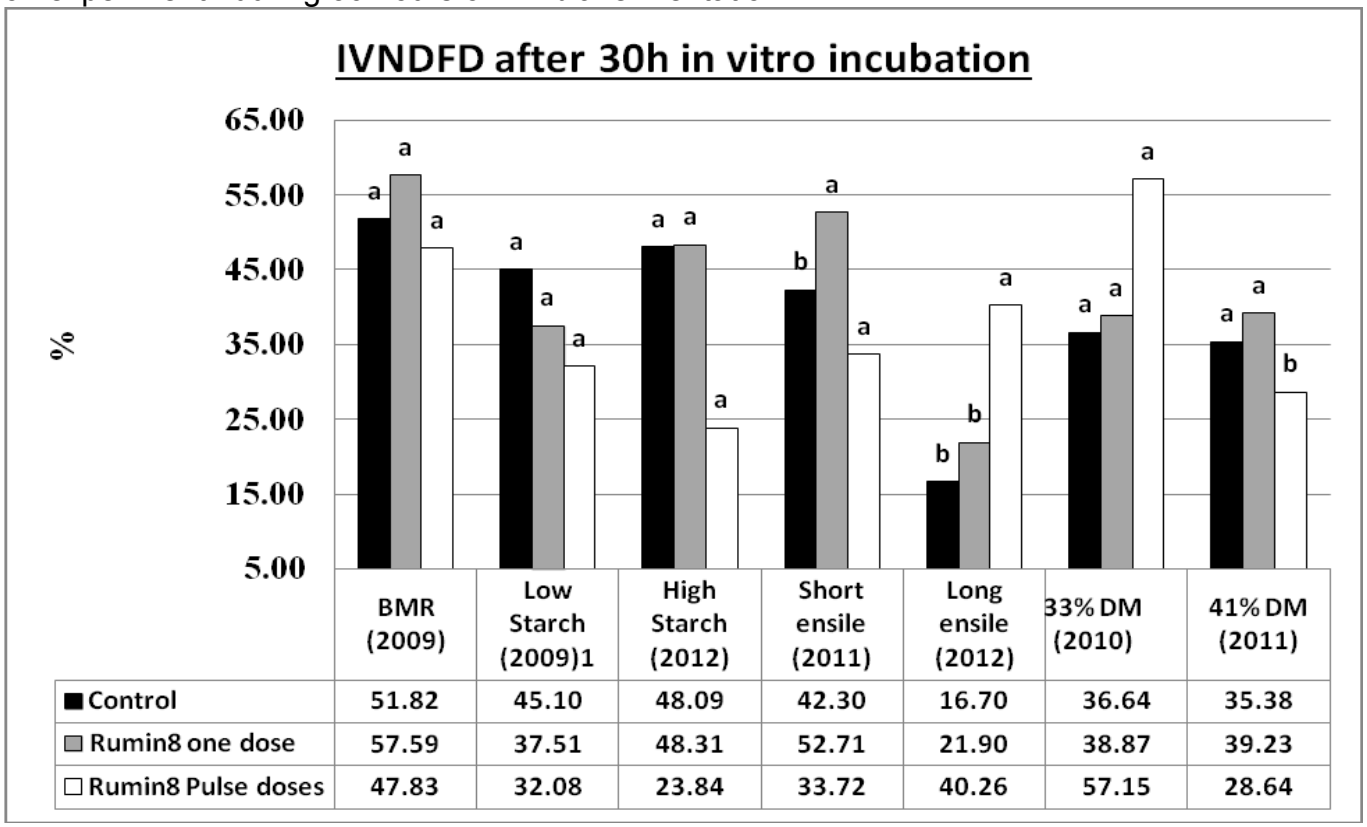
**Figure 7.** Effects of type of silage and pulse dose frequency of sugar on *in vitro* dry matter digestibility on experiment I during 30 hours of *in vitro* fermentation.




