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Methane from Lactating Dairy Cattle: Studies for Mitigation, Diurnal Variation, and Role in Energy Metabolism

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METHANE FROM LACTATING DAIRY CATTLE: STUDIES FOR MITIGATION, DIURNAL VARIATION, AND ROLE IN ENERGY METABOLISM

by

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METHANE FROM LACTATING DAIRY CATTLE: STUDIES FOR MITIGATION, DIURNAL VARIATION, AND ROLE IN ENERGY METABOLISM

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Reducing methane production in dairy cattle has received an increased interest due to environmental concerns associated with its potency as a greenhouse gas. Methane represents lost energy in cattle and reduction may increase animal efficiency and productivity. Experiment 1 evaluated strategies of mitigating methane production in lactating dairy cattle with inclusion of dried distillers grains and solubles (**DDGS**), DDGS with added corn oil, and DDGS with added calcium sulfate and effects on energy and nitrogen balance. Inclusion of DDGS, corn oil, and calcium sulfate, increased DMI and milk yield. Methane production was reduced with addition of corn oil and calcium sulfate to diets containing DDGS and these factors did not negatively affect production. When methane production was reduced, more energy was partitioned to milk production. Compared to zero control, cattle consuming DDGS had greater energy balance while nitrogen balance was not affected. Experiment 2 evaluated effects of increasing linolenic acid on methane production in lactating dairy cattle. Dry matter intake, digestibility, milk production and composition were not affected by increased linolenic acid. Increased linolenic acid did not reduce methane production as hypothesized. Results suggest that altering fatty acid profile has little if any influence on methane production. Furthermore, results suggest that previous observations reporting reductions in methane production were a result of fat content not fatty acid profile. Experiment 3 evaluated effects of

feeding frequency (once versus twice daily) on diurnal methane production and energy balance in lactating dairy cattle. Dry matter intake, nutrient digestibility, milk yield and composition were not affected by feeding frequency. Feeding twice daily did not affect total methane production; however, pattern of diurnal methane production was affected with greater methane production observed in the hours following the second feeding. Energy balance was not affected by feeding frequency with observed energy maintenance requirements near 146 kcal/kg $BW^{0.75}$ and k_1 of 0.76. Results emphasize the importance of sampling methane throughout the day to ensure accurate methane production values are obtained. Methane production can be affected by diet and ration-balancing strategies may be a powerful tool to reduce greenhouse gas production in the dairy industry.

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"The definition of insanity is doing the same thing over and over again and expecting different results" –Albert Einstein

"Winning isn't everything, it's the only thing." –Vince Lombardi

"Trust in the Lord with all thine heart; and lean not unto thine own understanding. In all thy ways acknowledge him, and he shall direct thy paths" –Proverbs 3:5-6

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INTRODUCTION

The Innovation Center for U.S. Dairy has set a goal to reduce total greenhouse gas (GHG) emissions for the dairy industry by 25% by the year 2020 (Innovation Center for U.S. Dairy, 2014). Among the most publicized GHG is methane (**CH_4**). According to the Environmental Protection Agency (2010), compared to carbon dioxide (**CO_2**) as a greenhouse gas, CH_4 is 21-25 times more potent. Lactating dairy cattle produce approximately 500 – 600 L/d of CH_4 representing 1.9 – 2.2% of the total GHG emissions in the U.S. (Thoma et al., 2013; Chase, 2014). Ruminants produce approximately 25% of the total CH_4 production, of which dairy cattle contribute to approximately 24.8% of enteric CH_4 production or 0.54% of GHG (Chase, 2014). Because of this CH_4 production, cattle production is one of the most scrutinized sectors of livestock production and is often blamed as a major contributor to climate change. Climate change is not the only reason CH_4 is important. The ruminant animal has a unique capability of utilizing feeds, such as forages not utilized by other species, turning them into highly valued meat and dairy foods. During the natural digestion process, the ruminant animal ferments feed and produces CH_4. Methane production represents an energetic loss for cattle of 2 to 12% (Johnson and Johnson, 1995). Reducing CH_4 production may allow this energy to be repartitioned to milk production or body gain. Practically, a 25% reduction in CH_4 production could increase milk production by approximately 1 L/d (Bruinenberg et al., 2002) or approximately 75 g/d BW gain (Nkrumah et al., 2006). Understanding how to manipulate the diet to reduce CH_4 production would be beneficial for producers who strive to produce food in the most sustainable manner.

One major concern with reducing CH_4 production in dairy cattle is the potential to also reduce milk production and negatively influence milk components, namely fat and protein. There are essentially three routes that are commonly believed to reduce CH_4 production; firstly, manipulation of the diet; secondly, modification of rumen fermentation; and thirdly, modification of production/management practices (Knapp et al., 2014). Many dietary manipulations have been tested in an attempt to reduce CH_4 production. These include altering type of carbohydrate, changing the forage-to-concentrate ratio, improving forage quality, forage processing, and adding a lipid or fatty acid supplement to the diet (Boadi et al., 2004). Altering the forage-to-concentrate ratio could have potential negative effects on milk components, particularly milk fat, thus may not be the best option for reducing CH_4.

The ethanol industry in the Midwest produces a high quality feed byproduct known as dried distillers grains and solubles (DDGS). Currently, ethanol plants are focused on added value from the distilling process and are removing fat from the product. Previous research has observed that inclusion of reduced-fat DDGS reduces CH_4 production in lactating dairy cattle (Foth et al., 2015). However, the effects of adding DDGS to high-forage diets have not been investigated.

A common feed ingredient used to increase the energy concentration of cattle diets is a lipid or fatty acid supplement. Interestingly, lipid supplementation has reduced CH_4 production (Beauchemin et al., 2007). In a study comparing lipid sources, Beauchemin et al. (2007) found that sunflower oil, an unsaturated fatty acid source, decreased CH_4 emissions compared to tallow, a saturated fatty acid source. A reduction in digestibility was observed when tallow and sunflower oil were supplemented, which

may have a negative impact on milk and milk fat production due to decreased acetate production from fiber-fermenting bacteria. Onetti et al. (2001) observed a decrease in the acetate-to-propionate ratio with fat supplementation due to the negative effects on rumen microbes. The dairy industry continuously implements new ideas to improve milk production, so any mitigation technique devised would need to be beneficial to the producer by not reducing production.

During rumen fermentation, acetate production causes the accumulation of hydrogen, which may negatively impact fermentation. One way to reduce the hydrogen concentration in the rumen is for methanogens to utilize hydrogen and produce CH_4. Hence, to effectively reduce CH_4 production, an alternative source would be needed that could compete for hydrogen. These alternatives to CH_4 production are known as hydrogen sinks. One such sink is sulfate, which may be added to the diet to reduce CH_4 production (Van Zijderveld et al., 2010). Addition of sulfate may reduce CH_4 production; however, there are concerns as high inclusion may result in excessive hydrogen sulfide gas accumulation in the rumen, which can cause polioencephalomalacia (Merck, 2010). However, the dairy NRC (2001) reported that there have been no observations of polioencephalomalacia in dairy cattle. Research is needed to understand how intake and milk production are affected by addition of sulfate in lactating dairy cattle diets, as the majority of the work done to our knowledge was in sheep.

Measuring CH_4 production in cattle can be a technically challenging process. These challenges include acquiring the instruments and equipment needed, adapting animals to experimental devices, and allocating the time and expertise needed to operate and maintain equipment. Research has illustrated that CH_4 produced in the rumen

accounts for about 87% of total enteric CH_4, whereas the large intestine accounts for about 8 to 13% of the total production (Torrent and Johnson, 1994; Boadi et al., 2004). However, approximately 89% of the CH_4 produced in the large intestine is excreted via the lungs (Murray et al., 1976). Total CH_4 excreted via lungs or eructation is nearly 99%. Therefore, the majority of the gases can be collected using headbox-style indirect calorimetry. One facet of CH_4 production that has not been described well is how it varies diurnally. Feeding multiple times during a day has altered rumen fermentation as evidenced by a reduced duration of pH under 5.8 and the corresponding potential for acidosis (Macmillan et al., 2017). Brask et al. (2015) observed that CH_4 production had a minor peak after cows were fed in the morning, but had a major peak after a second daily feeding in the evening. It is important to characterize diurnal variation in CH_4 production because if the variation is large, methods that employ single-time-point sampling may result in biased estimates. A more complete understanding of the diurnal variation is needed to better estimate total CH_4 production while also demonstrating potential influence on estimates of energy utilization.

In cattle, methane production is just one component of a very large energetic web. Correctly identifying each component of energy loss in lactating dairy cattle is laborious, challenging, and expensive. Calorimetry systems such as the headbox-style system can be utilized to measure energy metabolism of dairy cows while allowing the animals to be milked. Key components measured in the headbox include oxygen consumed, CO_2 and CH_4 produced/emitted, and indirectly, heat production. These values need to be measured to determine if CH_4 reduction techniques are affecting energy utilization. Dairy cattle have changed due to genetic selection and maintenance requirements have been observed

to increase (Moraes et al., 2015). Further investigation into maintenance energy requirements and how they may change with CH_4 mitigation is needed.

Therefore, the objectives of this research were to 1) determine how diets may be formulated to reduce CH_4 production in lactating dairy cattle, 2) study the influence of fat source and profile of fatty acids on CH_4 production in lactating dairy cattle, 3) evaluate the relationship between CH_4 production and overall energy utilization in lactating dairy cattle, and 4) describe the diurnal variation of CH_4 production and determine the influence of feeding frequency on it.

REFERENCES

Brask, M., M. R. Weisbjerg, A. L. F. Hellwing, A. Bannink, and P. Lund. 2015. Methane production and diurnal variation measured in dairy cows and predicted from fermentation pattern and nutrient or carbon flow. Animal 9(11):1795-1806.

Beauchemin, K. A., S. M. McGinn, and H. V. Petit. 2007. Methane abatement strategies for cattle: Lipid supplementation of diets. Can. J. Anim. Sci. 87:431-440.

Boadi, D., C. Benchaar, J. Chiquette, and D. Masse. 2004. Mitigation strategies to reduce enteric methane emissions from dairy cows: Update review. Can. J. Anim. Sci. 84:319-335.

Bruinenberg, M.H., Y. van der Honing, R.E. Agnew, T. Yan, A.M. van Vuuren, and H. Valk. 2002. Energy metabolism of dairy cows fed on grass. Livestock Prod. Sci. 75:117-128.

Chase, L.E. 2014. Carbon footprint and the dairy industry. Cornell Nutrition Conference Animal Science Conference Proceedings. Cornell Univ. Ithaca, NY.

Environmental Protection Agency. 2010. Methane and nitrous oxide emissions. U.S. Environmental Protection Agency, Washington, DC, USA.

Foth, A. J., T. Brown-Brandl, K. J. Hanford, P. S. Miller, G. Garcia Gomes, and P. J. Kononoff. 2015. Energy content of reduced-fat dried distillers grains with solubles for lactating dairy cows. J. Dairy Sci. 98:7142-7152.

Innovation Center for U.S. Dairy. 2013. Memorandum of Understanding between United States Department of Agriculture and Innovation Center for U.S. Dairy. Accessed September 26, 2017. https://www.usda/gov/sites/default/files/documents/usda-mou-innovation-center-us-dairy.pdf

Johnson, K. A., and D. E. Johnson. 1995. Methane emissions from cattle. J. Anim. Sci. 73:2483-2492.

Knapp, J. R., G. L. Laur, P. A. Vadas, W. P. Weiss, and J. M. Tricarico. 2014. Invited review: Enteric methane in dairy cattle production: Quantifying the opportunities and impact of reducing emissions. J. Dairy Sci. 97:3231-3261.

Macmillan, K., X. Gao, and M. Oba. 2017. Increased feeding frequency increased milk fat yield and may reduce the severity of subacute ruminal acidosis in higher-risk cows. J. Dairy Sci. 100:1045-1054.

Merck Veterinary Manual. 2010. Polioencephalomalacia. 10th ed. Merck & CO., Inc. Whitehouse Station, NJ.

Moraes, L.E., E. Kebreab, A.B. Strathe, J. Dijkstra, J. France, D.P. Casper, and J.G. Fadel. 2015. Multivariate and univariate analysis of energy balance data from lactating dairy cows. J. Dairy Sci. 98:4012-4029.

Murray, R.M., A.M. Bryant, and R.A. Leng. 1976. Rates of production of methane in the rumen and large intestine of sheep. Br. J. Nutr. 36:1-14.

National Research Council. 2001. Nutrient Requirements of Dairy Cattle. 7th rev. ed. Natl. Acad. Press, Washington, DC.

Nkrumah, J.D., E.K. Okine, G.W. Mathison, K. Schmid, C. Li, J.A. Basarab, M.A. Price, Z. Wang, and S.S. Moore. 2006. Relationships of feedlot feed efficiency, performance, and feeding behavior with metabolic rate, methane production, and energy partitioning in beef cattle. J. Anim. Sci. 84:145-153.

Onetti, S.G., R.D. Shaver, M.A. McGuire, and R.R. Grummer. 2001. Effect of type and level of dietary fat on rumen fermentation and performance of dairy cows fed corn silage-based diets. J. Dairy Sci. 84:2751-2759.

Thoma, G., J. Popp, D. Nutter, D. Shonnard, R. Ulrich, M. Matlock, D.S. Kim, Z. Neiderman, N. Kemper, C. East, and F. Adom. 2013. Greenhouse gas emissions from milk production and consumption in the United States: A cradle-to-grave life cycle assessment circa 2008. International Dairy Journal 31:S3 – S14.

Torrent, J., and D.E. Johnson. 1994. Methane production in the large intestine of sheep. Pages 391-394 in Energy metabolism of farm animals. EAAP Publication No. 76. CSIC. Publishing Service. Granada, Spain.

Van Zijderveld, S.M., W. J. J. Gerrits, J. A. Apajalahti, J. R. Newbold, J. Dijkstra, R. A. Leng, and H. B. Perdok. 2010. Nitrate and sulfate: Effective alternative hydrogen sinks for mitigation of ruminal methane production in sheep. J. Dairy Sci. 93:5856-5866.

CHAPTER 2

LITERATURE REVIEW

Calorimetry Methods

Calorimetry is best defined as the transfer of heat between a subject such as an animal and its environment (Nienaber et al., 2009). Calorimetry was first recognized by Lavoisier, who in 1777 developed and used direct and indirect calorimetry systems to explain oxygen's (O_2) role in life cycles and particularly oxidation (Brody, 1945). Direct calorimetry measures the heat lost from the animal directly, whereas indirect calorimetry measures the heat production of the animal (Nienaber et al., 2009). Both systems are generally accepted amongst the scientific community and results are similar unless some type of work is being performed by the animals (Nienaber et al., 2009). When work is performed by the animal, such as producing milk or gaining tissue, use of indirect calorimetry more accurately measures the change in heat production (Nienaber et al., 2009). These two systems are not generally compared against the other because of the different analytical principles and assumptions used (Johnson et al., 2003).

The primary purpose of using calorimetry in animal nutrition research is to quantify energy utilization by the animal compared to the energy supply of their diet. Johnson et al. (2003) described 3 main reasons for energy research in animals, which included the need to: 1) describe the relationship between heat production and the exchange of gases, 2) determine the different routes through which energy is expended, and 3) derive feeding values as they are related to energy requirements and expenditures.

However, calorimetry can be used to study the dynamics of thermoregulation and other factors involved in the environment of the animal (Nienaber et al., 2009).

Direct Calorimetry

Direct calorimetry was discovered when Lavoiseir and Laplace studied the amount of heat and CO_2 produced while ice surrounding a guinea pig melted (Brody, 1945). Direct calorimetry uses a water jacket that absorbs heat and collects the exhaled air. Direct calorimetry measures heat loss via radiation, convection and conduction that is normally measured using a whole animal or human chambers (Blaxter, 1989). According to Nienabar et al. (2009), by measuring both the evaporative and sensible heat losses, direct calorimetry can be used to measure total heat lost by the animal.

There are many types of direct calorimeters such as the respiratory, gradient layer, convection, and spot or local calorimeters as described by Nienaber et al. (2009). The respiratory calorimeter is also known as an adiabatic calorimeter because no heat is lost to or from the box. The gradient layer calorimeter measures the heat within the walls and as a result can be used to generate rapid measures from the animal. The gradient layer calorimeter has been used since the 1880's, but a major breakthrough occurred in the 1940's when Benzinger and Kintzinger (1949) were able to develop thermoelectric heat flow meters for humans. With this type of calorimeter system, heat loss can be rapidly measured and major changes can be observed in real-time. These changes are usually a result of physical movement, such as a change in body position (i.e. standing up vs. lying down), and the resulting effect of this activity is lost heat.

Another system that can be used is a spot or portable calorimeter, which was developed by Hillman et al. (2001) at Cornell University and used with dairy cattle. The device allows the investigator to measure the air confined in the defined sample area (76 mm × 102 mm) and also measures the temperature and relative humidity. This allows for the measurement of sensible and latent heat losses. This system could be beneficial in hot, humid outdoor environments where whole chambers are not commonly found. The convection calorimeter forces heat through a ventilation system and then determines the difference in temperature to calculate total heat produced. The heat produced in these calorimeters is considered sensible heat loss from the animal (Nienaber et al., 2009).

Indirect Calorimetry

Indirect calorimetry has been used since 1777 when Lavoisier and Laplace first used a guinea pig to demonstrate the relationship between the volume of gas and specifically carbon dioxide (**CO_2**) exhaled to the volume of ice melted around the animal (Brody 1945). This experiment was the first to indicate that O_2 consumption and CO_2 production are closely related to heat production. Consequently, indirect calorimetry is used to measure the gas exchange within the animal, which is a result of catabolism of body tissue or the metabolism of feed by measuring the rate of CO_2, methane (**CH_4**), and urinary nitrogen being produced, and O_2 being consumed (Nienaber et al., 2009). Indirect calorimetry, therefore, is closely based on the relationship between organic compounds that are oxidized, which relates to the O_2 consumed and the CO_2 produced (Young et al., 1975). Incomplete oxidation of proteins for example, will excrete urea. The indirect calorimetry method takes this into the calculation of heat production. This system is able to more robustly account for incomplete oxidation of feeds compared to direct

calorimetry. Direct calorimetry requires the transfer of heat to acquire heat produced, which usually has a lag time because the body sequesters heat, particularly in non-steady-state situations (Young et al., 1975).

Indirect Calorimetry Methods

There are two main types of indirect calorimetry systems. The first is known as closed-circuit system and was developed in France by Henri Regnault and Juels Reiset (College of France). The closed-circuit system recycles captured air back to the animal after different absorbents are used to remove water vapor and CO_2 (Blaxter, 1962). The closed-circuit system measures the mass of the absorbents to determine the amount of CO_2 produced. The system needs to measure the concentrations of both CO_2 and O_2 before and after the experiment to make sure they remain constant (Blaxter, 1962). The second type of system is known as an open-circuit system, which was developed by Max von Pettenkofer and Carl von Voit (University of Munich). Open-circuit calorimetry originally only measured CO_2, but was latter modified to also measure the concentrations of O_2, CO_2, and CH_4 going into the chamber (considered fresh air) and exiting the chamber (Blaxter, 1962; Van Soest, 1994). This modified system later became known as the Pettenkofer-Tigerstedt Apparatus (Blaxter, 1962). An open-circuit system needs precise measurement of the flow rates and properties of incoming air as well as the outgoing air. Proper ventilation rates are needed. If ventilation is low it could stimulate respiration in the animal due to the CO_2 accumulation and water vapor could also become a problem by increasing heat production (Blaxter, 1962). In this system, the gas may be collected in a bag and analyzed later for gases or these gases may be analyzed in real-time. Open-circuit systems can be further categorized by methods of animal confinement.

These include entire confinement, where the animal is fully enclosed in a respiration chamber and total gas exchange for the animal is measured, while other methods employ the use of masks, hoods, or tracheal cannula, where only gaseous exchange from the lungs is measured (Blaxter, 1989).

As described above, there are many types of indirect calorimetry methods that can determine gas exchange in the animal. These methods include enclosed whole-animal chambers, headbox-style calorimeters, the carbon dioxide entry rate technique (CERT), and comparative slaughter. Sample collection technique will vary by location as well as availability (Johnson and Johnson, 1995). Cattle that normally graze would have a greater stress imposed upon them when using a headbox or whole-body chamber because they are used to moving about freely.

Whole-animal chamber method. Historically, whole-animal chambers were the most common type of indirect calorimeter used in energy studies (Figures 2.1 to 2.3). The ability to control the environment allows for consistent, reliable, and stable measurements (Storm et al., 2012). These chambers are highly accurate and all gas emissions from the animal (from eructation and hindgut fermentation) are sampled and accounted for in measures of total gas production. Whole-animal chambers need to be well sealed and this may create a problem (i.e. suffocation) with airflow for the animal. To create an environment more suitable for the animal, the chambers are usually equipped with air conditioners, dehumidifiers and methods to remove the feces and urine while also providing feed and allowing the animal to move about somewhat normally (Johnson and Johnson, 1995). Depending on the design of the system, some restraint may be needed to ensure that the feces and urine are properly removed. Typically, when an animal is

removed from its environment, feed intake decreases and this is generally believed to be a result of stress involved in environmental change. The effect of the apparatus on the animal's feed consumption is important to note, as CH_4 production is positively correlated with feed intake, thus an abnormal drop in feed intake will lead to a decrease in overall CH_4 production (Johnson and Johnson, 1995). Because the system is open-circuit, it is difficult to prevent air leaks. The whole-animal chamber is designed to compensate or tolerate such leaks by creating a negative pressure inside the chamber, as was described by Young et al. (1975). The negative pressure forces air to move inwards through leaks, preventing expelled gases from leaking out of the system and not being measured. Gas recovery is measured to ensure that the leaks are minimal and that the system measurements are valid. The air line from the chamber to the analyzer needs to be under positive pressure. This positive pressure allows for small leaks, but the leakage is outward so the sample gas is not diluted by entry of fresh air. The ventilation rate is set to obtain a difference in O_2 and CO_2 content that can be determined by gas analysis. Gas samples from the chamber are also corrected to standard temperature and pressure, which are based upon the dry-bulb temperature, dew-point temperature, and pressure of the air in the chamber. The accuracy of the whole-animal chamber method depends largely on the ability to accurately measure the concentration of individual gases and overall gas volume. An additional source of error in determining heat production from whole-animal chamber systems includes gas temperature, while the pressure and moisture content inside the chamber are of much less significance.

The chamber is also designed to allow for the collection of urine and feces. Because of the large design, feed and water need to be placed inside the chamber. Some

chambers are large enough to accommodate multiple animals (McGinn et al., 2004), which may reduce the potential stress related to isolation. A major challenge with whole-animal chambers is the initial expense involved in building them, especially when trying to accommodate large animals such as lactating dairy cows. The cows also need to be milked and to do so the calorimeter must be shut down while the technician enters the chamber to conduct the chore. In general and in terms of indirect calorimetry, it is widely accepted that whole-animals chambers are the gold standard, however, many researchers that have compared it to other methods observed similar accuracies between methods (Young et al., 1975; Sahlu et al., 1988; Boadi and Wittenberg, 2002; Grainger et al., 2007).