| | BMR (2009) | Low Starch (2009)1 | High Starch (2012) | Short ensile (2011) | Long ensile (2012) | 33% DM (2010) | 41% DM (2011) |
|---|---|---|---|---|---|---|---|
| ■ Control | 76.52 | 75.19 | 84.64 | 81.85 | 78.06 | 76.02 | 78.08 |
| ▒ Rumin8 one dose | 79.33 | 71.76 | 84.71 | 85.13 | 79.43 | 76.87 | 79.39 |
| □ Rumin8 Pulse doses | 74.58 | 69.30 | 77.46 | 79.15 | 84.26 | 83.78 | 75.79 |

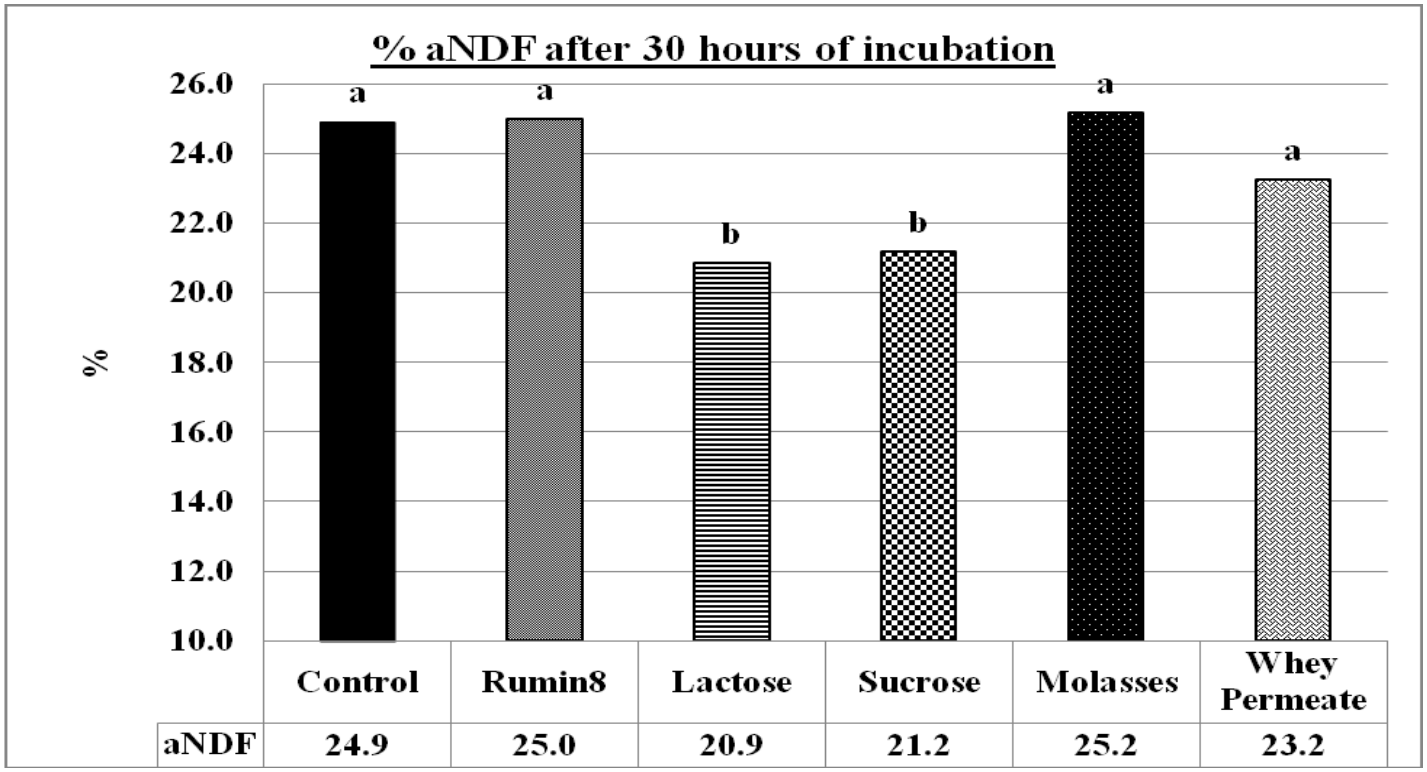
*P-value* = 0.05
SEM = 2.19

**Figure 8.** Effects of type of silage and pulse dose frequency of sugar on *in vitro* neutral detergent fiber digestibility on experiment I during 30 hours of *in vitro* fermentation.