Headbox-style calorimetry method. Headbox-style indirect calorimeters often referred to as hoods or headboxes, have received increased attention in the last few years (Figure 2.4). The headbox is designed to function similarly to that of the whole-animal chamber with the exception that only the animal's head is enclosed inside the hood. A slight disadvantage to the headbox method is that it accounts for gas produced via eructation, but it does not account for gases lost from hindgut fermentation. However, 90% of the gas produced after the rumen is absorbed back into the bloodstream and exhaled through the lungs (Boadi and Wittenberg, 2002) so there is only a minor portion of the hindgut production that is expelled outside of the headbox (Torrent and Johnson, 1994; Boadi et al., 2004).

The headbox system is under negative pressure, similar to that of the whole-animal chamber. Similarly, the accuracy of the method depends on the ability to accurately measure the gases as well as the gas flow in the headbox (Young et al., 1975).

Headboxes are much less expensive than whole-animal chambers, and have the potential to be moved to the animal. The ability to move the unit to the animal is a major advantage because it can reduce the added stress of being moved to a new environment and/or being in isolation. It is still important to adapt the animal to the headbox. The headbox is usually constructed out of polycarbonate or acrylic sheeting that surrounds the front and sides of the animal. This construction allows the animals to see what is going on around them and also gives the opportunity to see other animals (Kelly et al., 1994; Place et al., 2011). Another advantage of the headbox is that it allows the animal to be milked without an interruption in the gas analysis. Validation of the headbox method has been observed by Nienaber et al. (2009) by burning alcohol within the headbox and measuring the gas concentration in collection bags after a 2-h period. Recoveries for CO_2 and O_2 were within 98 to 103%, indicating proper recovery (Nienaber et al., 2009).

Carbon dioxide entry rate technique (CERT) method. The carbon dioxide entry rate technique (**CERT**) method has been used to indirectly measure CO_2 production in the body and, in cattle that are grazing, to estimate total heat production (Herselman et al., 1998). The method was first developed in the late 1950's and was a response to the lack of data available on animals not housed in confinement (Schürch and Wenk, 1970). The procedure involves the infusion of a ^{14}C isotope that is in the form of ^{14}C-bicarbonate (Sanchez and Morris, 1984). The ^{14}C is then allowed time to equilibrate with fluids within the animal, particularly those which represent major pools of CO_2. The body loses ^{14}C through many different routes, which include CO_2; these include respiration or rumen fermentation, feces and urine. After it has reached an equilibrium, a tube running through

the animals' cheek is then used to collect saliva samples from the parotid gland and this saliva is then deposited into a backpack and later analyzed for the labeled carbon.

In a study using sheep to validate the CERT method, Sahlu et al. (1988) compared the CERT to indirect respiration chambers. They observed that CO_2 production was similar between methods (20.6 vs. 20.3 L/kg of $BW^{0.75}$ for CERT vs. respiration chambers, respectively). They also did not observe any difference in estimates of heat production and the respiratory quotient. One concern when using the CERT method is there is potential for radioactive contamination as ^{14}C is infused into the animal (Sahlu et al., 1988). Nonetheless, the CERT method is generally accepted as a method that can be used to accurately predict CO_2 production in fed animals and may be used to predict heat production.

Comparative slaughter method. The comparative slaughter method is based on the principle that metabolizable energy (**ME**) is equal to the sum of retained energy (**RE**) and heat production (**HP**), but unlike calorimetry, which measures HP and ME and calculates RE, comparative slaughter measures RE and ME and calculates HP by difference (Nienaber et al., 2009). Retained energy and ME are determined by determining the energy contained in the gastrointestinal tract, liver, hind limb, gravid uterus, or fetus after the animal is slaughtered and then uses this body composition data to determine HP. There are several disadvantages to the comparative slaughter method; these include the requirements to slaughter the animals, time needed to conduct the experiments, large number of animals needed, and analytical care needed to accurately determine the energy contained in individual tissues (Kelly et al., 1994). Another disadvantage of this method is the challenge of properly determining the energy value for

a lactating dairy cow that is producing milk, thus, this method is not recommended for lactating dairy cows.

Gas Sampling Methods

There are many methods that can be used to determine gas exchange in the animal. These methods include enclosed whole-animal chambers, headbox-style indirect calorimetry, sulfur hexafluoride (SF_6) tracer, infrared lasers, and GreenFeed systems (C-Lock Inc., Rapid City, SD). Respiration chamber and headbox-style indirect calorimetry have been discussed above and are the standard for measuring gases in animals. Sample collection technique will vary by location as well as availability (Johnson and Johnson, 1995). Cattle that normally graze would have a greater stress imposed upon them when using a headbox or whole-body chamber because they are used to moving about freely. However, with the SF_6 or GreenFeed system, grazing cattle could move about and graze in a normal pattern so gas samples could be collected in their respective environment.

Sulfur hexafluoride (SF_6) method. The sulfur hexafluoride (SF_6) method has been used with cattle that are either grazing pasture or housed in free-stall barns (Figure 2.5). Based on indirect calorimetry, the method determines CH_4 production using a tracer gas that is diffused into the rumen and originates from a slow release from a permeation tube. The release of SF_6 is used to calculate gas production in the animal based on release rate and concentration of gases measured (Arbre et al., 2016). Before the release rate is determined, the SF_6 needs to equilibrate. If the release rate is not calibrated correctly, either an upward or downward bias may result (Vlaming et al., 2007). Hence, proper understanding of the release rate is crucial for proper measurement. According to this method, gas is collected from the nostril and deposited into a chamber that rests on the

back or neck of the animal. The sample is analyzed using a gas analyzer. Using this analysis, total CH_4 production is estimated based upon SF_6 concentration observed (Grainger et al., 2007).

Advantages to the SF_6 method include sampling cattle in their natural environment such as grazing conditions, it is relatively inexpensive compared to whole-animal chambers, individual measurements can be obtained for each animal, and a large number of cattle can be measured simultaneously (Beauchemin et al., 2012). The major disadvantage of the SF_6 tracer method is the large degree of expected variability that is observed within animals (Pinares-Patiño et al., 2011). Another potential disadvantage is that it does not measure CH_4 expelled from hindgut fermentation (Muñoz et al., 2012). Beauchemin et al. (2012) observed that the SF_6 method should not be used in conjunction with cannulated cattle as this increases the variability within measurements. If cannulated cattle were to be used, Beauchemin et al. (2012) concluded that the cannula should be sealed tightly to minimize leakage.

Because the tracer method results in variation both within and between animals, more animals are needed for accurate measurements (Boadi et al., 2002). When comparing the SF_6 tracer gas to whole-animal chambers, the coefficient of variation for individual cows was greater for the SF_6 tracer at 6.1 vs. 4.3% for the whole-animal chamber, and the coefficient of variation for treatment was also greater in the SF_6 vs. the whole chamber (19.6 vs. 17.8%, respectively) (Grainger et al., 2007). Even though there was more variation with the SF_6 method, mean total CH_4 production was similar between methods (331 vs. 322 g of CH_4/d for SF_6 vs. whole-animal chamber, respectively). When comparing the SF_6 method to a headbox, animal-to-animal variation was found to be

greater (11.8% vs. 1.6%). Methane production was similar between methods at 137 vs. 130 L/d for SF_6 vs. headbox, respectively, and CO_2 was found to be greater in the SF_6 method at 2,354 vs. 1,892 L/d compared to the headbox method (Boadi et al., 2002). The large increase in CO_2 production for the SF_6 method was likely caused by increased activity by those animals.

Increased CO_2 production is a potential limitation with the SF_6 method if CO_2 is of interest. Such error could be minimized by reducing the animals' excitement during the experiment. The SF_6 tracer method requires longer collection times because of this variation. Similar research by Arbre et al. (2016) observed that 3 days of collections were needed to get good repeatability. Similar to headbox-style chambers, a major concern with using the SF_6 tracer technique is the potential for losses that occur from hindgut fermentation to go unmeasured. The CH_4 produced from fermentation beyond the rumen accounts for about 13% of the total CH_4 produced (Murray et al., 1976). However, of the 13%, about 89% is reabsorbed into the bloodstream and this CH_4 is then passed into the lungs and expelled through the mouth, resulting in approximately 1% loss through the rectum (Torrent and Johnson, 1994). Boadi and Wittenberg (2002) found that under normal production settings, using the SF_6 tracer method achieved accurate measurements of enteric CH_4 production, but with high intakes, there may be greater variation, of which about 64% is caused by different feed intake that causes the difference in the CH_4 calculations. Methane production in sheep and cattle are 93 to 95% for the SF_6 tracer method compared to whole-animal chambers, likely caused by the small release of CH_4 from the rectum (Grainger et al., 2007). Muñoz et al. (2012) observed CH_4 production to be 3% lower for the tracer method and suggested that an adjustment be made when using

the SF_6 method due to production from hindgut fermentation, but also concluded that the method is reasonably accurate to measure or estimate CH_4 production.

GreenFeed method. Measuring the CO_2 and CH_4 produced by grazing cattle is inherently challenging and the GreenFeed system (C-Lock Inc., Rapid City, SD) represented an additional attempt to do so (Figure 2.6). The GreenFeed system is relatively convenient for getting gas concentration numbers for cattle that are grazing, but it can also be applied to animals in a barn. Animals are lured to the GreenFeed unit by a feed-pellet, which is dropped into a tray inside a partially enclosed hood of the unit (Hammond et al., 2016b). Each cow is assigned a unique ear tag, which is used to identify the animal and match gas measurements to the animal. By varying the frequency of the feed reward, the unit can be set up to vary the number of visits as stipulated by the user. The gas produced from the animal is drawn through an extractor fan, airflow is measured, and then subsamples are ultimately measured for CO_2 and CH_4. As background measurements are made on each measure, adjustments are made for all estimates of CO_2 and CH_4 production. The system is powered by electricity, but may be solar or battery powered, which is especially useful in grazing studies.

Comparing the GreenFeed method to the SF_6 and respiratory chamber method, Hammond et al. (2015) observed that the GreenFeed method failed to show differences between treatments that both the SF_6 and respiratory chambers were capable of detecting. The authors indicated that the GreenFeed system relies heavily on timing of the animal to arrive to the device and accuracy depends upon a large number of visits and consequently, measurements. Respiratory chambers and the SF_6 method measure samples continuously over the collection period, and this allows for these methods to collect

representative samples over time, especially capturing gas at times when CH_4 production is at a peak or a low. Respiratory chambers and the SF_6 tracer methods are also able to overcome the inherent variation that can occur in the cow because of rumination and eructation (Hegarty, 2013). Due to the circadian patterns of CH_4 production, many spot samplings are likely needed to accurately measure true CH_4 or gas production (Hammond et al., 2016). An alternative method to collect with the GreenFeed system would be to control the animal visits (Hammond et al., 2015). With a tie-stall barn, the investigator would easily be able to control visits and times of visits. During a 24-h feeding cycle, it is suggested that the animal visit the system at least 8 times a day for 3 days with staggering times throughout each day to collect a representative sample (Branco et al., 2015; Hristov et al., 2015). An additional disadvantage to the GreenFeed method is the high variation in gases between animals and within days. This is in part due to the fact that CH_4 production is episodic in cattle and as a result is inherently a challenge to measure properly (Hegarty, 2013). As a consequence, it has been suggested that to obtain accurate estimates of CH_4 production, 17-d measurements periods are needed (Arbre et al., 2016). This could be a great challenge if the rotation in the pasture is more frequent than 17 d. Another consideration with this method is the challenge of getting all animals to freely use the feeder. Hammond et al. (2016) observed that up to half of those cattle grazing chose not to visit the feeder. In response to this observation, a training period, in which animals could become acquainted with the device and the feed reward it offers, may be useful. Improving the palatability of the pellet may be a means by which animals may be more motivated to visit the device. In summary, when using the GreedFeed method, and compared to the SF_6 tracer or respiration chambers, more days are needed to collect gas

measures accurately. With increased number of sampling days, the GreenFeed unit may be an effective way to sample gas production in grazing cattle.

Gas collection. The number of days or time needed to collect a representative sample is an important element when attempting to estimate gas production, heat production, and energy utilization using indirect calorimetry. Historically, whole-animal chambers were used and gas was collected over 4 to 14 d (Blaxter et al., 1965). In more recent years, using headbox-style indirect calorimetry, shorter gas collection periods have been used. Using whole-animal chambers, Beauchemin et al. (2007b) collected gas over 4 d, but in the first 12 hr of these collections the CH_4 data collected was not used as it was assumed that during this time animals were still adapting to their surroundings. Freetly et al. (2006) used a 23 hr gas collection period and then multiplied the gas concentration by 24/23 to adjust them to a measurement by day. Itoh (1974) suggested that given the variation in the method of measurement, the minimum length of time to adequately calculate gas production is one day. Hence, it is generally recognized that at least one full day is needed for representative gas measurements, but longer collection periods would further reduce variation.

Gas recovery validation. Validation of the gas recovery system is needed to ensure reliability of data. This can be done by burning alcohol and then determining the recovery of CO_2 and O_2 (Nienaber et al., 2009). The respiratory quotient (**RQ**) is the ratio of CO_2 produced to O_2 consumed. When burning alcohol for gas recovery, the CO_2 produced to O_2 consumed should be approximately 2/3, resulting in a calculated RQ of 0.667. Recovery of CO_2 and O_2 should be close to 100%, but a range of 96 to 104% may be observed in well-calibrated systems (Nienaber et al., 1993). This method of validation

presents many challenges. Firstly, the burning of alcohol requires an open flame to be placed in a small area and as a consequence, increases the temperature inside the device. Once the alcohol is weighed in the lamp prior to burning, it must quickly be placed inside the chamber and commence the recovery test because of evaporation. After burning the alcohol for 2 hr, the airflow system must be shut off to extinguish the flame. This allows for an influx of air before the system finishes its purge of CO_2 from inside the system, which may alter the ratio of CO_2 to O_2. Alcohol is also highly flammable and evaporates quickly so caution should be practiced to prevent unwanted flames and ensure accurate measurement of alcohol used. Another method for gas recovery validation is to infuse CO_2 from a cylinder into the chamber/calorimeter. If the volume of the chamber is known, it is possible to calculate the gas recovery from the chamber/calorimeter (Derno et al., 2009). Although this method is less hazardless than burning alcohol, accurately determining the volume of the chamber and measuring the outgoing gas may be problematic and represent an additional source of error. When using this method, typically recoveries close to 100% are expected.

Energy Utilization

Energy balance. Measuring the amount of energy that animals consume, produce, and lose are all components of the energetic web known as energy balance. Energy balance is a method used to determine energy utilization in an animal. Distribution of energy starts with the energy in the feed and ends with product formation (Figure 2.7.) Flatt et al. (1967c) used 96 dairy cattle to describe energy utilization throughout each stage of lactation (Figure 2.8; Figure 2.9). Losses in feces, urine, CH_4, and heat are consistent during lactation and much greater than that occurring during the dry period.

Tissue energy is the energy available after subtracting heat energy and milk energy from metabolizable energy and is the energy available for tissue growth. Tissue energy is negative in early lactation, but increases throughout lactation. Assuming a 600 kg cow producing 40 kg of milk, Coppock (1985) estimated that approximately 35% of energy was lost in the feces, 30% lost as heat, 25% lost in milk, 5% lost in gas, and about 3% was lost in urine (Figure 2.10).

Total energy consumption from the feed that an animal consumes is based upon the energy density of the feed multiplied by the feed intake of the animal. This is commonly referred to as gross energy intake (**GEI**; equation 1; Table 2.1). The energy from the diet that the animal consumes will be digested and ultimately absorbed so the animal can utilize it for physiological function. If the feed is not digested it will pass through the animal undigested and be lost in the feces. Undigested material represents an energetic loss to the animal. Energy in the feces is subtracted from GEI to yield the total amount of digestible energy or the amount of energy assumed to be digested from the feed (**DE**; equation 2). Further energetic losses may occur as microorganisms produce CH_4 during ruminal digestion as well as the metabolism needed to produce urine to excrete waste products from the animal. When these along with digested energy are accounted for, it is referred to as metabolizable energy (**ME**; Equation 3) (DE minus urine and CH_4 energy). The heat increment is the amount of heat produced by the consumption of feed (Smith et al., 1978). It is estimated that approximately one-third of the ME is lost as part of the heat increment (VandeHaar, 1998). Energy remaining is then partitioned into the net energy requirements that include maintenance, growth, lactation, and pregnancy. The capacity of a feedstuff to be transformed into work, body tissue, and

milk is net energy (Brody, 1945). Lactating animals' net energy requirement is known as net energy of lactation, which includes the requirements for maintenance, lactation, growth, and pregnancy (**NE_L**; Equation 4). Digestion, absorption, and fermentation are biological functions in the animal's body that utilize energy and result in the production of heat. This heat produced represents an energetic loss to the animal known as heat production (**HP**), which is the difference between ME and NE_L (equation 4) and can be measured indirectly using calorimetry. Tissue energy (**TE**) is the difference between ME, HP, and lactation energy. Tissue energy is usually associated with the greatest error because it entails the cumulative error of ME, HP, and milk energy (Moe et al., 1971).

$$\text{GEI} = \text{diet energy} \times \text{dry matter intake} \quad [1]$$

$$\text{DE} = \text{GEI} - \text{fecal energy} \quad [2]$$

$$\text{ME} = \text{DE} - \text{urinary energy} - \text{CH}_4 \text{ energy} \quad [3]$$

$$\text{NE}_L = \text{ME} - \text{HP} \quad [4]$$

Energy inputs. The major portion of energy needed for milk production comes from dietary intake. However, during early lactation, cattle are often unable to consume enough feed to support their need for energy. Typically, milk production reaches its peak around 60 – 70 days while peak feed intake lags until approximately 90 days. This results in a major challenge for achieving needed energy consumption and consequently, lactating cattle catabolize body tissues for a supply of energy and this results in a negative energy balance. Prolonged duration of negative energy balance may lead to metabolic diseases such as ketosis. Nutritionists attempt to avoid negative energy balance by taking measures to stimulate animal intake of energy. However, there is large

variation in milk production and feed intake, which contribute to the challenge of partitioning of energy (Bauman et al., 1985).

The concentration of energy in a feed ingredient or its gross energy content can be determined by combusting the feed ingredient in a bomb calorimeter and then determining the amount of heat produced by measuring the temperature increase of the water. This factor does have limitations, as it does not fully explain the biological system within the animal (Blaxter, 1962). The challenge can be exacerbated by the extremely complex nature of energy digestion and utilization. Energy utilization is extremely complex because partitioning is dependent on type/nutrient profile of the ration, animal size, environmental conditions, and stage of lactation (Samma and Mao, 1993). Large variation in energy balance has been observed in lactating dairy cattle caused by differences in how individual cattle partition energy (Bauman et al., 1985). Variation in milk energy during lactation is likely the cause of the variation in energy balance (Samma and Mao, 1993). In order to get accurate energy estimates, total collections of feces, feed refusals, heat production, milk, and urine should be collected to calculate energy balance. The simple but laborious procedure is crucial for accurate measurements.

Efficiency. Efficiencies in dairy cattle have been studied for decades. Efficiency is a common measure used to explain the productivity of animals. Productive efficiency usually requires the knowledge of milk yield while accounting for nutritional costs of maintenance, milk fat and protein synthesis, and potential loss of body tissue during lactation and is expressed as the amount of some production variable such as milk production per unit of feed consumed (Bauman et al., 1985) or the ratio of energy content of the product to energy required for product synthesis (Moe et al., 1981). Efficiency can

increase with the manipulation of the diet as this affects digestion, nutrient absorption, and maintenance requirements. In practice, diets fed to lactating dairy cattle containing greater fiber digestibility, greater starch content, and potentially supplemental fat could improve efficiency of converting gross energy to net energy (VandHaar, 2016). Caution should however be exercised when explaining that efficiency is potentially increased due solely to increased digestibility. Tine et al. (2001) compared corn silage type and found that the increase in milk production resulted from increased feed intake and was not the result of increased energetic efficiency. Hence, nutritive entities within the ration may also influence efficiency.

Energetic efficiency can be explained in thermodynamic terms as the ratio of work being performed to the amount of free energy expended or, for animals, the amount of a certain product created such as milk or tissue per unit of the nutrient utilized (Brody, 1945). Gross energy carries the inherent burden of maintenance and as such, it will never be as productive as net efficiency (Brody, 1945). With increased milk production caused by increased feed intake, energetic efficiency of lactation may increase initially, but then decrease at a certain production level. This is similar to an automobile; for example, as a car accelerates from a stop, efficiency of gasoline use initially increases, but eventually reaches a speed at which efficiency decreases. However, there is still potential that an increase in milk production may improve productive efficiency because of the dilution effect on the maintenance requirement (VandeHaar et al., 2016). The dilution of maintenance occurs because the production of milk increases while the requirement for maintenance remains relatively constant. VandeHaar et al. (2016) gave the following example, cattle producing no milk and eating at maintenance have a gross feed efficiency

of zero, whereas a cow eating at twice her maintenance will have half the energy go to maintenance and the other half for milk production and becomes more efficient. The more feed consumed, the smaller the fraction of that feed that goes to maintenance and the cow becomes more efficient. The requirements for maintenance may increase slightly due to the additional load organs such as the liver, which may be explained by a slight increase in maintenance requirements over time (Moraes et al., 2015). When comparing lactating beef cow requirements to those of dairy cows, Freetly et al. (2006) observed similar overall efficiency of energy retention albeit, the beef cows were younger and had lower milk production.

Other measures of efficiency reported in the literature include: digestive efficiency, feed efficiency or residual feed intake, efficiency of converting DE to milk, energy lost per unit of milk produced, percent of efficiency of ME for milk production, or CH_4 produced per unit of feed intake or milk production (Moe and Tyrrell, 1974; VandeHaar, 1998; Van Zijderveld et al., 2011b; Xue et al., 2011; Benchaar et al., 2013; Reynolds et al., 2014; Foth et al., 2015; VandeHaar et al., 2016). Increased milk production may be supported by an increase in feed intake, which then results in decreased digestive efficiency (VandeHaar, 1998). The depression in digestibility is not normally observed in high producing dairy cattle because other biological efficiencies may increase (Bauman and Currie, 1980). Feed efficiency is defined as the proportion of the feed energy that is captured in products (VandeHaar et al., 2016). Residual feed intake measures the efficiency of a cow by predicting the amount of feed an animal needs based upon the animal's weight, expected weight gain, and milk production for lactating animals. If the residual feed intake is negative, the cow is more efficient at converting

gross energy to net energy or maintenance requirements are lower than expected for that cow.

Methane production represents an energetic loss to the animal, so improving efficiency could prove beneficial for the industry. When assigning environmental impact, looking just at total CH_4 production is misleading. In a study testing the effects of increased proportions of corn silage in the diet, Benchaar et al. (2013) observed total production of CH_4 increased linearly as corn silage was increased. However, when comparing the amount of CH_4 produced per unit of milk produced or feed intake, CH_4 production decreased linearly as corn silage was increased in the diet. If efficiencies were not taken into account, this increase in CH_4 production would be widely viewed as detrimental to the industry. For example, from 1944 to 2007 there was an increase of 175% in total CH_4 production per cow per day (Capper et al., 2009). When using the correct efficiency factor and assessing the amount of CH_4 per unit of milk produced, there was a 60% decrease in CH_4 production over this time period (Capper et al., 2009).

Production of short-chain fatty acids during milk secretion is more efficient than production of long-chain fatty acids in body tissue. Consequently, efficiency is greater for milk production than for growth and fat deposition (Brody, 1945; Blaxter, 1962, and Bauman et al., 1985). Efficiency of converting tissue energy to milk production is approximately 82% (Moe et al., 1981). Efficiency for converting ME to lactation energy is 0.61 – 0.68, but has been observed to be as high as 0.76 (Foth et al., 2015). The greater this value is, the more efficient the animal is at converting feed to lactation energy. Over-supplementation of nutrients may result in either increased or decreased efficiencies for the animal. Partial efficiency of converting dietary fat to milk fat is approximately 94 –

97% (Baldwin et al., 1985). Excess protein gets deaminated (broken down) and the nitrogen will be excreted in the urine as urea (Blaxter, 1962; Reed et al., 2017). Continued progress in the understanding of efficiencies and the components that play a key role in efficiencies, such as genetics and diet nutrient profile, may help improve overall efficiencies in the dairy industry (Bauman et al., 1985).

Maintenance. Maintenance has been defined in a variety of ways. Firstly, the process of keeping (maintaining) a nonproducing mature animal in the same energy state. Secondly, the amount of digestible or metabolizable energy that is needed to reach an equilibrium in adult nonproducing animals (Moe and Tyrrell, 1974). The 2001 Dairy NRC described the average daily maintenance requirement as 0.080 Mcal of NE_L × metabolic body weight, which is the weight of the cow raised to the 0.75 power (Equation 5). In the Holstein breed, for example, the maintenance requirement would result in about 10 Mcal of NE_L/d. In order to obtain this, the animal would need to consume approximately 25 Mcal of GE or approximately 6 kg of feed (VandeHaar, 2016). Measuring maintenance is challenging due to the inherent variation within and among animals (Coppock et al., 1964).

$$\text{Maintenance} = 0.08 \text{ Mcal} \times BW^{0.75} \quad [5]$$

Historically, whole-body maintenance energy expenditure has been divided into three main categories: work functions, cell component synthesis, and membrane transport (Baldwin et al., 1985). Work functions account for approximately 40 – 50% of energy expenditure and include work associated with heart, liver, nervous tissue, ion resorption in the kidney and muscle work for respiration. Synthesis of cell components accounts for

approximately 15 – 25% of energy expenditure and is comprised of mostly of protein and membrane lipid re-synthesis. Membrane transport accounts for approximately 25 – 35% of energy expenditure and is mainly comprised of the maintenance of membrane potentials such as the potassium ATPase or sodium ATPase. The large variation that occurs in basal metabolism could be accounted for partially by the different weights of tissues requiring high energy and turnover of cell components. McNamara (2015) reported that the variation in ion pumping and protein turnover could account for approximately 20% of variation observed in maintenance requirements for cows producing similar amounts of milk. Recent work has suggested that the maintenance energy requirement has increased to 0.1 Mcal/$BW^{0.75}$ (Moraes et al., 2015). This would indicate that animals genetically selected for milk production might require more energy per unit of metabolic body weight (VandeHaar, 2016). VandeHaar (2016) suggests that the increased maintenance requirement is associated with an increase in metabolic activity and increased digestive activity. Agnew and Yan (2005) suggested that the increase is due to greater intakes, digestive load, O_2 consumption, blood flow required for digestion, and delivery of nutrients to the mammary gland, which increase internal organ size. Additional energy required should be assigned to heat production associated with feeding and lactation and not heat of maintenance.

Maintenance in the literature is usually referred to as metabolizable energy for maintenance (**MEm**) as it is expressed as total kcal needed per kg of metabolic body weight and will be referred to as such for the duration of the review. Metabolizable energy required for maintenance of cattle ranges from 97 – 208 kcal of ME/kg $BW^{0.75}$ with an approximate mean at 137 ± 26 kcal of ME/kg $BW^{0.75}$ (Table 2.2). The large range

could result from a wide range of studies used in the analysis. Agnew and Yan (2005) suggested that the historical data underestimate the total requirement of lactating cattle compared to more resent research. Lactating dairy cattle had an approximate mean of 143 kcal of ME/kg $BW^{0.75}$ and non-lactating cattle were 120 kcal of ME/kg $BW^{0.75}$. The reason for increased maintenance is not clear, but may be due to increased digestive and metabolic activity (VandeHaar et al., 2016). As stated earlier, some believe that maintenance requirements are increasing (Evans et al., 2000; Moraes et al., 2015). It is possible that improvements in genetics have driven this change in increased maintenance due to increased production capability. The efficiency that ME is converted to lactation energy is approximately 64%, however, Coppock et al. (1964) observed a large range between 63 and 107%. Increasing the efficiency of converting ME to lactation energy could be a target for reducing energy loss in lactating dairy cows.

Factors that affect maintenance requirement include the extent of grazing activity, season, temperature, body condition of the animal, forage concentration in the diet, age, sex, and breed type (Byers and Carstens, 1991; Laurenz et al., 1991; Agnew and Yan, 2005; Reynolds and Tyrrell, 2000; Freetly et al., 2002). Grazing animals may have increased maintenance requirements and require 20 to 50% more energy for maintenance (Flatt et al., 1967b). The need to continuously graze to consume enough feed would increase maintenance compared to cattle that only need to get to a feed bunk. Comparing seasonal effects on maintenance requirements, Angus and Simmental cattle had increased maintenance requirements during the summer (122.6 vs. 91.4 and 145.9 vs. 109.3 kcal/ $BW^{0.75}$ for Angus and Simmental cattle, respectively) (Laurenz et al., 1991). Increased temperature and increased grazing activity may account for the increase in maintenance

requirements during the summer. Another possible explanation could be a change in body condition of the animals. If cattle are in the thermal-neutral zone (-0.5 to 20 °C), no additional energy is needed to maintain body temperature; however, as temperatures increase, more energy is expended to dissipate heat (West, 2003). With temperatures near 35 °C, maintenance increased by approximately 7 to 20% (NRC, 2001). Similarly, Collier and Beede (1985) observed increased energy maintenance requirements for heat-stressed animals due to elevated body metabolism to dissipate heat via panting. Panting may account for a 7 -25% increase in maintenance energy requirements depending on the severity of the heat (Collier and Beede, 1985). With prolonged temperatures below the thermal-neutral zone, there is an increase in basal metabolic activity, DMI, thermal insulation, and a potential alteration in function of the digestive tract resulting in increased maintenance requirements (Young, 1983).

Thompson et al. (1983) observed maintenance requirements to increase 2.7% for fat vs. thin Angus-Holstein cows. However, the author also observed a 6.1% decrease in maintenance requirement for fat vs. thin Angus-Hereford crossbred cattle. Angus and Simmental cattle had greater maintenance for thin cattle during the fall/winter seasons compared to spring/summer season. With increased body condition, maintenance requirements were greater during spring/summer compared to fall/winter. Further investigation may prove beneficial in determining how body condition affects maintenance requirements.

Forage concentration in the diet has the potential to increase or decrease fermentation and, therefore, reduce maintenance in the animal. Yan et al. (1997) increased the amount of silage in the diet at three concentrations: 50% of GE, 51-99% of

GE, and 100% of GE. In this study, maintenance requirements increased and were observed to be 141, 162, and 177 kcal/ $BW^{0.75}$. Even though maintenance increased with greater concentrations of forage in the diet, the efficiency of ME use for lactation (k_1), was not affected (0.62, 0.64, and 0.63 for silage as a percentage of GE at < 50, 51-99, and 100, respectively). Similarly, Dong et al. (2015b) observed increased maintenance requirements with increasing forage proportion in the diet (145, 156, 161, and 162 kcal/ $BW^{0.75}$ for < 30, 30-59, 60-99, and 100% forage in the diet, respectively). The increased maintenance requirement for a greater forage proportion may be a result of increases in the heat of fermentation or digestion. Agnew and Yan (2005) suggested that forages increase the energy needed for digestion due to increased production of saliva, bile, enzymes, salts and digestive juices. With increased forage inclusion, ration digestibility decreases and maintenance requirements increase (Flatt et al., 1967a). To compensate, increased energy content may need to be fed.

Research on the maintenance requirements for sex of the animal have observed increased requirements for bull vs. heifer calves. Ferrell and Jenkins (1985) observed greater maintenance requirements for bull vs. heifer calves (123 vs. 110 kcal/ $BW^{0.75}$ for bulls vs. heifers, respectively). However, Garrett (1970) observed similar maintenance requirements for Hereford steers and heifers. The literature is inconclusive at this time on the effect of breed and warrants further research into the potential differences in maintenance requirements for sex.

Different breeds of cattle may have different maintenance requirements. Lactating dairy cattle, for example, consume more feed, produce more milk than beef breeds, and may have greater maintenance requirements. However, Reynolds and Tyrrell (2000)

observed that lactating Hereford-Angus beef heifers had similar energy requirements as Holstein-Friesian cows (120 vs. 117 kcal/ $BW^{0.75}$, for beef vs. dairy, respectively). Within the beef breed, Ferrell and Jenkins (1985) compared Hereford and Simmental breeds and observed greater maintenance requirements for Simmentals vs. Herefords (126 vs. 106 kcal/ $BW^{0.75}$ for Simmental vs. Hereford respectively). Angus cows had lower maintenance requirements compared to Simmental cows (103.6 vs. 123.5 kcal/ $BW^{0.75}$ for Angus vs. Simmental, respectively) (Laurenz et al., 1991). In the dairy breed, despite differences in mature body size, Jersey and Holstein cows had similar metabolizable intake per unit of gross energy intake (55.6 vs. 56.3% ME/GE, for Jersey vs. Holsteins, respectively) (Tyrrell et al., 1990). Xue et al. (2011) compared primiparous Holsteins to crossbred Jersey-Holstein cattle and found no difference in maintenance requirements (170 vs. 160 kcal/ $BW^{0.75}$). Lactating dairy cows had greater maintenance requirements than non-lactating dairy cows (120 vs. 100 kcal/ $BW^{0.75}$ for lactating vs. non-lactating, respectively) (Moe et al., 1971). The increase in maintenance could be the result of increased organ weights. Baldwin et al. (1985) found a 50% increase in liver weight during lactation. Increased organ weight would increase the maintenance requirement of the animal.

Gas Exchange

Heat increment. The heat increment is composed of heat of fermentation, waste, digestion/assimilation, maintenance, and tissue formation. Coppock (1985) used a 600 kg lactating cow producing 40 kg of milk to illustrate the approximate distribution of the heat production (53, 24, 12, 8, and 3% for product formation, maintenance, digestion, fermentation, and waste, respectively; Figure 2.11). The heat increment has been

illustrated to be the second largest energy loss in a lactating animal. Heat associated with tissue or product formation accounted for the greatest amount of total heat produced at approximately 53%. Maintenance followed at 23%, heat of digestion at approximately 12%, heat of fermentation at 8% and heat of waste formation and excretion at 3%. Heat associated with digestion and assimilation makes up approximately 18 to 20% of the total heat increment for maintenance. After feeding, heat increment increases due to the increase in digestion and absorption of nutrients.

Heat production. An understanding of metabolic pathways of nutrients can aid in describing energy utilization and production. If the amounts of organic compounds oxidized in the body are known, by summing the enthalpies of oxidation, heat production (**HP**) can be calculated (Blaxter, 1989). According to the Law of Hess, the total enthalpy change for the reaction is independent of the pathway and is the sum of all. This allows for the indirect measurement of heat production without having to determine each individual pathway that the energy required to meet that physiological state (Saama and Mao, 1993). This allows for the heat of combustion to be predicted and this prediction is based upon carbon, hydrogen and O_2 present in feed consumed and respired air from the animal (Blaxter, 1962). Compared to amino acids, the oxidation of carbohydrates may either over- or underestimate total heat production. Carbon and nitrogen balance can also be used to indirectly measure retained energy (**RE**) in the animal and is measured in kilocalories by using equation 6.

$$RE = (12.55 \times \text{g C retained}) - (6.90 \times \text{g N retained}) \quad [6]$$

With using indirect calorimeters, heat production can be measured by taking into account the amount of O_2 consumed, CO_2 produced, CH_4 produced, and N excreted in urine (equation 7), where HP is measured in kcal; O_2, CO_2, and CH_4 are measured in liters; and urinary nitrogen is measured grams (Brouwer, 1965). Brower's equation theorizes that heat produced from oxidation of fats, proteins and carbohydrates and the production of urea is equal to total heat produced by the animal. Most energy-balance studies employ this methodology; however, complete accuracy is not achieved because of the assumption that all components of the diet are oxidized completely (Blaxter, 1962). Thus, the correction for urinary nitrogen as nitrogen is not completely oxidized, but converted into urea and excreted in the urine (Young et al., 1975). However, even with correction for urinary nitrogen loss, accurate measurements of gas concentration and production are crucial for proper measurements of heat production. Hence, using a highly accurate and precise gas analyzer for indirect estimates of heat production measurements is necessary (Young et al., 1975).

$$HP = 3.866 \times O_2 + 1.200 \times CO_2 - 0.518 \times CH_4 - 1.431 \text{ x urinary nitrogen} \quad [7]$$

Many factors influence the amount of heat produced. Genetic differences, breed, stage of production, housing environment type, temperature, age and weight can all have an effect on total heat produced (Albright, 1990). In a comparison between Jersey and Holstein cows, Ritzman and Benedict (1938) observed greater heat production in Jersey compared to Holstein cows (8170 vs. 6665 calories/500 kg empty BW for Jersey vs. Holstein, respectively). However, Münger (1991) observed decreased heat production of Jersey cows compared to Holstein-Friesian and Simmental cows (34.4, 36.7, and 36.5% of GE for Jersey, Holstein-Friesian and Simmental cattle, respectively). Rambouillet

ewes had lower heat production per unit of BW compared to Finnsheep ewes at any age during growth (Freetly et al., 2002). This observation was likely due to the increased growth rate of the Rambouillet ewes. Increased environmental temperatures cause an increased heat production as animals attempt to cool down. The amount of heat produced will increase in early lactation and slowly decrease over the lactation. With advancing age of sheep, heat production decreased per unit of BW (Freetly et al., 2002). Standing behavior of the animal also has a large impact on the amount of O_2 being consumed and, therefore, heat production. Kelly et al. (1994) found that as sheep lie down and ruminate, the total consumption of O_2 decreases. This cost is related to the increased energetic cost of standing or the posture of the animal. More energy is required to stand as more muscles have to conduct work.

An important physiological aspect in energy utilization is homeostasis of the animal. The most important function of homeostasis is to maintain body temperature of the animal (Albright, 1990). Animals produce heat as a by-product from growth, maintenance and production, which essentially results in a constant state of heat production (Albright, 1990). In times of extreme cold, the animal must consume more feed to produce more heat needed to maintain a constant body temperature. This usually occurs at the expense of other functions such as growth and production (Albright, 1990). During the summer when there are conditions of extreme heat, animals may consume less feed and have a lower production to limit the amount of heat that needs to be released into the environment (Albright, 1990). Heat-stressed animals may have increased maintenance requirements because of energy needed for panting and sweating (Wheelock et al., 2010)

Respiratory quotient. The respiratory quotient (**RQ**) is the ratio of CO_2 produced to O_2 consumed (Equation 8) (Kim et al., 2015). Understanding the RQ is important in conducting energy-balance studies, especially those that rely upon indirect calorimetry. The RQ can be used to determine metabolism of different substrates or be an indication of metabolic processes occurring within the animal. When carbohydrates such as glucose are the substrate being oxidized, the RQ will be close to 1.00, as 6 mols of CO_2 are produced when 6 mols of O_2 are consumed (Equation 9). When short-chain fatty acids are oxidized, RQ will be near 0.80, as 30 mols of CO_2 are produced when 37 mols of O_2 are consumed (Equation 10). Oxidation of long-chain fatty acids results in an RQ near 0.703 as 102 mols of CO_2 are produced when 145 mols of O_2 are consumed (Equation 11). Oxidation of combinations of short- and long-chain fatty acids results in an RQ of approximately 0.711. Oxidation of protein results in an RQ near 0.81, but will be dependent on amino acid profile. For example, alanine results in an RQ of approximately 0.83 as 5 mols of CO_2 are produced and 6 O_2 are consumed (Equation 12), whereas the RQ of leucine is 0.73. Synthesis of lipids will result in an RQ above 1.00 closer to 1.10 - 1.30 and is dependent on what fat is being synthesized (equations were derived from Brody, 1945, and Blaxter, 1989). Jakobsen and Thorbek (1970) observed a linear relationship between RQ and retained fat concentration in swine diets; specifically, as fat retention increased, RQ also increased.

$$\text{Respiratory quotient} = CO_2 \text{ produced (L)}/O_2 \text{ consumed (L)} \quad [8]$$

$$C_6H_{12}O_6 + 6O_2 = 6CO_2 + 6H_2O \quad [9]$$

$$RQ = 6CO_2/6O_2 = 1.00$$

$$C_3H_5(CH_3CH_2COO)_3 + 37O_2 = 30CO_2 + 26H_2O \quad [10]$$

$$RQ = 30CO_2/37O_2 = 0.80$$

$$2C_3H_5(CH_3(CH_2)_{15}COO)_3 + 145O_2 = 102CO_2 + 86H_2O \quad [11]$$

$$RQ = 102CO_2/145O_2 = 0.703$$

$$2CH_3CH(NH_2)_2COOH + 6O_2 = CO(NH_2)_2 + 5CO_2 + 5H_2O \quad [12]$$

$$RQ = 5CO_2/6O_2 = 0.83$$

Various metabolic pathways may alter the RQ in cattle. When cattle are acidotic or ketotic, excess CO_2 may be liberated and lower the RQ, whereas cattle with alkalosis store CO_2 and potentially increase RQ (Brody, 1945). Production of sugar from fat and protein or incomplete oxidation may also result in a low RQ (Brody, 1945). Breakdown of bicarbonates in the rumen and anaerobic fermentation produce large quantities of CO_2 in the rumen, but may not have metabolic significance as a measure of metabolism (Brody, 1945). If excess fermentation and breakdown of bicarbonates occurs, O_2 consumption is a better measure of metabolism (Brody, 1945).

Methane Production and Mitigation

Methane production. Lactating dairy cattle produce approximately 500 – 600 L/d of CH_4 per cow and contribute 1.9 – 2.2% of the total greenhouse gas (**GHG**) emissions in the U.S. (Thoma et al., 2013; Chase, 2014). Ruminants produce approximately 25% of the total CH_4 production, of which dairy cattle contribute approximately 24.8% of enteric CH_4 production or 0.54% of total U.S. GHG (Chase, 2014). Methane is of particular interest with environmentalists because it is approximately 25 times more effective in

trapping heat than CO_2, which causes concerns about global warming (Thoma et al., 2013). It is estimated that CH_4 production accounts for approximately 30 – 50% of total GHG production, of which enteric CH_4 production accounts for approximately 80% (Morgavi et al., 2010). In 2009, the Innovation Center for U.S. Dairy set a goal of reducing total GHG production by 25% by 2020, of which CH_4 has been a focus. Many see gas production from ruminants as a key problem of the industry. Environmental concerns are not the only reason CH_4 production is important in the dairy industry. Methane production may have a negative impact on metabolizable energy available for production and reduce the animals overall efficiency (Gill et al., 2010; Hynes et al., 2016). Energetic losses to the animal from CH_4 production are approximately 2 to 12% (Johnson and Johnson, 1995). Potential mitigation of CH_4 of 25% could increase growth in beef cattle by approximately 75 g/d BW gain (Nkrumah et al., 2006) or increase milk production by approximately 1 L/d (Bruinenberg et al., 2002). Hence, reducing CH_4 production in dairy cattle is of energetic importance for producers.

Fermentation of carbohydrates, proteins, and other organic compounds in the rumen by microbes results in various products, including volatile fatty acids, CO_2, and dihydrogen (**H_2**) (Figure 2.12; Morgavi et al., 2010). Methane production is one of the last steps in the long chain of fermentation as it uses the products of CO_2 and H_2 to produce CH_4 by a process called methanogenesis. Methanogenesis is performed by methanogenic archaea in the rumen, often referred to as methanogens (Morgavi et al., 2010). Methanogens use three major substrates for CH_4 production: CO_2, acetate, or a compound that contains a methyl group (Morgavi et al., 2010). The majority of known methanogens use CO_2 and H_2 to produce CH_4 (Hungate, 1967) in a pathway commonly

referred to as hydrogenotropic (Hook et al., 2010). At least four pathways are known for CH_4 production, and two are illustrated below in Figure 2.13. Concentration of H_2 in the rumen drives CH_4 production. Without the production of CH_4 in the rumen, alternative pathways would need to become more active so the partial pressure in the rumen would remain low. A high partial pressure in the rumen inhibits the normal function of microbes involved in electron transfer, particularly NADH dehydrogenase, which would increase NADH accumulation in the rumen and decrease fermentation (Morgavi et al., 2010).

Methane production is also closely associated with the breakdown of fibrous feed as the breakdown of fiber produces acetate, butyrate and H_2, with H_2 being converted into CH_4 (Morgavi et al., 2010). Production of CH_4 is determined by ration type, passage rate of rumen digesta out of the rumen, temperature, presence of lipids and ionophores (McAllister et al., 1996). Microbial species involved in CH_4 production include protozoa and fibrolytic bacteria. Defaunation has been observed to reduce CH_4 production. Morgavi et al. (2010) found that 47% of variation in CH_4 production could be explained by protozoa concentration in the rumen. Possible explanations include the fact that protozoa produce H_2 and become a host for methanogens because of interspecies H_2 transfer while also protecting them from O_2 toxicity. Interspecies H_2 transfer occurs when H_2 is produced by one microbial species and is captured or utilized by another microbial species, creating a syntrophic relationship between rumen microbes (Morgavi et al., 2010). Methanogens have been found in the liquid and solid fractions of the feed as well as the epithelium in the rumen (Pei et al., 2010). Methanogens have a diverse community in which they can survive and thrive, making them extremely prolific in the rumen.

Methane production in cattle varies depending on amount of feed intake, level of production, stage of production, carbohydrate concentration of the diet, forage processing, and change in the microbial population (Moe and Tyrrell, 1979; Johnson and Johnson, 1995; Alstrup et al., 2015). Any manipulation of these factors will influence CH_4 production. For example, as more feed is consumed, more substrate is available for rumen microbes to ferment feed and produce more CH_4. Shortly after calving, feed intake is normally suppressed as cattle transition from the dry period to lactation. As lactation progresses, intake increases until it reaches a peak, followed by a slight decrease for the remainder of lactation. This influences the total amount of fermentation. Changing particle size may increase rate of passage, which in turn will decrease the amount of fermentation to produce CH_4. Alstrup et al. (2015) found an increase in CH_4 production with increasing days in milk. This increase was consistent for CH_4 produced per unit of milk produced and per unit of feed intake.