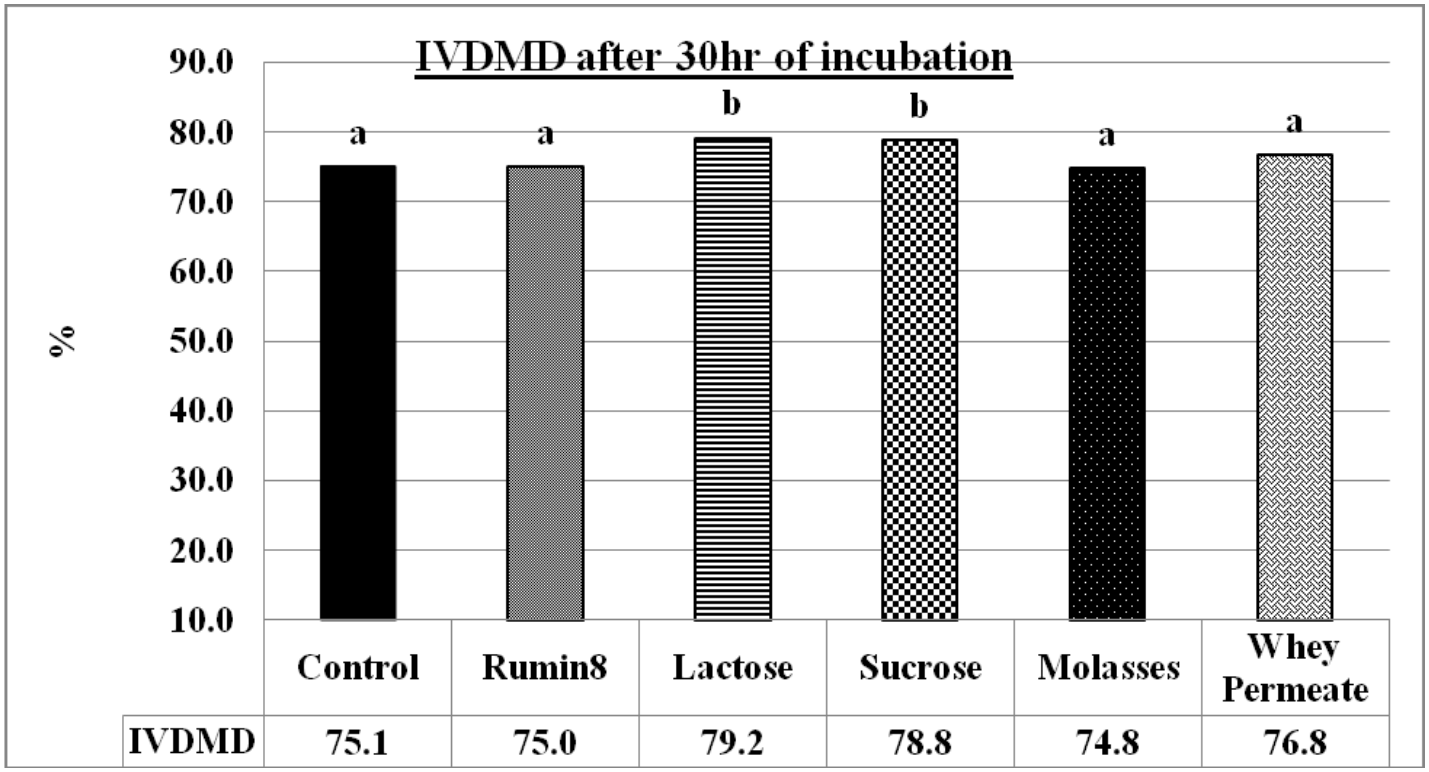

*P-value* = 0.05
SEM = 6.8

**Figure 9**. Effects of different sugar supplementation on neutral detergent fiber on experiment II. After 30 hours of *in vitro* fermentation.



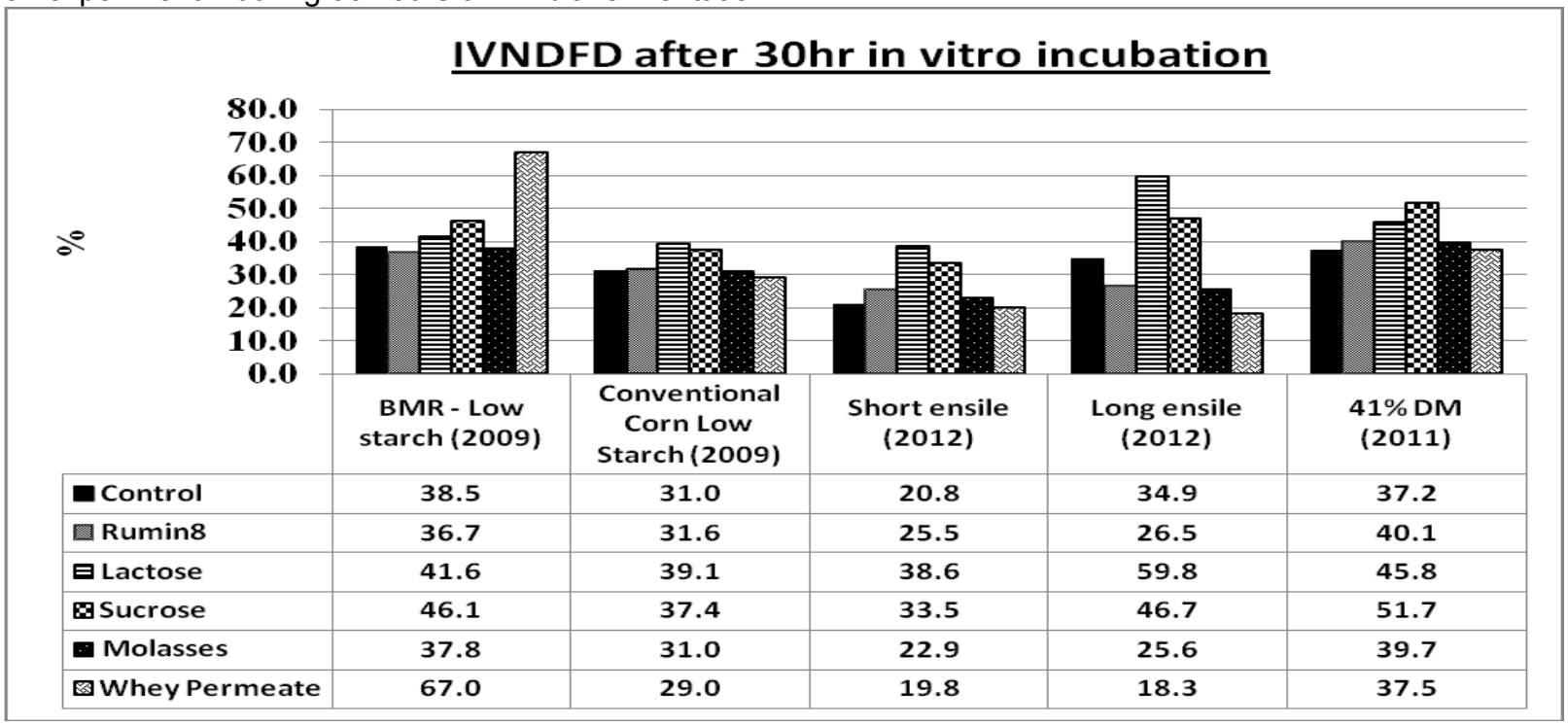

*P-value* < 0.001

**Figure 10.**Effects of different sugar supplementation on in vitro dry matter digestibility on experiment II. Thirty hours of in vitro fermentation.