Diurnal Variation

Collecting accurate measures of gas production is a laborious task. Methane production is episodic in cattle and may contribute to the challenge (Hegarty, 2013). Other factors known to affect gas production involve feeding practices. This includes time between feedings and frequency of feedings, but gas production may also be affected by the number of meals consumed, fermentation rate, and fermentation patterns (Brask et al., 2015). Methane production is dependent on feed consumption and digestion and can vary by as much as five-fold throughout the day (Crompton et al., 2010.)

In a recent study using lactating dairy cows (Brask et al., 2015), two peaks in CH_4 production were observed over a 24 h period. A minor peak occurred after morning

feeding and a major peak occurred after the evening feeding. Similarly, Hollmann et al. (2013) observed major peaks in CH_4 production following morning and afternoon feedings. Crompton et al. (2010) also observed increased CH_4 concentrations after cattle were fed in the morning and afternoon. The delay in peak CH_4 occurred approximately 1 to 2 h post-feeding for morning and afternoon feedings. In a study using sheep restricted to one meal per day, Van Zijderveld et al. (2010) found a single peak after feeding. Sheep were fed at 0800 h and the peak CH_4 production occurred around 4 to 6 h post feeding, after which it gradually declined until feeding time the next day. Interestingly, sheep supplemented with nitrate reduced peak CH_4 production and sheep supplemented with sulfate produced less CH_4 but the response was delayed until 10 h post-feeding. Similarly, in beef cattle, Hales et al. (2017) fed beef steers once daily and observed peak CH_4 production around 4 to 6 hours post feeding. Processing affected the time until peak CH_4 production, with diets containing steam-flaked corn reaching peak production 1 hour before dry-rolled corn diets. Therefore, diet type may alter the time to peak CH_4 production. Being able to measure CH_4 production accurately is needed for energy-balance studies. Together these studies indicate that there is diurnal variation in CH_4 production associated with feeding as well as time of day. Future research is needed to determine if the peaks illustrated are representative of whether there is a need for continuous sampling or if spot sampling is sufficient. Spot sampling may give skewed results of total CH_4 produced if it is calculated for the entire day based upon peak production hours. Thus, further research should determine the best sampling method for lactating dairy cows being fed either once daily or twice daily for CH_4 production.

Methane Mitigation

Many strategies have been devised in attempts to develop methods to mitigate CH_4 production, and these can be broadly categorized into three main methods, 1) nutritional or feed management, 2) modification of the rumen environment to directly inhibit methanogens, and 3) management practices that increase animal productivity (Knapp et al., 2014). Research on nutritional or feed management methods represents the greatest amount of research conducted. This includes changing the quality of the feed, particularly the forage, or altering the forage-to-concentrate ratio to increase feed efficiency, shifting the site of digestion from the rumen to the intestines (Beauchemin et al., 2008b), inclusion of rumen modifiers designed to control or inhibit methanogenesis, and even immunization and defaunation (Knapp et al., 2014). If the aim is to improve nutrient utilization while increasing production of the animal, improvement in animal genetics may represent an important method to do so.

Nutritional Techniques

Fiber digestion. Fiber digestion increases CH_4 production due to increased production of acetate and H_2 by fibrolytic bacteria. A method to avoid this phenomenon is to manipulate the rumen and promote the growth of non-H_2-producing fibrolytic bacteria such as *Fibrobacter succinogenes* (Morgavi et al., 2010). Using gnotobiotic lambs, H_2-producing vs. non-H_2-producing microorganisms were inoculated into the lambs and CH_4 production was lower in non-H_2-producing lambs (Chaucheyras-Durand et al., 2008). With reduced H_2 concentrations, there would be a reduction in CH_4 production because the partial pressure in the rumen would remain lower.

Forage-to-concentrate ratio. Addition of concentrate in the diet is also an effective way to reduce CH_4 production. This is because fibrolytic bacteria are less active and become less important in digestion of energy sources. A diet with greater concentrate inclusion may increase production due to an increase in available energy. With increased concentrate or carbohydrate in the diet, there may also be a change in the rumen microbiota as well as rumen pH (Hook et al., 2010). The reduction in CH_4 production would occur as the fermentation substrate changes from a fiber source to a starch source and more propionate-producing bacteria would thrive (Beauchemin et al., 2008a). The production of propionate utilizes hydrogens, which would reduce the partial pressure and CH_4 production. Methane reduction will likely occur when concentrate is fed at concentrations greater than 40% of dietary dry matter (Hristov et al., 2013a). In mid-to-late lactation Holstein-Friesian cattle, Hatew et al. (2015) increased the starch concentration from 10 to 20% of dry matter and observed an 8% decrease in CH_4 production. A limitation with increasing the concentrate in the diet is the potential to negatively affect milk quality that can occur when too much grain is fed. Increased starch or concentrate in the diet can decrease pH and inhibit fermentation of fiber, which ultimately leads to a decrease in milk quality. With increased starch concentrations, Hatew et al. (2015) observed no negative effects on milk quality or rumen pH, demonstrating that with precise nutrition programs, maximum efficiencies may be achieved without negative impacts on the animal.

Alternative H_2 sinks. Bacteria in the rumen can respire anaerobically and are capable of using alternative electron acceptors besides CO_2 to oxidize H_2 (Morgavi et al., 2010). Alternative H_2 sinks do not normally dominate the environment in the rumen, but

they can increase in number with the correct electron acceptor in the diet. Nitrate- and sulfate-reducing bacteria are two common electron acceptors with acetogenesis being another route that may be more desirable as using hydrogens (Weimer, 1998). Addition of lipids to the diet may also be an alternative H_2 sink (Beauchemin et al., 2008a).

Nitrate. Nitrate may compete with methanogenesis for available hydrogens and reduce CH_4 production (Olijhoek et al., 2016). Nitrate-reducing bacteria have the capability to utilize hydrogens in the conversion of nitrate to nitrite and then to ammonia. This process is more thermodynamically favorable than methanogenesis (Morgavi et al., 2010). Eight electrons are consumed during the reduction of nitrate to ammonia, which should lower production of CH_4 by one mol and should yield more energy (Van Zijerveld et al., 2010). Ammonia may serve to be beneficial to the animal as an alternative source of nitrogen, especially when diets are limiting in rumen-degradable protein (Dijkstra et al., 1998).

In vitro studies have observed a reduction in CH_4 by approximately 65% with the addition of nitrate (Iwamoto et al., 1999). In lambs, an experimental diet containing 2.6% nitrate reduced CH_4 production by 32% compared to a control diet containing no additional nitrate, without negatively affecting feed intake (Van Zijderveld et al., 2010). Reduction of nitrate to nitrite in the rumen proceeds quickly and often exceeds the reduction of nitrite to ammonia, leading to accumulation of nitrite, which may become toxic to the animal (Iwamoto et al., 1999). If nitrite accumulates, it may inhibit nitrate reductase and nitrate-reducing bacterial activity. This occurs more often in cattle that are not adapted to nitrate. Gradual adaption to nitrate lowers risk of toxicity (Lee and Beauchemin, 2014). Van Zijderveld et al. (2010) recommend a stepwise introduction of

nitrate into the ration to allow the microbial community to adapt and maximize reduction potential.

Using Holstein cattle, Olijhoek et al. (2016) increased calcium ammonium nitrate concentrations at 0, 5.3, 13.6, and 21.1 g of nitrate/ kg of dry matter and observed a linear decrease in CH_4 production (491, 468, 424, 396 L/d for 0, 5.5, 13.6, and 21.1 g of nitrate, respectively). Correspondingly, H_2 concentration increased linearly with nitrate supplementation (11.4, 27.4, 37.8, and 35.0 L/d, for 0, 5.5, 13.6, and 21.1 g of nitrate, respectively). Potential concerns with using alternative hydrogen sinks are that the reduction will not last very long or will affect production. Van Zijderveld et al. (2011b) used lactating dairy cows for 90 days and showed a sustained 16% reduction in CH_4 throughout the experiment with no significant effect on feed intake or milk production. In a study by Klop et al. (2016) with lactating dairy cattle consuming a low-forage diet, the addition of nitrate decreased CH_4 production compared to the control (263 vs. 363 g/d for nitrate vs. control, respectively), however, there was also a reduction in feed intake (15.7 vs. 16.5 kg/d) and milk production (25.1 vs. 27.85 kg/d for nitrate vs. control diet, respectively). Therefore, nitrate has the potential to reduce CH_4 production, but effects on cow performance vary. With proper adaption to the nitrate, negative effects of nitrate toxicity may be minimized.

Sulfate. Sulfate-reducing bacteria have a greater affinity for H_2 than methanogens and, compared to CO_2 reduction, provide more energy to the animal (Weimer, 1998). Sulfate has the potential to act as an electron acceptor and produces dihydrogen sulfide (**H_2S**). Sulfate reducers can function under lower partial pressures than methanogens and hence are able to outcompete methanogens for H_2 (Mathison et al., 1998). The rumen

environment provides an excellent reducing power to reduce dietary sulfate to sulfite and ultimately sulfide (Lewis, 1954). Methane production may also decrease with additional sulfur in the diet because sulfite can be toxic to methanogenic bacteria (Mathison et al., 1998). Methane production may decrease by 16.7 g for every 100 g of sulfate if a full reduction takes place according to stoichiometry (Van Zijderveld et al., 2010). This is dependent on the medium or substrate available for the sulfate reducers (Isa et al., 1986). However, the methanogens may be able to adapt to the effect of sulfur over time. The major concern with adding sulfate to the diet is that it may result in the condition known as polioencephalomalacia. Polioencephalomalacia affects the central nervous system due to the highly toxic effects of H_2S on energy production (Merck, 2010). Also, diets high in sulfate can cause a thiamin deficiency leading to polioencephalomalacia. The concern may be exacerbated by adding sulfur to diets containing distillers grains due to the higher concentration of sulfur as a result of cleaning and control of pH in DDGS production (Schingoethe et al., 2009). According to the Dairy NRC (2001), the risks of toxicity are greatest when cattle consume very high grain diets, such as those fed to beef cattle. One method that may allow more sulfate to be fed is to feed supplemental thiamin, as it helps reduce the effects of polioencephalomalacia (Dairy NRC, 2001).

Using lactating Holstein-Friesian dairy cows, Van Zijderveld et al. (2011a) tested the effects of feeding diallyl disulfide, unsaturated fatty acids, and medium-chain fatty acids on CH_4 and milk production. Diets contained approximately 66% grass and corn silage with a concentrate mix containing 2 different concentrations (56 vs. 200 mg/kg of DM) of diallyl disulfide, yucca powder, calcium fumerate, unsaturated fatty acids or medium-chain fatty acids. Diallyl sulfide did not reduce CH_4 production compared to

calcium fumarate, an extruded flaxseed product or alternative fat sources containing unsaturated or medium-chain fatty acids. A potential reason for the lack of difference between diets may have resulted from less reduction occurring for sulfide compared with sulfate. Van Zijderveld et al. (2010) tested the addition of magnesium sulfate to high corn silage diets of sheep showed a 16% reduction in CH_4 without affecting feed intake and an increase in sulfate-reducing bacteria. This indicates a competition between methanogens and sulfur-reducing bacteria for the hydrogens produced in the rumen. Similarly, Silivong et al. (2011) fed goats sodium sulfate and observed a 14% reduction in CH_4 without affecting digestibility or N retention. However, Pesta (2015) fed sulfate to finishing steers and observed no reduction on total CH_4 production; sulfate did, however, reduce CH_4 per unit of feed intake. Relatively little research is available on the effects of additional sulfate supplementation in dairy cattle and the effects on CH_4 production. With lactating cattle, addition of a compound containing calcium and sulfate may prove beneficial in reducing CH_4 while also contributing to the calcium demand of the animal.

Acetogenesis. Acetogenesis converts CO_2 and H_2 into acetate through a reductive process and has been found in the gastrointestinal tract of non-ruminants, but has recently gained more interest in ruminants (Morgavi et al., 2010). Acetate is formed when reductive acetogenic bacteria oxidize H_2 while reducing 2 mol of CO_2 (Le Van et al., 1998). Two major advantages of acetogenesis are the production of acetate, which is an energy source used readily by the animal and the availability of electron acceptors (Weimer, 1998). The major challenge for acetogenesis is a reduced affinity for H_2 compared to methanogens. The low affinity of acetogenesis makes it challenging to outcompete methanogens, as the reaction is thermodynamically less favorable ($\Delta G^{\circ\prime}$ = -

104.6 vs. -135.6 kJ for acetogenesis vs. methanogenesis, respectively; Joblin, 1999). Acetogens have great versatility in metabolizing energy; this is a potential explanation for the poor affinity for H_2. Possible solutions to increase acetogenesis would include inhibition of methanogens combined with a yeast or probiotic, or genetic engineering to modify the acetogen to have a higher affinity for H_2, but further research is needed to determine the efficacy of manipulating acetogenesis as a technique to mitigate CH_4 production.

Lipid supplementation and biohydrogenation. Biohydrogenation represents an additional hydrogen sink in the rumen. Unsaturated fatty acids (**FA**) have the potential to be biohydrogenated in the rumen and use hydrogens that become available when the double bonds are broken (Beauchemin et al., 2008a). The process of biohydrogenation of fat may serve to utilize hydrogen in the rumen and potentially compete with methanogens in the rumen (Johnson and Johnson, 1995). Early research with linolenic acid supplementation in sheep observed nearly complete biohydrogenation of unsaturated fatty acids in the rumen (Czerkawski et al., 1966). With the biohydrogenation, there was a decrease in CH_4 production.

The degree of saturation for fatty acids may increase the amount of biohydrogenation and decrease CH_4 production by reducing H_2 concentration. Beauchemin et al. (2009) investigated the effects changing fat source to manipulate fatty acid profile by including crushed sunflower, flax, or canola seeds on CH_4 production in lactating dairy cows. The control ration was high in C16:0 and C 18:1, the sunflower ration was high in C18:1 (70.1 g/100 g of FA), the flaxseed ration was high in C18:3, and the canola ration was high in C18:1 and moderately high in 18:2. Experimental rations

were similar in total fat concentration. Compared to a zero control, diets containing sunflower, flaxseed, and canola meal all reduced CH_4, but were not different from one another (293, 264, 241, and 265 g/d for control, sunflower, flaxseed, and canola, respectively). One potential reason for no significant difference being observed amongst the different unsaturated FA rations could be the amount of FA in the diet. Nonetheless, less CH_4 was produced per unit of fat-corrected milk for flaxseed compared with sunflower and canola (10.5, 11.7, and 11.4 for flaxseed, sunflower, and canola, respectively). Digestibility was decreased when feeding sunflower and flaxseed compared with the control and canola, which is a major concern for practical application. Experimental diets were relatively low in total fat and the degree of biohydrogenation may have been too low and not affected CH_4 production.

Lipid supplementation is another method used to reduce CH_4 production. Lipid supplementation may decrease CH_4 production up to 40% depending upon the inclusion level in the diet, but reductions of 10 – 25% are more likely (Beauchemin et al., 2008a). Methane may be reduced by 10 to 20% when 2 to 4% fat is added to the diet (Beauchemin et al., 2008b). Practically, and because of its negative effect on fiber digestion, lipid concentrations usually do not exceed 7% of diet dry matter. When too much fat is fed, a reduction in feed intake and digestibility are possible and there are increased risks of milk fat depression in lactating dairy cows. High supplementation of lipid sources could negatively influence gastrointestinal function as well as production (Llonch et al., 2017). Lipid supplementation is believed to decrease CH_4 production because of being toxic to rumen microbes or, through its effects on biohydrogenation, decreasing organic matter fermentation, or increasing propionate concentration with

reduced acetate concentration (Johnson and Johnson, 1995; Beauchemin et al., 2009; Llonch et al., 2017). However, the effectiveness of reducing CH_4 production by using fats depends on concentration of fat in the ration, fat source, fatty acid profile, form of fat (whether it is a refined oil or in whole seeds), and diet type (Beauchemin et al., 2008a). Persistency of the CH_4 mitigation effectiveness is a major concern. Alstrup et al. (2015) measured the long-term effects of fat in lactating dairy cows and observed a persistent decrease in CH_4 production throughout lactation while using fat. Grainger and Beauchemin (2011) observed that lipid supplementation is effective for long duration, but there is a significant amount of variation between studies. Hence, further investigation on long-term effects of lipid supplementation is needed.

Fat concentration. Increasing fat content in the diet has the potential to affect CH_4 production. In a meta-analysis, Beauchemin et al. (2008a) found that for every 1% increase in dietary fat, there is a 5.6% reduction in CH_4. In the analysis, there was a high variation between some fat sources. High CH_4 reduction occurred with coconut oil reducing CH_4 production by 68% at an inclusion of 7% of dietary dry matter and myristic acid supplementation decreasing CH_4 production by 58% with inclusion of 5% of dietary dry matter. When feeding coconut oil to wethers at either 3.5 or 7% of dietary dry matter, Machmüller and Kreuzer (1999) observed CH_4 reductions of 28 and 73%, respectively, which indicates that increased fat concentrations may reduce CH_4 production. However, with increased fat supplementation, feed intake decreased. Similarly, Hollmann et al. (2013) increased coconut oil concentrations (0.0, 1.3, 2.7, 3.3, and 4.0%) and observed a linear decrease in CH_4 production as well as a linear decrease in feed intake. Milk production initially increased with dietary inclusion of 1.3% coconut oil, but then

decreased at greater inclusion levels. Hence, high inclusion of coconut oil is not recommended as a method to control CH_4 in dairy cattle due to the reduction in feed intake and milk production. In beef steers fed a finishing diet, Hales et al. (2017) supplemented corn oil at 0, 2, 4, and 6% of dietary dry matter and observed a linear decrease in CH_4 production without affecting feed intake. Therefore, increased concentrations of fat may reduce CH_4 production without affecting feed intake, but fat concentrations that are increased past the inclusion threshold will result in decreased feed intake.

Fat source and type. Fat sources used in cattle rations often depend upon geographical region. Depending on the fat, fatty acid profile will be different. For example, flaxseed products contain greater amounts of omega-3 fatty acids compared to other fat sources such as tallow. Many different fat sources have been used in an attempt to reduce CH_4 production, but effectiveness is highly variable due to the source and type of fat (Beauchemin et al., 2008b). Common sources include tallow, sunflower oil, whole sunflower seeds, flaxseed oil, flaxseed meal, soy oil, corn oil, fish oil, canola oil, rapeseed meal, camelina oil, and coconut oil (Machmüller and Kreuzer 1999; Beauchemin et al., 2008a; Grainger and Beauchemin 2011; Klop et al., 2016). Beauchemin et al. (2007b) studied adding tallow, sunflower oil, and whole sunflower seeds to rations to determine their effects on CH_4 production in Angus heifers and found a 33% decrease using sunflower seeds whereas tallow and sunflower oil each resulted in a 14% decrease. Digestibility was decreased 15 and 20% for tallow and sunflower seeds, respectively, compared to the control whereas sunflower oil only numerically decreased digestibility (12%). Decreased digestibility is likely the main factor for the decrease in

CH_4 while using tallow and sunflower seeds. Feed intake was decreased with sunflower seed supplementation, however, average daily gain was not affected by fat source.

Coconut oil is high in medium-chain fatty acids and has potential to decrease CH_4 production. When feeding coconut oil, CH_4 was reduced 3, 33, and 45% for dietary inclusions of 1.3, 2.7, and 3.3% (Hollmann et al., 2012). Feed intake decreased linearly with increasing fat inclusion while milk production and milk fat initially increased, but then decreased with higher inclusion. The decrease in CH_4 production with increased coconut oil also results in dramatic negative effects on milk production, milk fat yield and feed intake. Hence, coconut oil is not recommended as a CH_4 mitigation technique. Camelina oil is another fat source that is high in unsaturated fatty acids and may decrease CH_4 production. Compared to a control, a 30% decrease in CH_4 production was illustrated for camelina oil (Bayat et al., 2015), but milk production, milk fat, and feed intake decreased with camelina oil supplementation. The increased concentration of unsaturated fatty acids may have been toxic to rumen microbes, causing the decrease in CH_4 production while also decreasing feed intake. Alstrup et al. (2015) measured the effects of whole cracked rapeseed and found decreased CH_4 per unit of feed intake and energy corrected milk. Milk production increased with the inclusion of rapeseed but feed intake decreased. There was no effect on milk fat, although there was a slight decrease in milk protein. Inclusion of rapeseed may be a viable option to decrease CH_4 production.

Corn oil may be a practical fat source due to the large volume of distillers grains produced throughout the Midwest. In finishing beef steers, corn oil was increased at 0, 2, 4, and 6%, which resulted in a reduction in CH_4 without affecting feed intake (Hales et al., 2017). Digestibility of NDF increased from 0 to 4% inclusion of corn oil and then

decreased at 6% inclusion. Relatively little research has been conducted in lactating dairy cattle using corn oil to reduce CH_4 production. Including corn oil in rations could be of benefit to the industry if CH_4 reduction occurs without decreasing intake and milk production.

Flaxseed products have gained considerable attention as a feed source for dairy cattle due to potential benefits in reproduction and omega-3 in milk. Flaxseed can be fed to cattle as crude, extruded or oil products. Martin et al. (2008) used a control product containing no flaxseed, crude flaxseed, extruded flaxseed and flaxseed oil products to compare potential CH_4 differences between products. Rations containing flaxseed were balanced for FA and contained approximately 5.7% of dietary DM. Methane production was reduced for all rations containing flaxseed compared to the control. Specifically, total CH_4 production was decreased by 12% for crude flaxseed, 38% for extruded flaxseed, and 64% by flaxseed oil. Extruded flaxseed and flaxseed oil rations decreased feed intake and milk production compared to the control and crude flaxseed, but intakes and milk production for control and crude flaxseed were not different. Digestibility was also decreased by rations containing flaxseed. Flaxseed may have potential to reduce CH_4, however, lowered digestibility may result in decreased milk production and feed intake. Total fat content of the diet was not equal in this experiment as rations containing flaxseed had a greater fat content compared to the control (6.8, 7.0, 8.4, and 2.6% of DM for crude, extruded, oil, and control rations, respectively).

Whole oilseeds are potentially less toxic to the microbial population compared to crushed oilseeds and extracted oil because of decreased readily available fat (Beauchemin et al., 2008a), which may explain the reduction in feed intake as well as the drastic

reduction in CH_4 production. In a study to determine the effects of supplementing flaxseed oil in either corn silage or red clover silage-based diets (Benchaar et al, 2015), flaxseed oil supplementation to diets containing red clover silage decreased CH_4 production by 9% without affecting digestibility, whereas flaxseed oil supplementation to corn silage decreased CH_4 production by 26%, but total fiber digestibility was negatively affected. Total protozoa numbers decreased with the addition of flaxseed oil to corn silage but were not decreased in red clover silage. The decrease in protozoa changed the microbial community in the rumen and may attribute to the reduction in CH_4 as well as the decreased digestibility of the diet. Hence, the degree to which flaxseed products reduce CH_4 will be dependent on source.

Overall, fats have great potential to reduce CH_4 production in dairy cattle. They are also beneficial in changing the FA composition of milk. However, effects of fat on feed intake are generally complicated. The degree of reduction will depend on fat source and inclusion in the diet. Modification of the rumen environment may decrease overall digestibility due to potential toxic effects on the microbial community.

Distillers grains. Feeding dried distillers grain and solubles (**DDGS**) has increased in popularity over the years and has been illustrated to potentially reduce CH_4 production. Dietary inclusion of corn DDGS has been illustrated to increase feed intake in dairy rations without affecting milk components (Janicek et al., 2008). In lactating Holstein cattle, replacing corn and soybean meal with corn DDGS decreased CH_4 by 14% (Birkelo et al., 2004). Increasing corn DDGS from 0 to 30% of dietary dry matter, CH_4 production decreased linearly (495, 490, 477, 475 g/d for 0, 10, 20, and 30% corn DDGS diets, respectively) as well as CH_4 per unit of milk produced (Benchaar et al., 2013). Milk

production increased linearly with increasing corn DDGS (32.6, 35.1, 35.8, and 36.6 for 0, 10, 20, and 30% corn DDGS, respectively), however, energy corrected milk was not different as the production of milk fat and protein decreased linearly with increasing corn DDGS. Additionally, feed intake tended to increase linearly with increased corn DDGS (24.2, 24.6, 24.4, and 25.3 for 0, 10, 20, and 30% corn DDGS, respectively). Rumen fermentation characteristics indicate a linear decrease in the rumen acetate-to-propionate ratio with increasing corn DDGS. This could be the result of a negative effect on rumen protozoa.

When Hereford beef steers were fed a diet containing 65% silage with either 35% DDGS or barley grain, McGinn et al. (2009) found a 20% reduction in CH_4 production with corn DDGS. When comparing corn vs. wheat DDGS, Hünerberg et al. (2013) observed that corn DDGS decreased CH_4 production by approximately 17% and compared to the control it decreased CH_4 production by 13%. When adding oil to the wheat DDGS diet, CH_4 production was similar compared to the corn DDGS. Wheat DDGS has a lower fat content compared to corn DDGS so the addition of oil may have been the cause for the reduction in CH_4. Historically, corn DDGS typically contained 10 to 12% fat. It is possible that the reduction in CH_4 when using corn DDGS was caused by increased dietary fat from the DDGS as unprotected fat has a negative effect on rumen protozoa (Benchaar et al., 2013). However, typical corn DDGS available now contain less than 8% fat.

Manufacturing of corn DDGS has evolved and now includes additional extraction of corn oil from the grain, creating reduced-fat DDGS (Mjoun et al., 2010). Using lactating dairy cows, Mjoun et al. (2010) found no difference using DDGS to replace

soybean feedstuffs at an inclusion of 30% of dietary dry matter for feed intake or milk production, but found an increase in milk fat percent and yield. Using lactating Holstein and Jersey cattle, Foth et al. (2015) used a corn silage and alfalfa-based diet with the addition of corn and soybean meal to compare the effects of feeding DDGS in lactating cattle diets. Feed intake was not affected by feeding DDGS but milk production increased from 29.8 to 30.9 kg/d with the inclusion of DDGS. Methane production was reduced from 504 to 472 L/d with inclusion of DDGS. This indicates that nutritive entities within the DDGS are able to reduce CH_4 production. Fat content in the DDGS is still approximately 6%, which may still play a role in reducing CH_4 production, but not likely to the same degree as rations containing DDGS. Another potential role in reduced CH_4 is the increased digestibility of DDGS. The NDF in DDGS is highly digestible (Janicek et al., 2008) and may contribute to the reduction in CH_4 production. Knapp et al. (2014) observed that DDGS are highly digestible compared to forages and produce half to one-third the CH_4 per unit of digested dry matter. Similarly, Drehmel (2017) observed a 32% decrease in CH_4 per unit of digested NDF with increased concentrations of hemicellulose in the diet of lactating dairy cows. Further investigation should determine the effects of adding additional oil to the DDGS to determine CH_4 reduction potential.

Management

Management. Management decisions have one of the greatest impacts on CH_4 production. Forage type, genetics, culling, disease reduction, and facility type and design are all management decisions that affect CH_4 production (Knapp et al., 2014). Feed management may increase the productivity of the animal so they make more milk per unit of feed intake and reduce CH_4 production (Shibata and Terada, 2010). Generally, when

the fiber content of the diet increases CH_4 production is increased, whereas an increase in protein content of the diet leads to a decrease in CH_4 production (Johnson and Johnson, 1995; Shibata and Terada, 2010).

Increasing the efficiency of the animal to improve the energy utilization as well as productivity may help reduce total CH_4 production. Utilizing improved genetics, a long-term reduction in CH_4 may be possible with increased efficiencies from cattle (Knapp et al., 2014; Van Middelaar et al., 2015). Increasing the efficiency of converting feed to milk as well as increasing total milk production could decrease CH_4 produced per unit of feed intake as well as per unit of milk produced. Increasing the longevity of the herd may reduce total CH_4 production, but further research is still needed to verify potential benefits.

Rumen Modifiers

Rumen modifiers and feed additives. Rumen modifiers affect the microbial community and alter the production of CH_4. For example, monensin is an ionophore commonly used to alter the microbial community and the production of volatile fatty acids, which has been illustrated to reduce CH_4 production (Odongo et al., 2007). Concerns have arisen with the potential benefits of long-term use of monensin on reducing CH_4 production. There is potential for different microbes to emerge when specific members of the communities are suppressed. However, Odongo et al. (2007) sustained a 7% decrease in CH_4 production during a 6-month study using monensin. Appuhamy et al. (2013) observed a greater effect of monensin with beef steers compared to dairy cattle. This is likely the result of increased forage amounts in dairy cattle. Long-term effects will be dependent on diet type, cattle type, and inclusion rate.

In an avenue related to nitrate, 3-nitrooxypropanol (3NOP) is a CH_4 inhibitor that affects rumen archaea by inhibiting the methyl coenzyme B reductase, which is the final step in methanogenesis (Hristov et al., 2015). 3-nitrooxypropanol offers CH_4 reduction capability without the potentially negative effects of nitrate. To determine the effectiveness of 3NOP, Haisan et al. (2014) dosed 2,500 mg/d 3NOP to lactating Holstein cattle and observed a reduction in CH_4 production of approximately 60% (17.8 to 7.18 g/kg of DMI for control and 3NOP, respectively). Milk production and feed intake were not affected by 3NOP but acetate production was reduced. However, Reynolds et al. (2014) dosed mid-lactation Holstein-Friesian dairy cows with either 500 or 2,500 mg/d 3NOP and observed a reduction in CH_4 of 6.6 and 9.8% per day, unlike the 60% decrease previously reported. Analysis of volatile fatty acids in the rumen indicated a decreased ratio of acetate to propionate with a significant decrease in acetate concentration at the higher dose of 3NOP. Milk production and feed intake were only numerically reduced. A major challenge with CH_4 mitigation is the ability of the method to work with persistency or long-term effectiveness. Hristov et al. (2015) determined the effectiveness of a control, 40, 60, and 80 g/d of 3NOP in a 12-wk experiment, and found a persistent reduction in CH_4 (25, 31, and 32% compared to the control for 40, 60, and 80 g/d, respectively) throughout the experiment without affecting milk production or feed intake. Similarly, Lopez et al. (2016) found a 31% decrease in CH_4 production when supplementing 3NOP at the 60 g/d concentration. In beef cattle, supplementation of 3NOP decreased CH_4 production 33% at an inclusion of 4.50 mg/kg BW (Romero-Perez et al., 2014). Additionally, CH_4 production decreased linearly with increased 3NOP supplementation.

Use of 3NOP appears to be a potential method to reduce CH_4 production both short-term and long-term without negatively affecting milk production or feed intake.

Plant compounds. Plant compounds such as tannins, saponins and essential oils may also be used to reduce CH_4 production. The reduction is believed to be caused by decreased availability of H_2, which indirectly inhibits methanogenesis as well as directly inhibiting the methanogens (Hook et al., 2010). In goats, tannins have reduced CH_4 by 47% (Puchala et al., 2005) and grazing dairy cattle by 32% (Woodward et al., 2004). Lactating dairy cows fed sainfoin (a high-tannin silage) have been observed to produce less CH_4 (19.4 vs. 20.6 g/kg of feed intake for sainfoin vs. control, respectively), decrease digestibility (71.8 vs. 74.7% for sainfoin vs. control, respectively), and increase milk production (24.1 vs. 22.0 kg/d for sainfoin vs. control, respectively) (Huyen et al., 2016). However, in growing Angus beef steers and heifers, feeding tannins at 2% of dietary dry matter did not reduce CH_4 production (Beauchemin et al., 2007a). The potential decreases in the studies with grazing dairy cattle, the sheep and the goats may have been caused by differences in digestibility. However, in the growing beef cattle study, forage quality remained consistent with only a change in tannin concentration. Increased tannin supplementation decreased digestibility in lactating dairy cow diets, which is a potential concern for incorporation in the dairy industry. Further research is needed to determine the potential benefits of tannin supplementation on CH_4 reduction in dairy cattle.

In vitro studies have demonstrated an inhibition of protozoa with saponin supplementation, which reduces the availability of H_2 for methanogenesis (Hook et al., 2010). Essential oils are believed to act similarly to monensin by inhibiting gram-positive bacterial and also possess antimicrobial activity, all of which could reduce

methanogenesis (Cobellis et al., 2016). The antimicrobial activity may be caused by the presence of a carbonyl group, which can disrupt the cell membrane of the microbe and inhibit microbial enzymes. Phenolic compounds are found in essential oils, which also have antimicrobial activity. A major concern with essential oil supplementation is the potential decrease in digestibility of fiber (Patra and Yu, 2012). In a study to determine the effects of different essential oil supplements on CH_4 production, oregano, rosemary, Ceylon cinnamon, cinnamon leaves, cinnamon bark, dill seeds, and eucalyptus where found to decrease the abundance of protozoa, archaea and some bacteria in an in vitro experiment (Cobellis et al., 2016). In this study, a reduction in total gas production and CH_4 production was observed. However, the nutrient profile of the essential oils was not listed, so it is difficult to determine the reason CH_4 was reduced with inclusion. Dry matter digestibility decreased with the use of essential oils except for a combination of Ceylon cinnamon-dill seeds-eucalyptus. Results are inconsistent with the use of essential oils and potential benefits may be dependent on essential oil type to reduce CH_4 production without affecting digestibility and production, but further research is needed.

SUMMARY OF LITERATURE

In the study of dairy cattle nutrition, calorimetry is generally considered the highest standard in studies designed to measure the energetic value of feed and overall energy utilization. Calorimetry is the process of measuring heat of biological reactions and can be further classified into two methods, namely direct and indirect. Direct calorimetry measures the heat lost by the animal and this heat ends up in the environment. Indirect calorimetry measures gases produced and this is then used to indirectly estimate the amount of heat produced by the animal. A number of different

apparatuses may be used to conduct studies involving indirect calorimetry, including respiration calorimeters and tracer gases. Respiration calorimeters can be either full body, in which the animal is fully enclosed, or headbox-style, in which the animal's head is secured inside the device. Headbox-style calorimeters are well suited for studies involving lactating dairy cattle, as they allow continuous gas collection during milking. The use of tracer gases allow cattle to be mobile, thus this technique is advantageous for grazing cattle. Ultimately, calorimetry is a method used to measure the amount of heat produced by animals and measure energy utilization.

Measuring energy utilization is often challenging, as energy is lost via heat, urine, feces and gas, and, as a result, precise and complete sampling is necessary to correctly account for each route of loss. Energy may be analytically partitioned into four different fractions: 1) gross energy, 2) digestible energy, 3) metabolizable energy, and 4) net energy for maintenance, lactation, growth, and conceptus. Together, these different fractions are sources of inherent biological variation in energy balance. Also contributing to the biological variation is stage of lactation. A negative energy balance, which is common in cows in early lactation, is a result of the inability of the animal to consume sufficient feed to support the total amount of milk produced. To compensate, cattle mobilize different body energy stores. Utilization of either feed or body stores have different efficiencies, which may attribute to the biological variation in efficiency of utilization. Genetic improvements have increased milk production and, therefore, increased efficiencies in cattle. Precise management of feed has also led to increased efficiency for feed.

Correctly deriving the amount of energy needed for maintenance in dairy cattle is also challenging. Large biological variation occurs from cow to cow, which makes it difficult to identify an exact value for maintenance. Stage of lactation may also affect energy requirements for maintenance. Additionally, experimental data would suggest that dry cows have lower maintenance requirements for energy than lactating cows. Over time, it is generally believed that the requirements for maintenance energy have increased, and this may partially be due to genetic selection for increased milk production.

Lactating dairy cattle produce approximately 500 L of CH_4 daily, and CH_4 is 25 times more potent as a greenhouse gas compared to CO_2. Consequently, mitigation of CH_4 production has been a topic of increasing scientific interest. Methane production also represents a 2 to 12% energetic loss to the animal. Methane is produced during the formation of volatile fatty acids during fermentation. It is produced in response to an excess of hydrogen and a need to keep the partial pressure low and ultimately to maintain normal rumen fermentation. Fiber digestion increases acetate production, which due to the availability of hydrogens, increases CH_4 production. A linear relationship also exists between feed intake and CH_4 production. As cattle consume more feed, there is an increase in CH_4 produced. Additionally, CH_4 production is very episodic and, as such, there is inherent variation throughout the day. Usually peak CH_4 occurs a few hours post feeding. When feeding multiple times during the day, multiple peaks have been observed, but published studies have conflicting results as to when the greater peak will occur.

Many methods exist to reduce CH_4 production and are usually categorized in three areas: 1) genetics, 2) rumen modifiers, and 3) feeding and nutrition management.

Genetic improvement focuses on management decisions that increase milk production and dilute CH_4 produced per unit of milk produced. Rumen modifiers include 3NOP, monensin, and essential oils, which alter the microbial community in an attempt to decrease methanogenesis. The majority of the mitigation work has been focused in feed and nutrition management. Managing fiber digestion may decrease CH_4 production if less hydrogen-producing bacteria are present. Manipulating the forage-to-concentrate ratio increases the amount of propionate produced and decreases acetate, resulting in H_2 and consequently CH_4. Feeding alternative H_2 sinks may also lead to a reduction in CH_4 production. These sinks include nitrate, sulfate, and fat. These sinks compete for the hydrogens and ultimately reduce CH_4 production. Feeding fat has reduced CH_4 production, but the effect is dependent on type of fat and concentration in the diet. Few studies have investigated the effects of fat type while maintaining constant dietary fat concentrations. Hence, omega-3 fatty source needs to be compared against an alternative fat source at similar dietary fat concentrations to determine if fat source affects CH_4 production in dairy cattle. Feeding distillers grains may be another method to reduce CH_4 production, as it is a highly digestible feed source with a high concentration of fat.

Many of these feed management strategies have been observed to reduce CH_4 production, however, most have not been studied in combination with DDGS or in diets containing greater than 55% forage. Dairy cattle require forage for production of milk fat. Utilizing some of these dietary methods in combination with diets greater than 55% forage diets are needed. Also, CH_4 is very episodic and represents an energetic loss to the animal. Further investigation is needed to accurately describe the diurnal variation of CH_4

production. Additionally, the relationship needs to be explored as to the effects of reducing CH_4 production on energy balance in dairy cattle.

REFERENCES

Agnew, R.E., and T. Yan. 2005. Calorimetry. Pages 421-442 In Quantitative aspects of ruminant digestion and metabolism. CABI Publishing, Oxfordshire, UK.

Albright, L.D. 1990. Environment control for animals and plants. Page 453 St. Joseph, MI. American Society of agricultural Engineers.

Alstrup, L., A.L.F. Hellwing, P. Lund, and M.R. Weisbjerg. 2015. Effect of fat supplementation and stage of lactation on methane production in dairy cows. Animal Feed Sci. and Tech. 207:10-19.

Appuhamy, J.A.D.R.N, A.B. Strathe, S. Jayasundara, C. Wagner-Riddle, J. Dijkstra, J. France, and E. Kebreab. 2013. Anit-methanogenic effects of monensin in dairy and beef cattle: A meta-analysis. J. Dairy Sci. 96:5161-5173.

Arbre, M., Y. Rochette, J. Guyader, C. Lascoux, L. M. Gómez, M. Eugéne, D. P. Morgavi, G. Renand, M. Doreau, and C. Martin. 2016. Repeatability of enteric methane determinations from cattle using either the SF6 tracer technique or the GreenFeed system. Animal Production Science, 56:238-243.

Baldwin, R.L., N.E. Forsberg, and C.Y. Hu. 1985. Potential for altering energy partition in the lactating cow. J. Dairy Sci. 68:3394-3402.

Bauman, D.E., and W.B. Currie. 1980. Partitioning of nutrients during pregnancy and lactation: A review of mechanisms involving homeostasis and homeorhesis. J. Dairy Sci. 63:1514-1529.

Bauman, D.E., S.N. Mccutcheon, W.D. Steinhour, J. Eppard, S.J. Sechen, and P.J. Eppard. 1985. Sources of variation and prospects for improvement of productive efficiency in the dairy cow. J. Anim. Sci. 60:583–592.

Bayat, A.R., P. Kairenius, T. Stefanski, H. Leskinen, S. Comtet-Marre, E. Forano, F. Chaucheyras-Durand, and K.J. Shingfield. 2015. Effects of camelina oil or live yeasts (Saccharomyces cerevisiae) on ruminal methane production, rumen fermentation, and milk fatty acid composition in lactating cows fed grass silage diets. J. Dairy Sci. 98:3166-3181.

Beauchemin, K. A., S. M. McGinn, T.F. Martinez, and T.A. McAllister. 2007a. Use of condensed tannin extract from quebracho trees to reduce methane emissions from cattle. J. Anim. Sci. 85:1990-1996.

Beauchemin, K. A., S. M. McGinn, and H. V. Petit. 2007b. Methane abatement strategies for cattle: Lipid supplementation of diets. Can. J. Anim. Sci. 87:431-440.

Beauchemin, K.A., M. Kreuzer, F. O'Mara, and T. A. McAllister. 2008a. Nutritional management for enteric methane abatement: A review. Australian Journal of Experimental Ag. 48:21-27.

Beauchemin, K.A., S.M. McGinn, and C. Grainger. 2008b. Reducing methane emissions from dairy cows. WCDS Advances in Dairy Technology 20:79-93.

Beauchemin, K.A., S.M. McGinn, C. Benchaar, and L. Holtshausen. 2009. Crushed sunflower, flax, or canola seeds in lactating dairy cow diets; Effects on methane production, rumen fermentation, and milk production. J. Dairy Sci. 92:2118-2127.

Beauchemin, K. A., T. Coates, B. Farr, and S. M. McGinn. 2012. Technical note: Can the sulfur hexafluoride tracer gas technique be used to accurately measure enteric methane production from ruminally-cannulated cattle? J. Anim. Sci. 90:2727-2732.

Benchaar, C., F. Hassanat, R. Gervais, P.Y. Chouinard, C. Julien, H.V. Petit, and D.I. Massè. 2013. Effects of increasing amounts of corn dried distillers grains with solubles in dairy cow diets on methane production, ruminal fermentation, digestion, N balance, and milk production. J. Dairy Sci. 96:2413-2427.

Benchaar, C., F. Hassanat, R. Martineau, and R. Gervais. 2015. Linseed oil supplementation to dairy cows fed diets based on red clover silage or corn silage: Effects on methane production, rumen fermentation, nutrient digestibility, N balance and milk production. J. Dairy Sci. 98:7991-8008.

Benzinger, T.H., and C. Kitzinger. 1949. Direct calorimetry by means of the gradient principle. Rev. Sci. Instrum. 20:849-860.

Birkelo, C.P., M.J. Brouk, and D.J. Schingoethe. 2004. The energy content of wet corn distillers grains for lactating dairy cows. J. Dairy Sci. 87:1815-1819.

Blaxter, K. L. 1962. The Energy Metabolism of Ruminants. 2nd ed. Hutchinsom & Co. Ltd., London, UK.

Blaxter, K. L. 1965. Energy Metabolism. Proceedings from the third symposium. Academic Press, London, UK.

Blaxter, K. 1989. Energy metabolism in animals and man. Pg 42-43. Cambridge University Press, Cambridge, Great Britain.

Boadi, D. A., K. M. Wittenberg, and A. D. Kennedy. 2002. Validation of the sulphur hexafluoride (SF_6) tracer gas technique for measurement of methane and carbon dioxide production by cattle. Can. J. Anim. Sci. 82:125-131.

Boadi, D. A., and K. M. Wittenberg. 2002. Methane production from dairy and beef heifers fed forages differing in nutrient density using the sulphur hexafluoride (SF6) tracer gas technique. Can. J. Anim. Sci. 82:201–206.

Boadi, D., C. Benchaar, J. Chiquette, and D. Masse. 2004. Mitigation strategies to reduce enteric methane emissions from dairy cows: Update review. Can. J. Anim. Sci. 84:319-335.

Branco, A.F., F. Giallongo, T. Frederick, H. Weeks, J. Oh, and A.N. Hristov. 2015. Effect of technical cashew nut shell liquid on rumen methane production and lactation performance of dairy cows. J. Dairy Sci. 98:4030-4040.

Brask, M., M. R. Weisbjerg, A. L. F. Hellwing, A. Bannink, and P. Lund. 2015. Methane production and diurnal variation measured in dairy cows and predicted from fermentation pattern and nutrient or carbon flow. Animal 9(11):1795-1806.

Brody, S. 1945. Bioenergetics and Growth. Reinhold Publishing Corporation, New York, NY.

Brouwer, E. 1965. Report of Sub-committee on Constants and Factors. Pages 441-443 in Energy Metabolism. K.L. Blaxter, editor. Academic Press Inc. Ltd., London, UK.

Bruinenberg, M.H., Y. van der Honing, R.E. Agnew, T. Yan, A.M. van Vuuren, and H. Valk. 2002. Energy metabolism of dairy cows fed on grass. Livestock Prod. Sci. 75:117-128.

Byers, F.M. and G.E. Carstens. 1991. Seasonality of maintenance requirements in beef cows. Pages 450-453 in Energy Metabolism of Farm Animals, EAAP Publ. No. 58, Kartause Ittengen, Switzerland.

Capper, J.L., R.A. Cady, and D.E. Bauman. 2009. The environmental impact of dairy production: 1944 compared with 2007. J. Anim. Sci. 87:2160-2167.

Chaucheyras-Durand, F., S. Masseglia, G. Fonty, and E. Forano. 2008. Development of hydrogenotrophic microorganisms and H_2 utilisation in the rumen of gnotobiotically-reared lambs. Influence of the composition of the cellulolytic microbial community and effect of the feed additive Saccharomyces cerevisiae I-1077. In: Proceedings of the 6th INRA-RRI symposium. Gut microbiome: functionality, interaction with the host and impact on the environment, Clermont-Ferrand, France, pp. 48–49.