*P-value* < 0.0001
SEM = 1.7

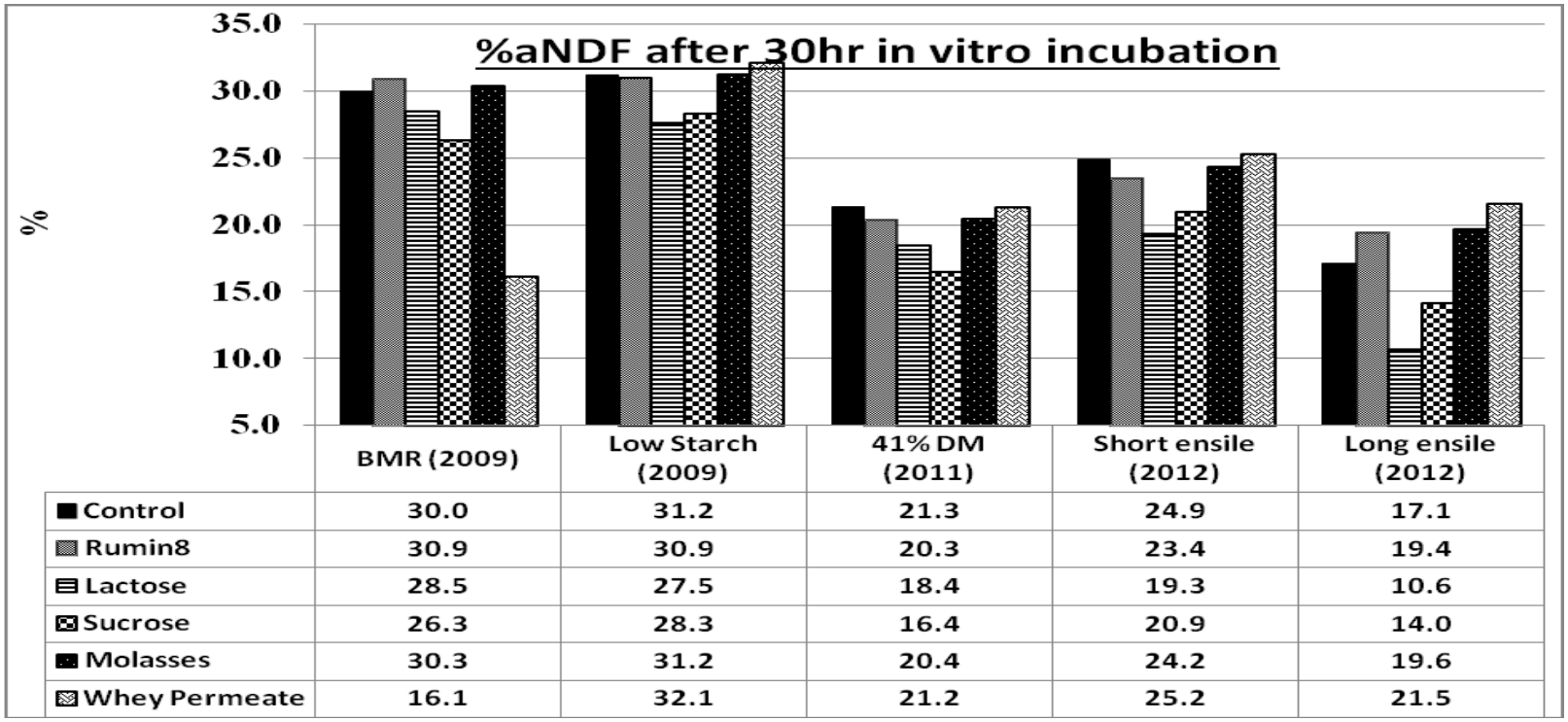
**Figure 11.** Effects of type of silage and different supplemental sugars on *in vitro* neutral detergent fiber digestibility on experiment II during 30 hours of *in vitro* fermentation.