Chase, L.E. 2014. Carbon footprint and the dairy industry. Cornell Nutrition Conference Animal Science Conference Proceedings. Cornell Univ. Ithaca, NY.

Cobellis, G., M. Trabalza-Marinucci, M. C. Marcotullio, and Z. Yu. 2016. Evaluation of different essential oils in modulating methane and ammonia production, rumen fermentation, and rumen bacteria in vitro. Anim. Feed Sci. Tech. 215:25-36.

Collier, R.J., and D.K. Beede. 1985. Thermal stress as a factor associated with nutrient requirements and interrelationships. In Nutrition of Grazing Ruminants. (ed) by L. McDowell. Academic Press, New York, NY. pgs. 59-71.

Coppock, C.E., W.P. Flatt, L.A. Moore, and W.E. Stewart. 1964. Effect of hay to grain ratio on utilization of metabolizable energy for milk production by dairy cows. J. Dairy Sci. 47:1330-1338.

Coppock, C.E. 1985. Energy nutrition and metabolism of the lactating dairy cow. J. Dairy Sci. 68:3403-3410.

Crompton, L.A., J.A.N. Mills, C.K. Reynolds, and J. France. 2010. Fluctuations in methane emission in response to feeding pattern in lactating dairy cows. In: Sauvant, D., J. Van Milgen, P. Faverdin, N. Friggens, (Eds.), Modelling Nutrient Digestion and Utilization in Farm Animals. Wageningen Academic Publishers, Wageningen, the Netherlands, pp. 176-180.

Czerkawski, J.W., K.L. Blaxter, and F.W. Wainman. 1966. The metabolism of oleic, linoleic, and linolenic acids by sheep with reference to their effects on methane production. Br. J. Nutr. 20:349-362.

De Campeneere, S. and N. Peiren. 2014. ILVO's ruminant respiration facility. In Technical manual on respiration chamber designs. Chapter 3. Ed. By Cesar Pinares and Garry Waghorn. Melle, Belgium.

Derno, M., W. Jentsch, M. Schweigel, S.Kuhla, C.C. Metges, and H.D. Matthes. 2005. Measurements of heat production for estimation of maintenance energy requirements of Hereford steers. J. Anim. Sci. 83:2590-2597.

Derno, M., H.G. Elsner, E.A. Paetow, H. Scholze, and M. Schweigel. 2009. Technical note: A new facility for continuous respiration measurements in lactating cows. J. Dairy Sci. 92:2804-2808.

Dijkstra, J., J. France, and D.R. Davies. 1998. Different mathematical approaches to estimating microbial protein supply in ruminants. J. Dairy Sci. 81:3370-3384.

Dong, L.F., C.P. Ferris, D.A. McDowell, and T. Yan. 2015a. Comparison of maintenance energy requirement and energetic efficiency between lactating Holstein-Friesian and other groups of dairy cows. J. Dairy Sci. 98:1136-1144.

Dong, L.F., C.P. Ferris, D.A. McDowell, and T. Yan. 2015b. Effects of diet forage proportion on maintenance energy requirement and the efficiency of

metabolizable energy use for lactation by lactating dairy cows. J. Dairy Sci. 98:8846-8855.

Drehmel, O.R. 2015. Effect of fat and fiber on methane production and energy utilization in lactating dairy cows. Thesis. Retrieved from http://digitalcommons.unl.edu/animalscidiss/145/.

Evans, B.J.L., B.L. Golden, and B.L. Hough. 2000. A new genetic prediction for cow maintenance energy. Pages 79-88 in Beef Improv. Fed. Symp. Proc., Oklahoma State Univ. Stillwater, OK.

Ferrell, C.L., and T.G. Jenkins. 1985. Energy utilization by Hereford and Simmental males and females. Anim. Prod. 41:52-61.

Flatt, W.P., C.E. Coppock, L.A. Moore, and R.W. Hemken. 1965. Energy balance studies with dry, non-pregnant dairy cows consuming pelleted forages. Pages 131-144 in Energy Metabolism ed. By K.L. Blaxter. London: Academic Press. (Proc. 3rd symp. Energy Metab., Troon, Scotland).

Flatt, W.P., P.W. Moe, and R.R. Oltjen. 1967a. Energy metabolism studies with dairy cows receiving purified diets. Pages 109-121 in Energy Metabolism of Farm Animals. ed. By K.L. Blaxter. London: Academic Press. (Proc. 4th symp. Energy Metab., Warsaw, Poland).

Flatt, W.P., P.W. Moe, A.W. Munson, and T. Cooper. 1967b. Energy utilization by high producing dairy cows. II. Summary of energy balance experiments with lactating dairy cows. Pages 221-234 in Energy Metabolism of Farm Animals. ed. By K.L. Blaxter. London: Academic Press. (Proc. 4th symp. Energy Metab., Warsaw, Poland).

Flatt, W.P., P.W. Moe, A.W. Munson, and T. Cooper. 1967c. Energy utilization by high producing dairy cows. II. Summary of energy balance experiments with lactating dairy cows. Pages 235-251 in Energy Metabolism of Farm Animals. ed. By K.L. Blaxter. London: Academic Press. (Proc. 4th symp. Energy Metab., Warsaw, Poland).

Foth, A.J., T. Brown-Brandl, K.J. Hanford, P.S. Miller, G. Garcia Gomez, and P.J. Kononoff. 2015. Energy content of reduced-fat dried distillers grains with solubles for lactating dairy cows. J. Dairy Sci. 98:7142-7152.

Freetly, H.C., and J.A. Nienaber. 1998. Efficiency of energy and nitrogen loss and gain in mature cows. J. Anim. Sci. 76:896-905.

Freetly, H.C., J.A. Nienaber, and T. Brown-Brandl. 2002a. Relationships among heat production, body weight, and age in Finnsheep and Rambouillet ewes. J. Anim. Sci. 80:825-832.

Freetly, H.C., J.A. Nienaber, and T. Brown-Brandl. 2006. Partitioning of energy during lactation of primiparous beef cows. J. Anim. Sci. 84:2157-2162.

Garrett, W.N. 1970. The influence of sex on the energy requirements of cattle for maintenance and growth. Page 101-104 in Energy Metabolism of Farm Animals, EAAP Publ. No. 13, Vitznau, Switzerland.

Gill, M., P. Smith, and J.M. Wilkinson. 2010. Mitigating climate change: The role of domestic livestock. Animal 4:323-333.

Grainger, C, C.W. Holmes, and Y.F. Moore. 1985. Performance of Friesian cows with high and low breeding indexes. Anim. Prod. 40:389-400.

Grainger, C., T. Clarke, S. M. McGinn, M. J. Auldist, K. A. Beauchemin, M. C. Hannah, G. C. Waghorn, H. Clark, and R. J. Eckard. 2007. Methane emissions from dairy cows measured using the sulfur hexafluoride (SF6) tracer and chamber techniques. J. Dairy Sci. 90:2755–2766.

Grainger, C., and K.A. Beauchemin. 2011. Can enteric methane emissions from ruminants be lowered without lowering their production? Anim. Feed Sci. and Tech. 166-167:308-320.

Haisan, J., Y. Sun, L.L. Guan, K.A. Beauchemin, A. Iwaasa, S. Duval, D.R. Barreda, and M. Oba. 2014. The effects of feeding 3-nitrooxypropanol on methane emissions and productivity of Holstein cows in mid lactation. J. Dairy Sci. 97:3110-3119.

Hales, K.E., and N.A. Cole. 2017. Hourly methane production in finishing steers fed at different levels of dry matter intake. J. Anim. Sci. 95:2089-2096.

Hales, K.E., A.P. Foote, T.M. Brown-Brandl, and H.C. Freetly. 2017. The effects of feeding increasing concentrations of corn oil on energy metabolism and nutrient balance in finishing beef steers. J. Anim. Sci. 95:939-948.

Hammond, K.J., D.J. Humphries, L.A. Crompton, C. Green, and C.K. Reynolds. 2016a. Methane emissions from cattle: Estimates from short-term measurements using a GreenFeed system compared with measurements obtained using respiration chambers or Sulphur hexafluoride tracer. Animal Feed Sci. and Technology 203:41-52.

Hammond, K. J., G. C. Waghorn, and R. S. Hegarty. 2016b. The GreenFeed system for measurement of enteric methane emission from cattle. Animal Production Science 56:181-189.

Hatew, B., S.C. Podesta, H. Van Laar, W.F. Pellikaan, J.L. Ellis, J. Dijkstra, and A. Bannink. 2015. Effects of dietary starch content and rate of fermentation on methane production in lactating dairy cows. J. Dairy Sci. 98:486-499.

Hayasaka, K., N. Takasuri, and N. Yamagishi. 1995. Energy metabolism in lactating Holstein cows (in Japanese, with English abstract). Animal Science and Technology 66:374-382.

Hegarty, R.S. 2013. Applicability of short-term emission measurements for on-farm quantification of enteric methane. Animal 7:401-408.

Herselman, M. J., T. Sahlu, S. P. Hart, and A. L. Goetsch. 1998. Energy expenditure by dry and lactating Alpine does estimated by entry rate of carbon dioxide. J. Dairy Sci. 81:2469-2474.

Hillman, P. E., K. G. Gebremedhin, A. Parkhurst, J. Fuquay, and S. Willard. 2001. Evaporative and convective cooling of cows in a hot humid environment. In Livestock Environment VI, Proc. 6th Intl. Livestock Environment Symposium, 343-350. St. Joseph, Mich.: ASAE.

Hollmann, M., W.J. Powers, A.C. Fogiel, J.S. Liesman, N.M. Bello, and D.K. Beede. 2012. Enteric methane emissions and lactational performance of Holstein cows fed different concentrations of coconut oil. J. Dairy Sci. 95:2602-2615.

Hollmann, M., W.J. Powers, A.C. Fogiel, J.S. Liesman, and D.K. Beede. 2013. Response profiles of enteric methane emissions and lactational performance during habituation to dietary coconut oil in dairy cows. J. Dairy Sci. 96:1769-1781.

Hook, S.E., A.D.G. Wright, and B.W. McBride. 2010. Methanogens: Methane producers of the rumen and mitigation strategies. Archaea. Article ID 945785.

Hristov, A.N., J. Oh, J.L. Firkins, J. Dijkstra, E. Kebreab, G. Waghorn, H.P.S. Makkar, A.T. Adesogan, W. Yang, C. Lee, P.J. Gerber, B. Henderson, and J.M. Tricarico. 2013a. Special Topics- Mitigation of methane and nitrous oxide emissions from animal operations: I. A review of enteric methane mitigation options. J. Anim. Sci. 91:5045-5069.

Hristov, A.N., J. Oh, F. Giallongo, T.W. Frederick, M.T. Harper, H.L. Weeks, A.F. Branco, P.J. Moate, M.H. Deighton, S.R.O. Williams, M. Kindermann, and S. Duval. 2015. An inhibitor persistently decreased enteric methane emissions from dairy cows with no negative effect on milk production. Proc. Nat. Acad. Sci. U.S.A., http://dx.doi.org/10.1073/pnas.15041124112.

Huhtanen, P., E.H. Cabezas-Garcia, S. Utsumi, and S. Zimmerman. 2015. Comparison of methods to determine methane emissions from dairy cows in farm conditions. J. Dairy Sci. 98:3394-3409.

Hünberberg, M., S.M. McGinn, K.A. Beauchemin, E.K. Okine, O.M. Harstad, and T.A. McAllister. 2013. Effects of dried distillers' grains with solubles on enteric methane emissions and nitrogen excretion from finishing beef cattle. Can. J. Anim. Sci. 93:373-385.

Hungate, R.E. 1967. Hydrogen as an intermediate in the rumen fermentation. Archives of Microbiology 59:158-164.

Huyen, N.T., O. Desrues, S.J.J. Alferink, T. Zandstra, M.W.A. Verstegen, W.H. Hendriks, and W.F. Pellikaan. 2016. Inclusion of sainfoin (Onobyrchis viciifolia) silage in dairy cow rations affects nutrient digestibility, nitrogen utilization, energy balance, and methane emissions. J. Dairy Sci. 99:3566-3577.

Hynes, D.N., S. Stergiadis, A. Gordon, and T. Yan. 2016. Effects of concentrate crude protein content on nutrient digestibility, energy utilization, and methane emissions in lactating dairy cows fed fresh-cut perennial grass. J. Dairy Sci. 99:8858-8866.

Innovation Center for U.S. Dairy. 2013. Memorandum of Understanding between United States Department of Agriculture and Innovation Center for U.S. Dairy. Accessed September 26, 2017. https://www.usda/gov/sites/default/files/documents/usda-mou-innovation-center-us-dairy.pdf

Isa, Z., S. Grusenmeyer, and W. Verstraete. 1986. Sulfate reduction relative to methane production in high anaerobic digestion: Microbiological aspects. Appl. Environ. Microbiol. 51:580-587.

Itoh, M. 1974. Reliability of digestibility of energy and nitrogen, nitrogen balance and gas production with cattle. In Energy Metabolism of Farm Animals. E. By K.H. Menke, H.J. Lantzsch, and J.R. Reichl. European Association for animal production Publication 14. (Proc. 6th symp. Energy Metabl. Hohenheim, Germany).

Iwamoto, M., N. Asanuma, and T. Hino. 1999. Effects of nitrate combined with fumarate on methanogenesis, fermentation, and cellulose digestion by mixed ruminal microbes in vitro. Anim. Sci. J. 70: 471-478.

Jakobsen, K., and G. Thorbek. 1970. The respiratory quotient in relation to fat retention from carbohydrates or lipids in growing pigs. Pages 126-129 in Energy Metabolism of Farm Animals, EAAP Publ. No. 58, Kartause Ittengen, Switzerland.

Janicek, B.N., P.J. Kononoff, A.M. Gehman, and P.H. Doane. 2008. The effect of feeding dried distillers grains plus solubles on milk production and excretion of urinary purine derivatives. J. Dairy Sci. 91:3544-3553.

Joblin, K.N. 1999. Ruminal acetogens and their potential to lower ruminant methane emissions. Aust. J. Agric. Res. 50:1307-1313.

Johnson, K. A., and D. E. Johnson. 1995. Methane emissions from cattle. J. Anim. Sci. 73:2483-2492.

Johnson, D. E., C. L. Ferrell, and T. G. Jenkins. 2003. The history of energetic efficiency research: Where have we been and where are we going? J. Anim. Sci. 81:E27–E38.

Kelly, J.M., B. Kerrigan, L.P. Milligan and B.W. McBride. 1994. Development of a mobile, open-circuit indirect calorimetry system. Can. J. Anim. Sci. 74:65-71.

Kim, D.H., K.R. McLeod, A.F. Koontz, A.P. Foote, J.L. Klotz and D.L. Harmon. 2015. Effect of intake on fasting heat production, respiratory quotient and plasma metabolites measured using the washed rumen technique. Animal 9:58-66.

Kirkland, R.M., and F.J. Gordon. 1999. The metabolizable energy requirement for maintenance and the efficiency of use of metabolizable energy for lactation and tissue gain in dairy cows offered a straw/concentrate ration. Livestock Prod. Sci. 61:23-31.

Klop, G., B. Hatew, A. Bannink, and J. Dijkstra. 2016. Feeding nitrate and docosahexaenoic acid affects enteric methane production and milk fatty acid composition in lactating dairy cows. J. Dairy Sci. 99:1161-1172.

Knapp, J. R., G. L. Laur, P. A. Vadas, W. P. Weiss, and J. M. Tricarico. 2014. Invited review: Enteric methane in dairy cattle production: Quantifying the opportunities and impact of reducing emissions. J. Dairy Sci. 97:3231-3261.

Laurenz, J.C., F.M. Byers, G.T. Schelling, and L.W. Greene. 1991. Effects of season on the maintenance requirements of mature beef cows. J. Anim. Sci. 69:2168-2176.

Lee, C., and K.A. Beauchemin. 2014. A review of feeding supplementary nitrate to ruminant animals: Nitrate toxicity, methane emissions, and production performance. Can. J. Anim. Sci. 94:557-570.

Le Van, T.D., J.A. Robinson, J. Ralph, R.C. Greening, W.J. Smolenski, J.A.Z. Leedle, and D.M. Schaefer. 1998. Assessment of reductive acetogenesis with indigenous ruminal bacterium populations and acetitomaculum ruminis. Applied and Envir. Microbiology 64:3429-3436.

Lewis, D. 1954. The reduction of sulfate in the rumen of sheep. Biochem. J. 56:391-399.

Lieber, D. J., J. Catlett, N. Madayiputhiya, R. Nandakumar, M. M. Lopez, W. W. Metcalf, and N. R. Buan. 2014. A multienzyme complex channels substrates and

electrons through acetyl-coA and methane biosynthesis pathways in Methanosarcina. PLoS ONE 9(9): e107563. doi:10.1371/journal.pone.0107563.

Llonch, P., M.J. Haskell, R.J. Dewhurst, and S.P. Turner. 2017. Review: current available strategies to mitigate greenhouse gas emissions in livestock systems: an animal welfare perspective. Animal 11L274-284.

Lopez, J.C., L.F. de Matos, M.T. Harper, F. Giallongo, J. Oh, D. Gruen, S. Ono, M. Kindermann, S. Duval, and A.N. Hristov. 2016. Effect of 3-nitrooxypropanol on methane and hydrogen emissions, methane isotopic signature, and ruminal fermentation in dairy cows. J. Dairy Sci. 99:5335-5344.

Martin, C., J. Rouel, J.P. Jouany, M. Doreau, and Y. Chilliard. 2008. Methane output and diet digestibility in response to feeding dairy cows crude linseed, extruded linseed, or linseed oil. J. Anim. Sci. 86:2642-2650.

Machmüller, A., and M. Kreuzer. 1999. Methane suppression by coconut oil and associated effects on nutrient and energy balance in sheep. Can. J. Anim. Sci. 79: 65-72.

Mathison, G.W., E.K. Okine, T.A. McAllister, Y. Dong, J. Galbraith, and O.I.N. Dmytruk. 1998. Reducing methane emissions from ruminant animals. J. Appl. Res. 14:1-28.

McGinn, S. M., K. A. Beauchemin, T. Coates, and D. Colombatto. 2004. Methane emissions from beef cattle: effects of monensin, sunflower oil, enzymes, yeast, and fumaric acid. J. Anim. Sci. 82:3346-3356.

McGinn, S.M., Y.H. Chung, K.A. Beauchemin, A.D. Iwaasa, and C. Grainger. 2009. Use of corn distillers' dried grains to reduce enteric methane loss from beef cattle. Can. J. Anim. Sci. 89:409-413.

McNamara, J.P. 2015. Triennial lactation symposium: Systems biology of regulatory mechanisms of nutrient metabolism in lactation. J. Anim. Sci. 93:5575-5585.

Merck Veterinary Manual. 2010. Polioencephalomalacia. 10th ed. Merck & CO., INC. Whitehouse Station, NJ.

Mjoun, K., K.F. Kalscheur, A.R. Hippen, D.J. Schingoethe, and D.E. Little. 2010. Lactation performance and amino acid utilization of cows fed increasing amounts of reduced-fat dried distillers grain with solubles. J. Dairy Sci. 93:288-303.

Moe, P. W., H. F. Tyrrell, and W. P. Flatt. 1970. Partial efficiency of energy use for maintenance, lactation, body gain and gestation in the dairy cow. Page 65 in Energy Metabolism of Farm Animals, EAAP Publ. No. 13, Vitznau, Switzerland.

Moe, P.W., H.F. Tyrrell, and W.P. Flatt. 1971. Energetics of body tissue mobilization. J. Dairy Sci. 54:548-553.

Moe, P.W., and H.R. Tyrell. 1974. Efficiency of conversion of digested energy to milk. J. Dairy Sci. 58:602-610.

Moe, P. W., and H. F. Tyrrell. 1979. Methane production in dairy cows. J. Dairy Sci. 62:1583-1586.

Moe, P.W. 1981. Energy metabolism of dairy cattle. J. Dairy Sci. 64:1120-1139.

Moraes, L.E., E. Kebreab, A.B. Strathe, J. Dijkstra, J. France, D.P. Casper, and J.G. Fadel. 2015. Multivariate and univariate analysis of energy balance data from lactating dairy cows. J. Dairy Sci. 98:4012-4029.

Morgavi, D.P., E. Forano, C. Martin, and C.J. Newbold. 2010. Microbial ecosystem and methanogenesis in ruminants. Animal 4:1024-1036.

Münger, A. 1991. Milk production efficiency in dairy cows of different breeds. Pages 292-295 in Energy Metabolism of Farm Animals, EAAP Publ. No. 58, Kartause Ittengen, Switzerland.

Muñoz, C., T. Yan, D. A. Willis, S. Murray, and A. W. Gordon. 2012. Comparison of the sulfur hexafluoride tracer and respiration chamber techniques for estimated methane emissions and correction for rectum methane output from dairy cows. J. Dairy Sci. 95:3139-3148.

Murray, R. M., A.M. Bryant, and R.A. Leng. 1976. Rates of production of methane in the rumen and large intestines of sheep. Br. J. Nutr. 36: 1–14.

National Research Council. 2001. Nutrient Requirements of Dairy Cattle. 7th rev. ed. Natl. Acad. Press, Washington, DC.

Nienaber, J.A., J.H. Eisemann, J.T. Yen, and G.B. Huntington. 1993. Technical Note: Comparison of Techniques for measurement of oxygen uptake by cattle. J. Anim. Sci. 71:2756-2759.

Nienaber, J. A., J. A DeShazer, H. Zin, P. Hillman, J. T. Yen, and C. F. Ferrell. 2009. Chapter 4: Measuring energetics of biological processes. Pages 73-112 in Livestock Energetics and Thermal Environmental Management. St. Joseph, MI.

Nkrumah, J.D., E.K. Okine, G.W. Mathison, K. Schmid, C. Li, J.A. Basarab, M.A. Price, Z. Wang, and S.S. Moore. 2006. Relationships of feedlot feed efficiency, performance, and feeding behavior with metabolic rate, methane production, and energy partitioning in beef cattle. J. Anim. Sci. 84:145-153.

Odongo, N.E., R. Bagg, G. Vessie, P. Dick, M.M. Or-Rashid, S.E. Hook, J.T. Gray, E. Kebreab, J. France, and B.W. McBride. 2007. Long-term effects of feeding monensin on methane production in lactating dairy cows. J. Dairy Sci. 90:1781-1788.

Olijhoek, D.W., A.L.F. Hellwing, M. Brask, M.R. Weisbjerg, O. Højberg, M.K. Larsen, J. Dijkstra, E.J. Erlandsen, and P. Lund. 2016. Effect of dietary nitrate level on enteric methane production, hydrogen emission, rumen fermentation, and nutrient digestibility in dairy cows. J. Dairy Sci. 99:6191-6205.

Oliviera, A. S. 2015. Meta-analysis of feeding trials to estimate energy requirements of dairy cows under tropical condition. Anim. Feed Sci. and Tech. 2010:94-103.

Ortiques, I., M. Petit, J. Agabriel, and M. Vermorel. 1993. Maintenance requirements in metabolizable energy of adult, nonpregnant, nonlactatating Charolais cows. J. Anim. Sci. 71:1947-1956.

Patle, B.R., and V.D. Mudgal. 1977. Utilization of dietary energy for maintenance, milk production and lipogenesis by lactating crossbred cows during their midstage of lactation. British J. Nutr. 37(1):23-33.

Patra, A.K. and Z. Yu. 2012. Effects of essential oils on methane production and fermentation by, and abundance and diversity of, rumen microbial population. Appl. Environ. Microbiol. 78:4271-4280.

Pei, C.X., S.Y. Mao, Y.F. Cheng, and W.Y. Zhu. 2010. Diversity, abundance and novel 16S rRNA gene sequences of methanogens in rumen liquid, solid and epithelium fractions of Jinnan cattle. Animal 4:20-29.

Pesta, A.C. 2015. Dietary strategies for mitigation of methane production by growing and finishing cattle. Dissertation. Retrieved from http://digitalcommons.unl.edu/animalscidiss/109.

Pinares-Patiño, C. S., K. R. Lassey, R. J. Martin, G. Molano, M. Fernandez, S. MacLean, E. Sandoval, D. Luo, and H. Clark. 2011. Assessment of the sulphur hexafluoride (SF6) tracer technique using respiration chambers for estimation of methane emissions from sheep. Animal Feed Sci. and Tech. 166-167: 201-209.

Place, S. E., Y. Pan, Y. Zhao, and F.M. Mitloehner. 2011. Construction and operation of a ventilated hood system for measuring greenhouse gas and volatile organic compound emissions from cattle. Animals 1:433-446.

Puchala, R., B.R. Min, A.L. Goetsch, and T. Sahlu. 2005. The effect of a condensed tannin-containing forage on methane emission by goats. J. Anim. Sci. 83:182-186.

Reed, K.F., H.C. Bonfá, J. Dijkstra, D.P. Casper, and E. Kebreab. 2017. Estimating the energetic cost of feeding excess dietary nitrogen to dairy cows. J. Dairy Sci. 100:7116-7126.

Reynolds, C.K., and H.F. Tyrrell. 2000. Energy metabolism in lactating beef heifers. J. Anim. Sci. 78:2696–2705.

Reynolds, C.K., D.J. Humphries, P. Kirton, M .Kindermann, S. Duval, and W. Steinberg. 2014. Effects of 3-nitrooxypropanol on methane emission, digestion, and energy and nitrogen balance of lactating dairy cows. J. Dairy Sci. 97:3777-3789.

Ritzman, E.G. and F.G. Benedict. 1938. Nutritional physiology of the adult ruminant. Pages 87-157. Publ. Carneg. Instn, No. 494.

Romero-Perez, A., E.K. Okine, S.M. McGinn, L.L. Guan, M. Oba, S.M. Duval, M Kindermann, and K.A. Beauchemin. 2014. The potential of 3-nitrooxypropanol to lower enteric methane emissions from beef cattle. J. Anim. Sci. 92:4682-4693.

Saama, P.M. and I.L. Mao. 1993. Sources of variation in partitioning of intake energy for lactating Holstein cows. J. Dairy Sci. 76:1334-1341.

Sahlu, T., H. G. Jung, J. A. Nienaber, and J. G. Morris. 1988. Development and validation of a prediction equation estimating heat production by carbon dioxide entry rate technique. J. Anim. Sci. 66:2036-2043.

Sanchez, M. D., and J. G. Morris. 1984. Energy expenditure of beef cattle grazing annual grassland. Can. J. Anim. Sci. 64(5):332-334.

Schingoethe, D.J., K.F. Kalscheur, A.R. Hippen, and A.D. Garcia. 2009. Invited review: The use of distillers products in dairy cattle diets. J. Dairy Sci. 92:5802-5813.

Schürch, A., and C. Wenk. 1970. Energy Metabolism of Farm Animals. Proceedings from the 5th symposium. Swiss Federal Institute of Technology, Zurich, Switzerland.

Shibata, M., and F. Terada. 2010. Factors affecting methane production and mitigation in ruminants. Animal Science Journal 81: 2-10.

Silivong, P., T.R. Preston, and R.A. Leng. 2011. Effect of sulphur and calcium nitrate on methane production by goats fed a basal diet of molasses supplemented with Mimosa (Mimosa pigra) foliage. Livestock Research for Rural Development. Volume 23, Article #58. Retrieved August 22, 2017, from http://www.lrrd.org/lrrd23/3/sili23058.htm.

Smith, R.R., G.L. Rumsey, and M.L. Scott. 1978. Heat increment associated with dietary protein, fat, carbohydrate and complete diets in salmonids: comparative energetic efficiency. J. Nutr. 108:1025-1032.

Storm, I. M. L. D., A. L. F. Hellwing, N. I. Nielsen, and J. Madsen. 2012. Methods for measuring and estimating methane emission from ruminants. Animals 2:160-183.

Thoma, G., J. Popp, D. Nutter, D. Shonnard, R. Ulrich, M. Matlock, D.S. Kim, Z. Neiderman, N. Kemper, C. East, and F. Adom. 2013. Greenhouse gas emissions from milk production and consumption in the United States: A cradle-to-grave life cycle assessment circa 2008. International Dairy Journal 31:S3–S14.

Thompson, W.R., J.C. Meiske, R.D. Goodrich, J.R. Rust, and F.M. Byers. 1983. Influence of body composition on energy requirements of beef cows during winter. J. Anim. Sci. 56(5):1241-1252.

Tine, M.A., K.R. Mcleod, R.A. Erdman, and R.L. Baldwin. 2001. Effects of brown midrib corn silage on the energy balance of dairy cattle. J. Dairy Sci. 84:885-895.

Torrent, J., and D.E. Johnson. 1994. Methane production in the large intestine of sheep. Pages 391-394 in Energy metabolism of farm animals. EAAP Publication No. 76. CSIC> Publishing Service. Granada, Spain.

Tyrrell, H. F., and C. K. Reynolds. 1989. Effect of stage of growth on utilization of energy by beef heifers. In: Proceedings of the 11th symp. Energy Metabolism of Farm Animals. EAAP Publ. No. 43. pp 17−20. Pudoc, Wageningen, The Netherlands.

Tyrrell, H.F., C.K. Reynolds, and H.D. Baxter. 1990. Energy metabolism of Jersey and Holstein cows fed total mixed diets with or without whole cottonseed. J. Dairy Sci. 73(suppl. 1):abstract 192.

Unsworth, E.F. 1991. The efficiency of utilisation of metabolizable energy for lactation from grass silage based diets. Pages 329-332 in Energy Metabolism of Farm Animals, EAAP Publ. No. 58, Kartause Ittengen, Switzerland.

Unsworth, E.F., C.S. Mayne, A. Cushnahan, and F.J. Gordon. 1994. The energy utilisation of grass silage diets by lactating dairy cows. In: Aguilera, J.F. (ed.) Energy Metabolism of Farm Animals. European Association for Animal Production Publication No. 76, Mojacar, Spain, pp. 179-181.

VandeHaar, M.J. 1998. Efficiency of nutrient use and relationship to profitability on dairy farms. J. Dairy Sci. 81:272-282.

VandeHaar, M.J. 2016. Understanding the physiological aspects to improving feed efficiency in dairy cows. Proc. Tri-State Dairy Nutrition Conference, pgs. 27-34

VandeHaar, M.J., L.E. Armentano, K. Weigel, D.M. Spurlock, R.J. Tempelman, and R. Veerkamp. 2016. Harnessing the genetics of the modern dairy cow to continue improvements in feed efficiency. J. Dairy Sci. 99:4941-4954.

Van Es, A.J.H. 1970. Page 97-100 in Energy Metabolism of Farm Animals, EAAP Publ. No. 13, Vitznau, Switzerland.

Van Es, A.J.H. 1975. Feed evaluation for dairy cows. Livestock Prod. Sci. 2:95-107.

Van Middelaar, C.E., P.B.M. Berentsen, J. Dijkstra, J.A.M. Van Arendonk, and I.J.M. De Boer. 2015. Effect of feed-related farm characteristics on relative values of genetic traits in dairy cows to reduce greenhouse gas emissions along the chain. J. Dairy Sci. 98:4889-4903.

Van Soest, P.J. 1994. Nutritional Ecology of the Ruminant. 2nd ed. Cornell University Press, Sage House, Ithaca, New York.

Van Zijderveld, S. M., W. J. J. Gerrits, J. A. Apajalahti, J. R. Newbold, J. Dijkstra, R. A. Leng, and H. B. Perdok. 2010. Nitrate and sulfate: Effective alternative hydrogen sinks for mitigation of ruminal methane production in sheep. J. Dairy Sci. 93:5856-5866.

Van Zijderveld, S.M., J. Dijkstra, H.B. Perdok, J.R. Newbold, and W.J.J. Gerrits. 2011a. Dietary inclusion of diallyl disulfide, yucca powder, calcium fumarate, an extruded linseed product, or medium-chain fatty acids does not affect methane production in lactating dairy cows. J. Dairy Sci. 94:3094-3104.

Van Zijderveld, S.M., W.J.J. Gerrits, J.Dijkstra, J.R. Newbold, R.B.A. Hulshof, and H.B. Perdok. 2011b. Persistency of methane mitigation by dietary nitrate supplementation in dairy cows. J. Dairy Sci. 94:4028-4038.

Vlaming, J. B., I. M. Brookes, S. O. Hoskin, C. S. Pinare-Patiño, and H. Clark. 2007. The possible influence of intra-ruminal sulphur hexafluoride release rates on calculated methane emissions from cattle. Can. J. Anim. Sci. 87:269-275.

Weimer, P.J. 1998. Manipulating ruminal fermentation: a microbial ecological perspective. J. Anim. Sci. 76:3114-3122.

West, J.W. 2003. Effects of heat stress on production in dairy cattle. J. Dairy Sci. 86:2131-2144.

Wheelock, J.B., R.P. Rhoads, M.J. VanBaale, S.R. Sanders, and L.H. Baumgard. 2010. Effects of heat stress on energetic metabolism in lactating Holstein cows. J. Dairy Sci. 93:644-655.

Woodward, S.L., G.C. Waghorn, and P. Laboyre. 2004. Condensed tannins in birdsfoot trefoil (Lotus corniculatus) reduce methane emissions from dairy cows. Proc. N. Z. Soc. Anim. Prod. 64:160-164.

Xue, B. T. Yan, C.F. Ferris, and C.S. Mayne. 2011. Milk production and energy efficiency of Holstein and Jersey-Holstein crossbred dairy cows offered diets containing grass silage. J. Dairy Sci. 94:1455-1464.

Yan, T., F.J. Gordon, R.E. Agnew, M.G. Porter, and D.C. Patterson. 1997. The metabolisable energy requirement for maintenance and the efficiency of utilisation of metabolisable energy for lactation by dairy cows offered grass silage-based diets. Livest. Prod. Sci. 51:141–150.

Young, B.A., B. Kerrigan, and R.J. Christopherson. 1975. A versatile respiratory pattern analyzer for studies. Can. J. Anim. Sci. 55:17–22.

Young, B.A. 1983. Ruminant cold stress: Effect on production. J. Anim. Sci. 57(6):1601-1607.

TABLES AND FIGURES

Table 2. 1. Equations for energy balance.

Response	ID	Equation[1]
Gross energy intake (GEI)	1	Diet energy × dry matter intake
Digestible energy (DE)	2	GEI – fecal energy
Metabolizable energy (ME)	3	DE – urinary energy – CH_4 energy
Net energy of lactation (NE_L)	4	ME – heat production
Maintenance	5	0.08 Mcal × $BW^{0.75}$
Retained energy (RE)	6	(12.55 × grams of C retained) – (6.90 × grams of N retained)
Heat Production (HP)	7	3.866 × O_2 + 1.200 × CO_2 – 0.518 × CH_4 – 1.431 × Urinary N
Respiratory quotient	8	CO_2 produced (L) / O_2 consumed (L)
Oxidation of glucose	9	$C_6H_{12}O_6 + 6O_2 = 6CO_2 + 6H_2O$
Oxidation of short chain fatty acids	10	$C_3H_5(CH_3CH_2COO)_3 + 37O_2 = 30CO_2 + 26H_2O$
Oxidation of long chain fatty acids	11	$2C_3H_5(CH_3(CH_2)_{15}COO)_3 + 145O_2 = 102CO_2 + 86H_2O$
Oxidation of Alanine	12	$2CH_3CH(NH_2)_2COOH + 6O_2 = CO(NH_2)_2 + 5CO_2 + 5H_2O$

[1]GEI is Mcal/d; DE is Mcal/d; HP, ME, Metabolizable energy is Mcal/d; RE, NE_L is Mcal/d; Maintenance is Mcal/d; Recovered energy is Mcal/d; Heat production is Mcal/d where O_2 and CO_2 are L/d and N is g/d; Respiratory quotient is L/L

Table 2. 2. Energy balance studies and determined mean (SEM) energy values (kcal ME/kg $BW^{0.75}$) and mean (SEM) efficiency of converting metabolizable energy for lactation (k_l) of cattle.

Author[1]	Maintenance Energy Value (kcal ME/kg $BW^{0.75}$)	k_l	Cow Breed	Lactation Status
Flatt et al., 1965	110			Lactating
Flatt et al., 1967a	133 (0.02)	0.700	Holstein	Lactating
Flatt et al., 1967b	110-120		Holstein	Non-Lactating
Flatt et al., 1967c	143 (0.01)	0.660	Holstein	Mixed
Moe et al., 1970	123 (1.97)	0.647		Lactating
Moe et al., 1970	101 (1.64)			Non-Lactating
Van Es et al., 1970	117	0.620		Lactating
Van Es, 1975	117	0.600		Lactating
Patle and Mudgal, 1977	139 (0.66)	0.663	Brown Swiss	Lactating
Grainger et al., 1985	190		Friesian	Lactating
Grainger et al., 1985	178		Friesian	Non-Lactating
Tyrrell and Reynolds, 1989	109		Hereford, Angus	Non-Lactating
Münger, 1991	130 (0.88)	0.616	Holstein-Friesian	Lactating
Münger, 1991	112 (0.88)	0.583	Simmental	Lactating
Unsworth et al., 1991	132 (5.67)	0.640	Friesian	Lactating
Ortiques et al., 1993	117		Charolais	Non-Lactating
Unsworth et al., 1994	153 (0.27)	0.670	Friesian	Lactating
Hayasaka et al., 1995	141	0.640	Holstein	Lactating
Yan et al., 1997	160 (0.01)	0.630 (0.030)	Holstein-Friesian	Lactating
Freetly and Nienaber, 1998	119		MARC III	Non-Lactating
Kirkland and Gordon, 1999	146 (0.42)	0.590 (0.010)	Holstein-Friesian	Lactating
Reynolds and Tyrrell, 2000	120 (2.01)		Hereford, Angus	Mixed
Birkelo et al., 2004	136	0.620	Holstein	Lactating
Derno et al., 2005	99 (2.10)		Hereford	Non-Lactating
Freetly et al., 2006	146 (8.00)	0.720 (0.037)	MARC III	Lactating
Xue et al., 2011	165 (3.50)	0.581 (0.014)	Holstein	Lactating
Dong et al., 2015a	163 (3.80)	0.641 (0.003)	Holstein-Friesian	Lactating
Dong et al., 2015b	156 (4.10)	0.636 (0.002)	Holstein-Friesian	Lactating
Foth et al., 2015	208	0.760	Jersey, Holstein	Lactating
Moraes et al., 2015	144	0.603	Holstein	Lactating
Oliviera, 2015	154 (13.66)	0.588 (0.024)	Bos Taurus	Unknown
Lactating[2]	143	0.643		
Non-lactating[3]	120			
Other[4]	139	0.588		

[1]Values for each paper is averaged for lactating or non-lactating cattle where applicable.
[2]Lactating maintenance value determined by the raw mean of studies with lactating cattle.
[3]Non-lactating maintenance value determined by the raw mean of studies with non-lactating cattle.
4Maintenance value for mixed (Lactating and non-lactating cattle) and unknown lactation status.

Figure 2. 1. Open-circuit whole-animal chamber, which is a method of indirect calorimetry, located at Beltsville, MD (Blaxter, 1962).

Figure 2. 2. Whole-animal respiration chamber, which is a method of indirect calorimetry, located at Melle, Belgium (De Campeneere and Peiren, 2014).

Figure 2. 3. Whole-animal respiration chamber, which is a method of indirect calorimetry, located at Aarhus University, Aarhus, Denmark (Storm et al., 2012).

Figure 2. 4. Collection of gases from a Holstein cow using a headbox-style, indirect calorimeter (Place et al., 2011).

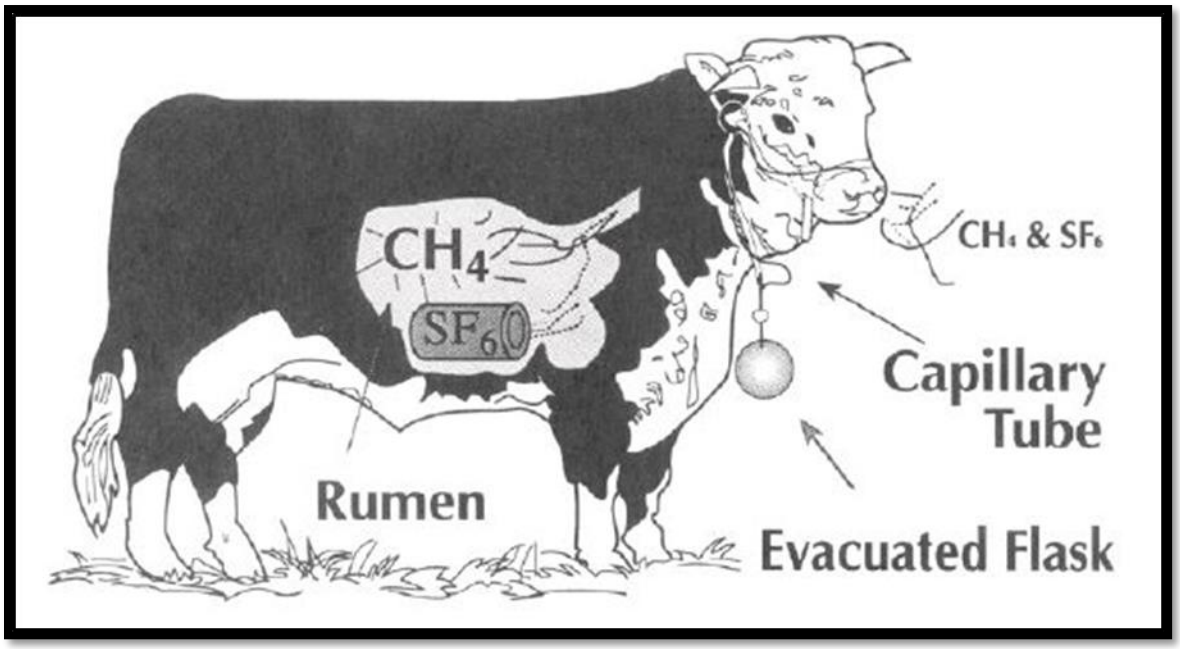

Figure 2. 5. Sampling apparatus for sulfur hexafluoride (SF_6) for indirect measurements of methane (Storm et al., 2012).

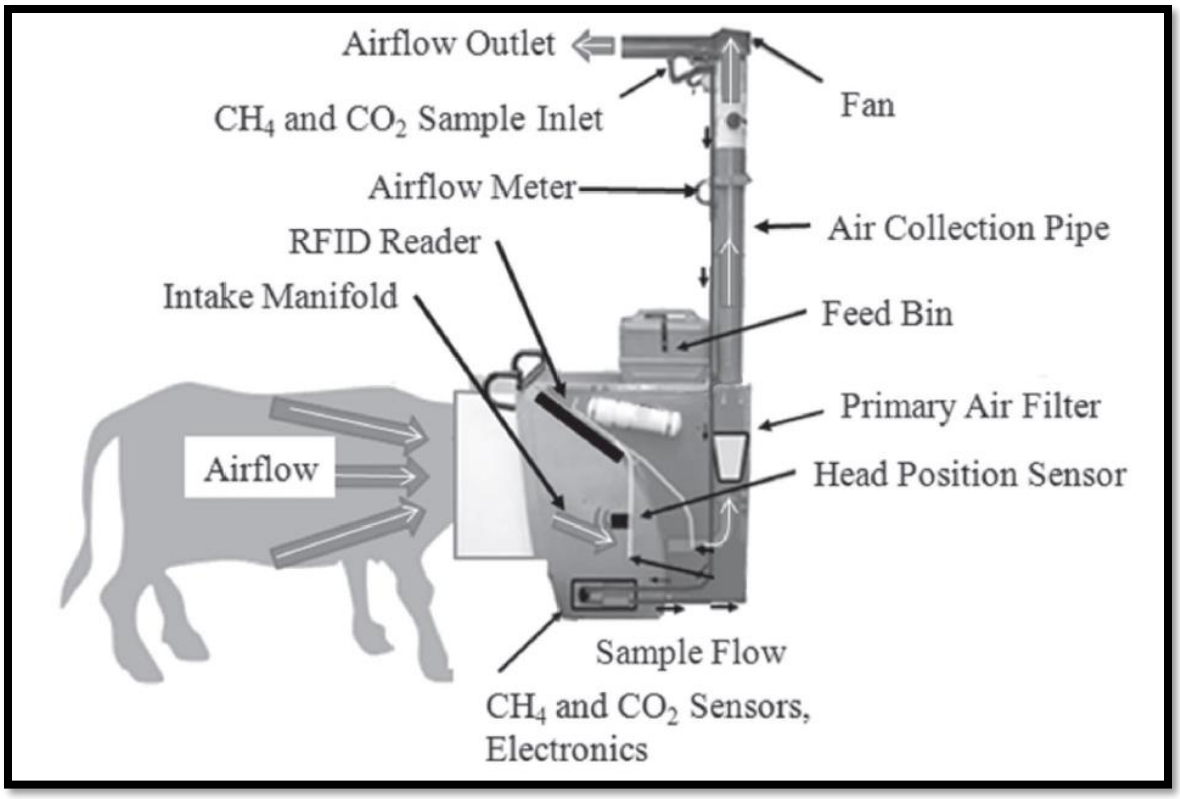

Figure 2. 6. Sampling apparatus for the GreenFeed system (C-Lock Inc., Rapid City, SD) (Huhtanen et al., 2015).