| | BMR - Low starch (2009) | Conventional Corn Low Starch (2009) | Short ensile (2012) | Long ensile (2012) | 41% DM (2011) |
|---|---|---|---|---|---|
| Control | 38.5 | 31.0 | 20.8 | 34.9 | 37.2 |
| Rumin8 | 36.7 | 31.6 | 25.5 | 26.5 | 40.1 |
| Lactose | 41.6 | 39.1 | 38.6 | 59.8 | 45.8 |
| Sucrose | 46.1 | 37.4 | 33.5 | 46.7 | 51.7 |
| Molasses | 37.8 | 31.0 | 22.9 | 25.6 | 39.7 |
| Whey Permeate | 67.0 | 29.0 | 19.8 | 18.3 | 37.5 |

*P-value* < 0.0001
SEM = 4.8

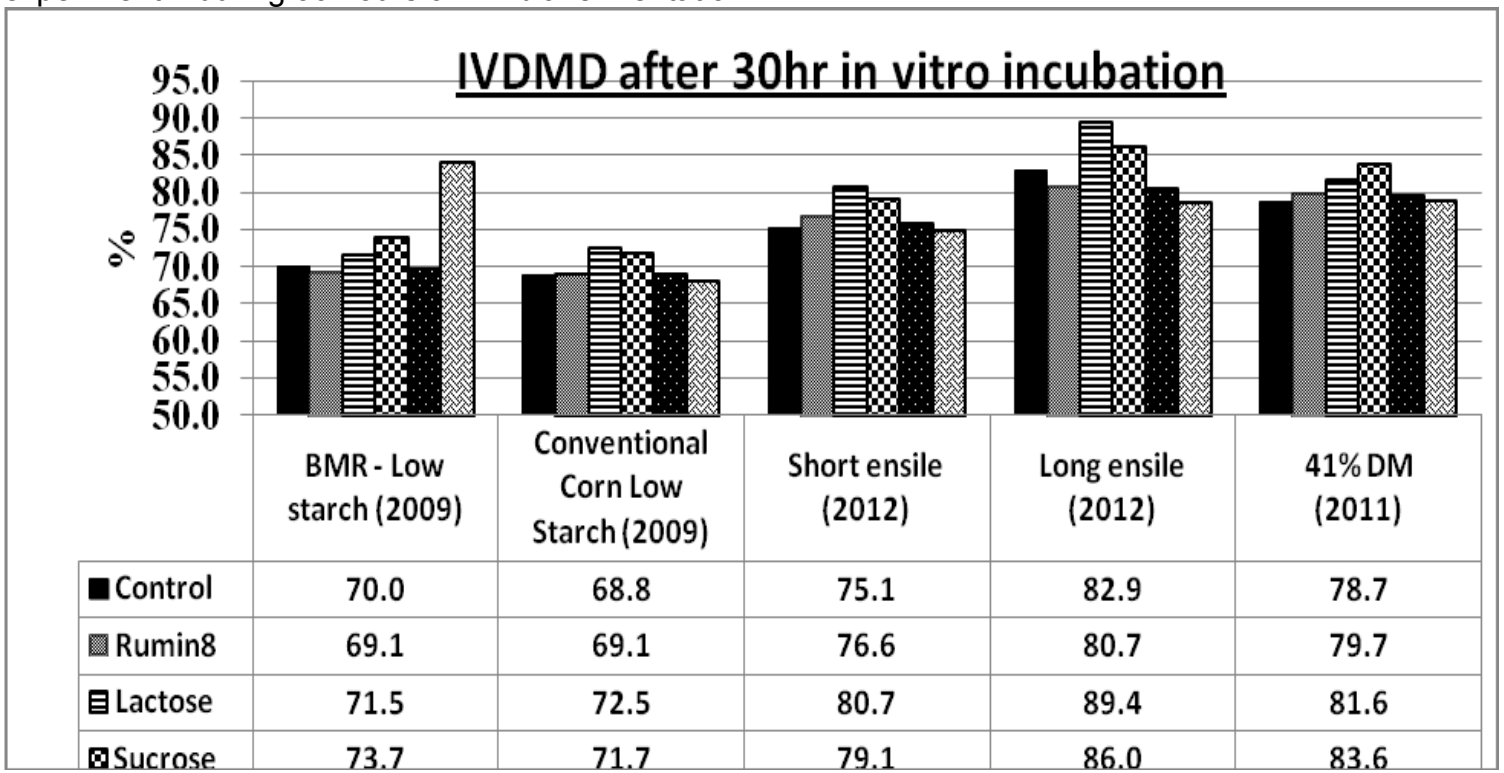
**Figure 12.** Effects of type of silage and different supplemental sugars on neutral detergent fiber on experiment II after 30 hours of *in vitro* fermentation.




| | BMR (2009) | Low Starch (2009) | 41% DM (2011) | Short ensile (2012) | Long ensile (2012) |
|---|---|---|---|---|---|
| Control | 30.0 | 31.2 | 21.3 | 24.9 | 17.1 |
| Rumin8 | 30.9 | 30.9 | 20.3 | 23.4 | 19.4 |
| Lactose | 28.5 | 27.5 | 18.4 | 19.3 | 10.6 |
| Sucrose | 26.3 | 28.3 | 16.4 | 20.9 | 14.0 |
| Molasses | 30.3 | 31.2 | 20.4 | 24.2 | 19.6 |
| Whey Permeate | 16.1 | 32.1 | 21.2 | 25.2 | 21.5 |

*P-value* < 0.001
SEM = 1.7

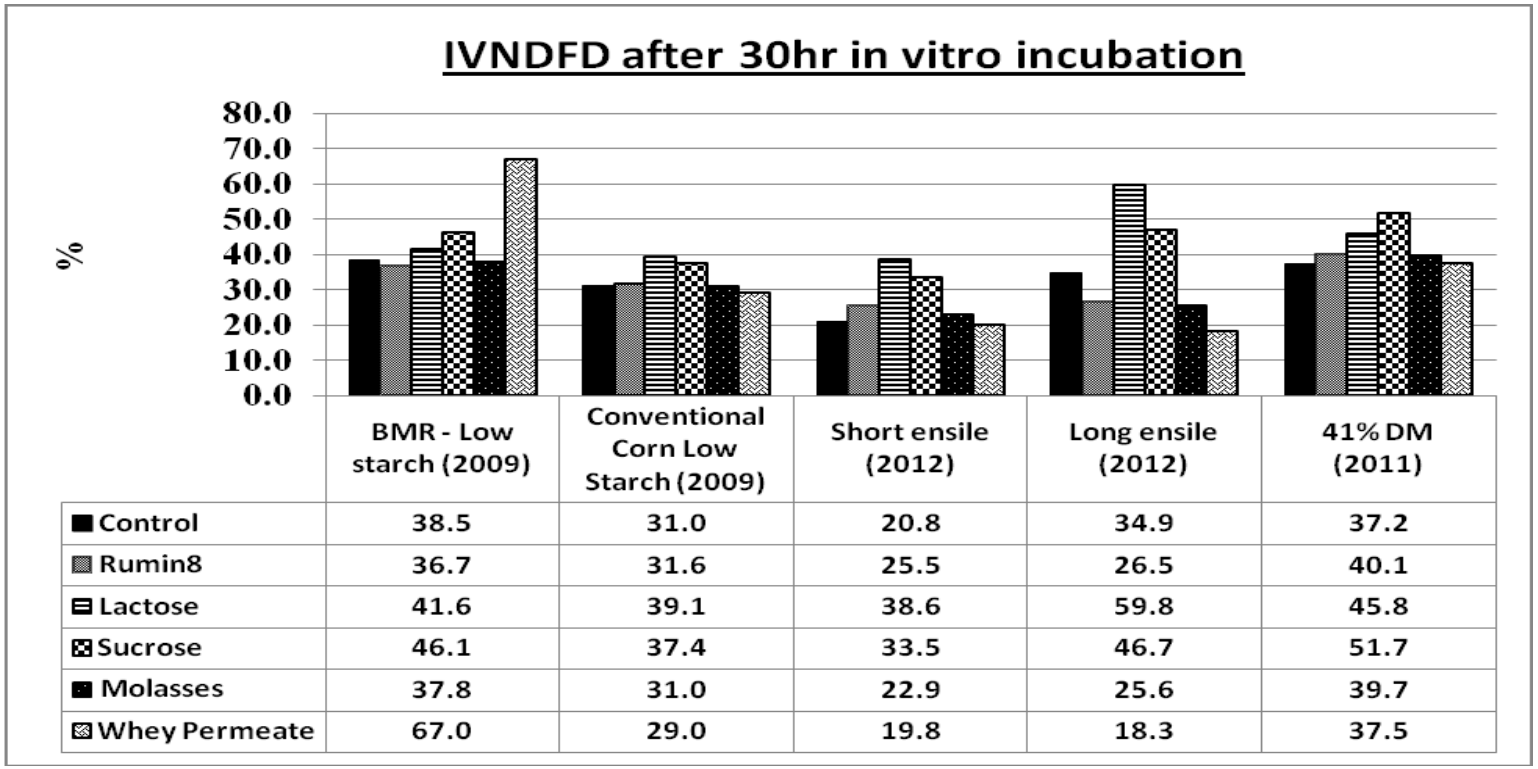
**Figure 13**. Effects of type of silage and different supplemental sugars on *in vitro* dry matter digestibility on experiment II during 30 hours of *in vitro* fermentation.




| | BMR - Low starch (2009) | Conventional Corn Low Starch (2009) | Short ensile (2012) | Long ensile (2012) | 41% DM (2011) |
|---|---|---|---|---|---|
| Control | 70.0 | 68.8 | 75.1 | 82.9 | 78.7 |
| Rumin8 | 69.1 | 69.1 | 76.6 | 80.7 | 79.7 |
| Lactose | 71.5 | 72.5 | 80.7 | 89.4 | 81.6 |
| Sucrose | 73.7 | 71.7 | 79.1 | 86.0 | 83.6 |

*P-value <0.05*
SEM = 1.7

**Figure 14**. Effects of different sugar supplementation on *in vitro* dry matter digestibility on experiment II. Thirty hours of *in vitro* fermentation *in vitro.*




| | BMR - Low starch (2009) | Conventional Corn Low Starch (2009) | Short ensile (2012) | Long ensile (2012) | 41% DM (2011) |
|---|---|---|---|---|---|
| Control | 38.5 | 31.0 | 20.8 | 34.9 | 37.2 |
| Rumin8 | 36.7 | 31.6 | 25.5 | 26.5 | 40.1 |
| Lactose | 41.6 | 39.1 | 38.6 | 59.8 | 45.8 |
| Sucrose | 46.1 | 37.4 | 33.5 | 46.7 | 51.7 |
| Molasses | 37.8 | 31.0 | 22.9 | 25.6 | 39.7 |
| Whey Permeate | 67.0 | 29.0 | 19.8 | 18.3 | 37.5 |

*P-value* < 0.0001
SEM = 4.8

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