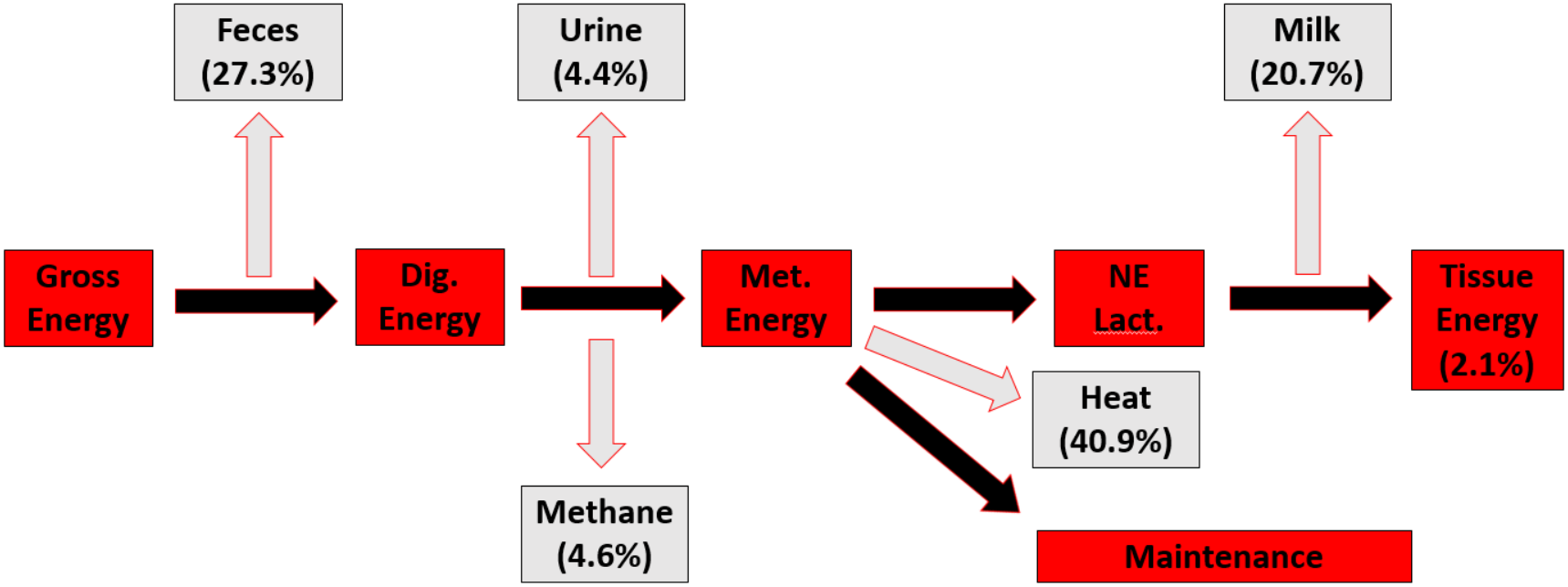

Figure 2. 7. Energy distribution diagram in animals adapted from Flatt et al., 1967.

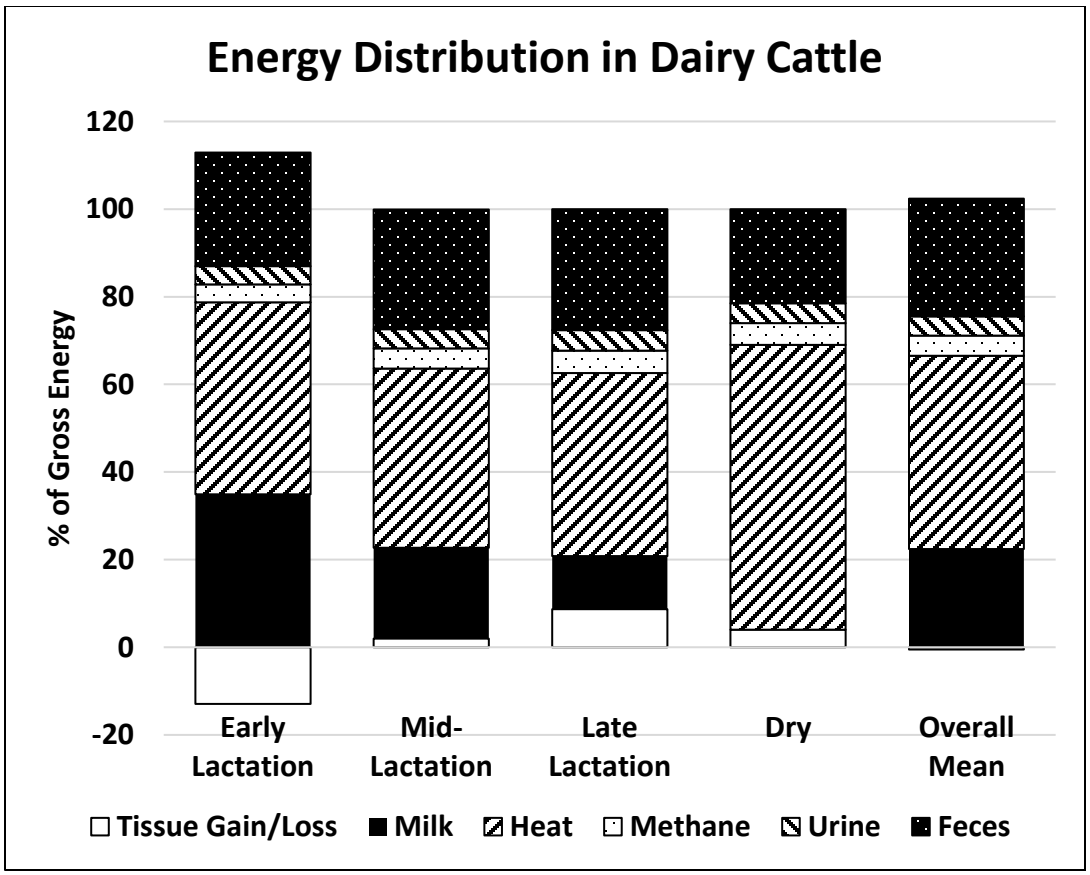

Figure 2. 8. Effect of stage of lactation on the utilization of energy by dairy cows (adapted from Flatt et al., 1967c).

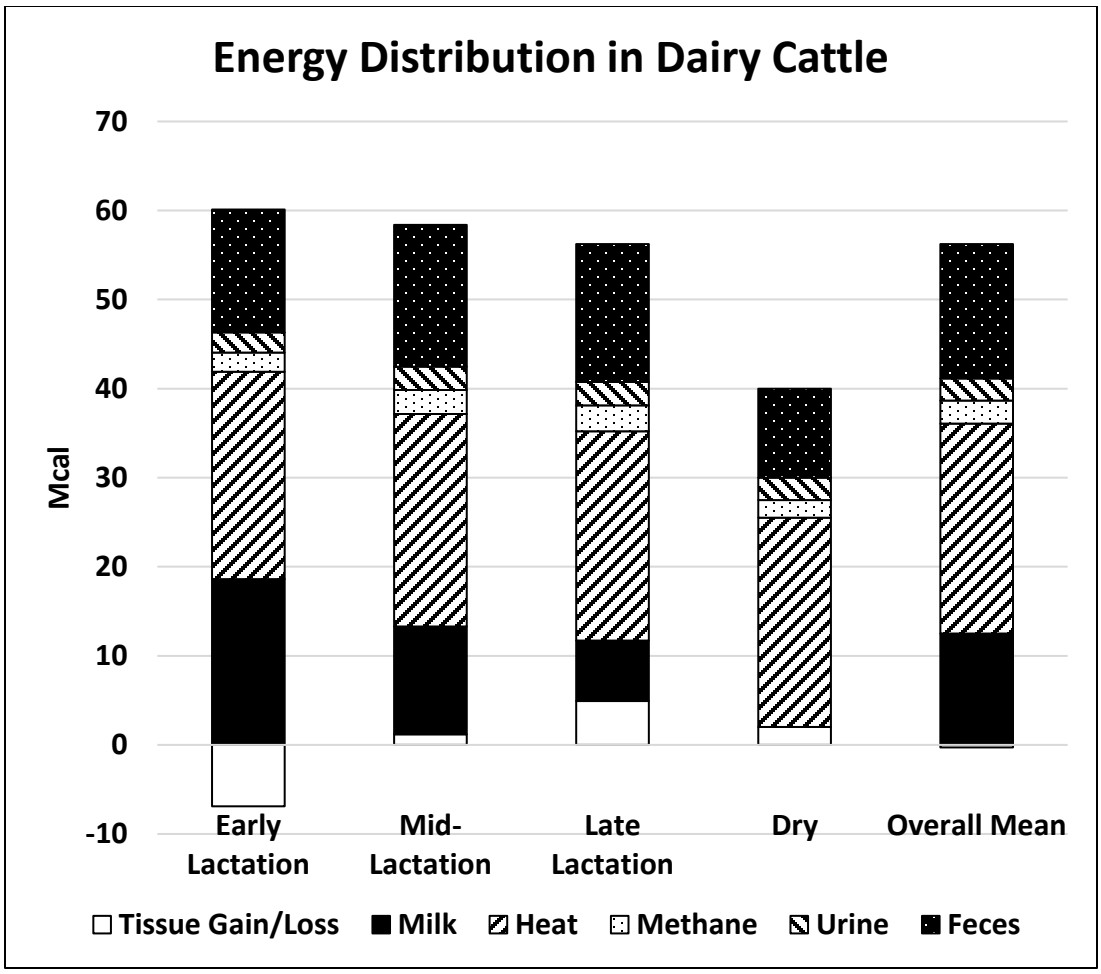

Figure 2. 9. Effect of stage of lactation on the utilization of energy by dairy cows (adapted from Flatt et al., 1967c).

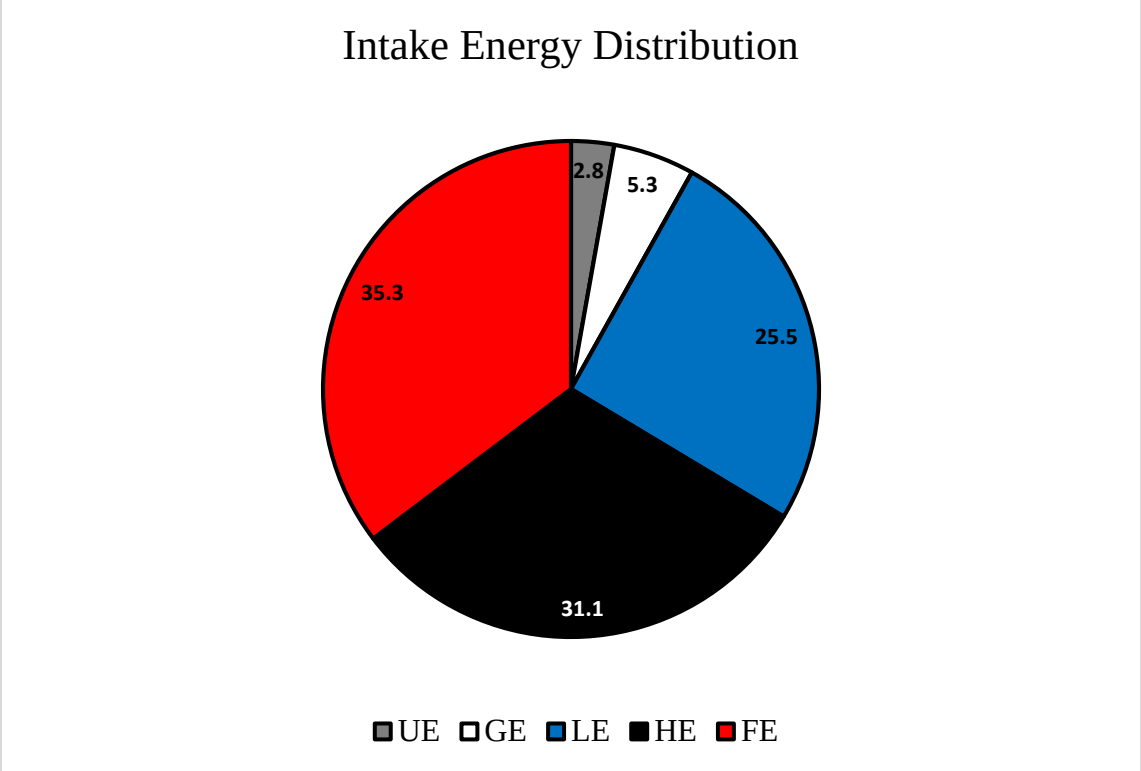

Figure 2. 10. Intake energy distribution of a 600 kg cow producing 40 kg of milk. UE = urinary energy, GE =Gaseous energy, LE = lactation energy, HE = heat production, FE = fecal energy (Coppock, 1985).

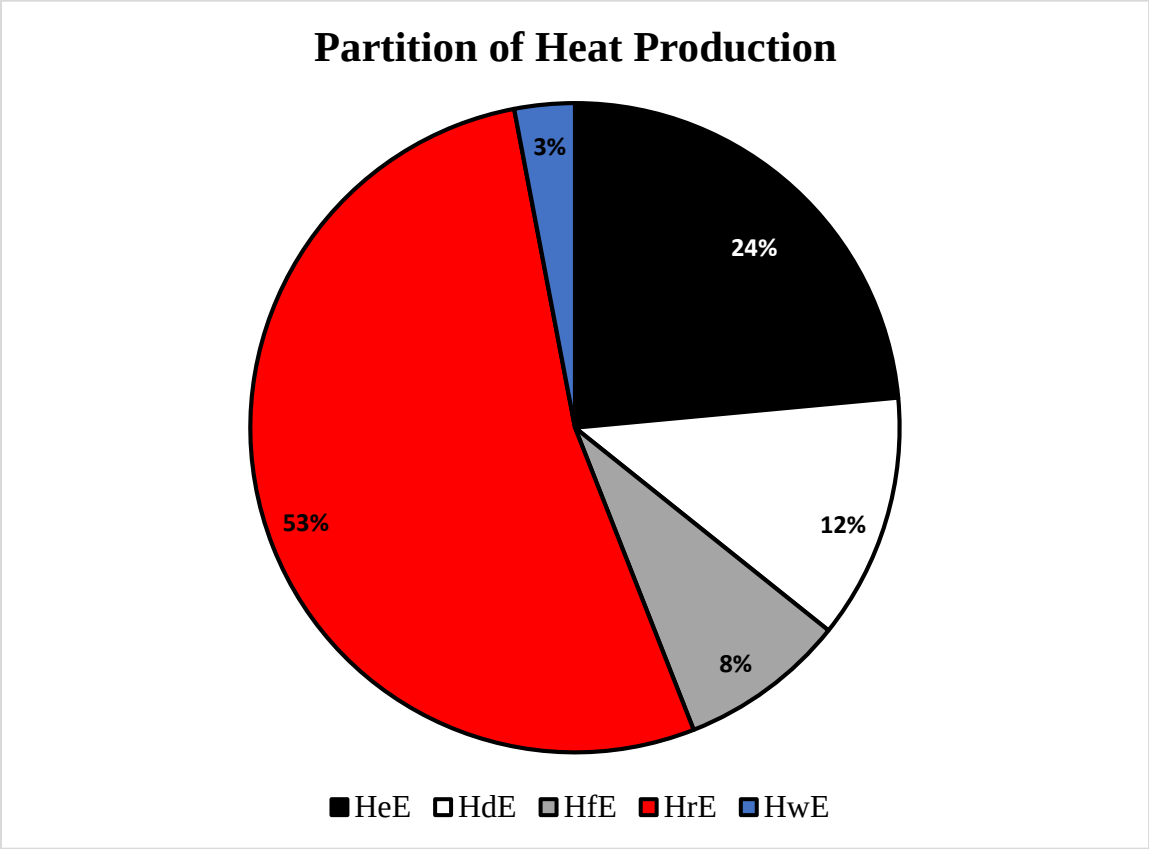

Figure 2. 11. Partition of heat production by a 600 kg cow producing 40 kg of milk. HwE = heat of waste formation and excretion, HrE = heat of product formation, HfE = heat of fermentation, HdE = heat of digestion, HeE = heat associated with maintenance (Coppock, 1985).

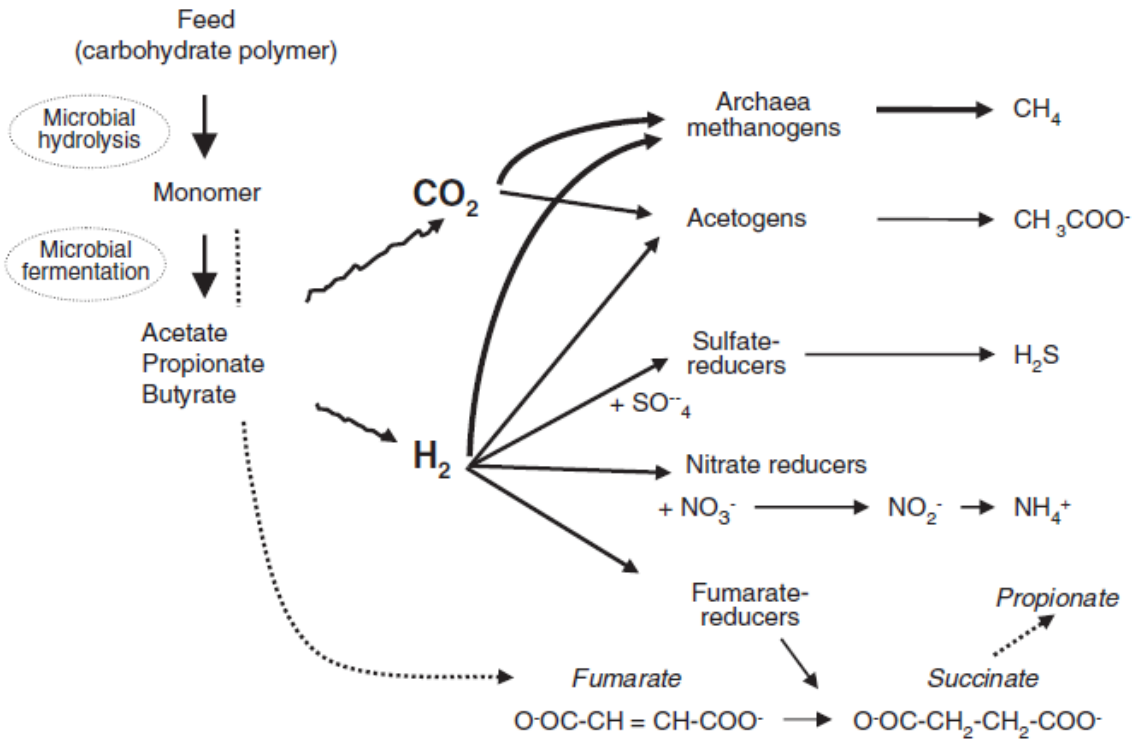

Figure 2. 12. Schematic microbial fermentation of feed polysaccharides and H_2 reduction pathways in the rumen (found in Morgavi et al., 2010).

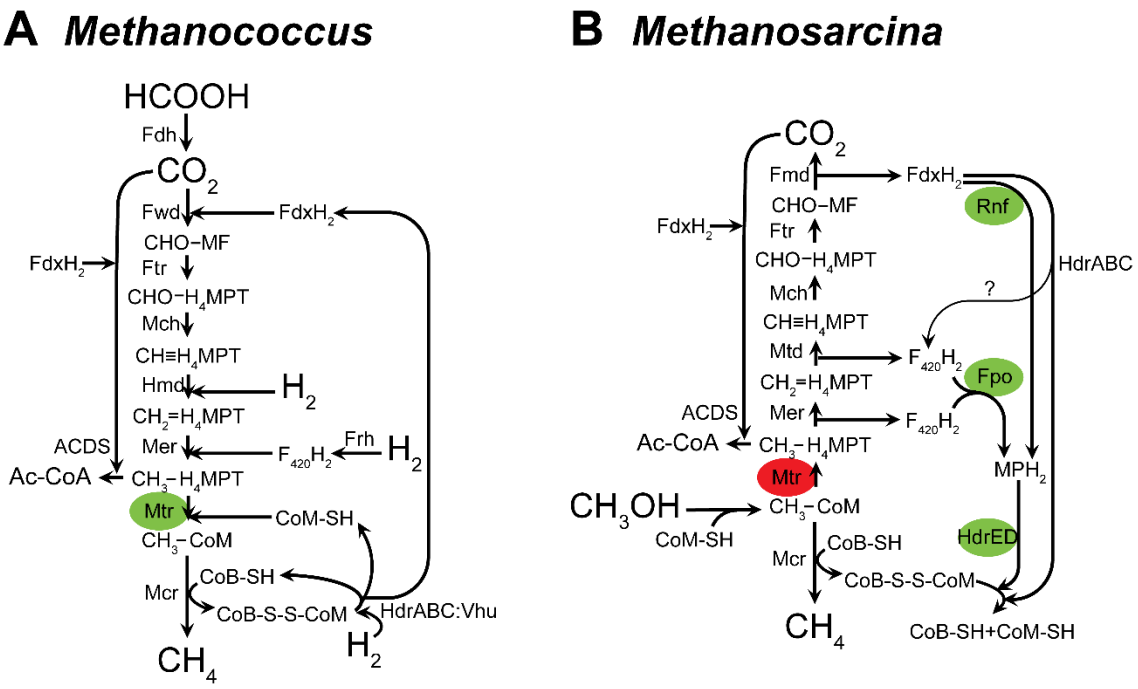

Figure 2. 13. Two pathways utilized by different groups of methanogens (Leiber et al., 2014).

CHAPTER 3

Methane mitigation with corn oil and calcium sulfate, responses on whole-animal energy and nitrogen balance in dairy cattle consuming reduced-fat dried distillers grains with solubles[1]

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ABSTRACT

Addition of fat and calcium sulfate to diets fed to ruminants has resulted in a reduction in methane production, but these factors have not illustrated effects on energy balance. A study using indirect calorimetry and 16 multiparous (8 Holstein and 8 Jersey; 78 ± 15 DIM; mean ± SD) lactating dairy cows was conducted to determine how mitigating methane by adding corn oil or calcium sulfate to diets containing reduced-fat distillers grains, affects energy and nitrogen balance in dairy cattle. A replicated 4 × 4 Latin square design with 35-d periods (28-d adaption and 4 d collections) was used to compare 4 different dietary treatments. Treatments were composed of a control (**CON**) diet, which did not contain reduced-fat distillers grain and solubles (**DDGS**), and treatment diets containing 20% (DM basis) DDGS (**DG**), 20% DDGS with 1.38% (DM basis) added corn oil (**CO**), and 20% DDGS with 0.93% (DM basis) added calcium sulfate (**CaS**). Methane was measured using headbox-style indirect calorimeters. Compared to CON, DMI was greater for DG and CO, but was not affected by CaS (19.1, 20.1, 20.0, and 19.3 ± 0.37 kg/d, for CON, DG, CO, and CaS, respectively). Milk production was increased for diets containing DDGS compared to the CON (26.3 vs. 27.8 ± 0.47 kg/d for CON vs. DDGS, respectively). Compared to CON, ECM was greater in DG and CO (30.1 vs. 31.4, 31.7, and 31.0 ± 0.67 kg/d for CON, DG, CO, and CaS, respectively). Addition of CaS reduced and CO tended to reduce methane production compared to CON diet (421.6, 429.5, 394.7, and 381.4 ± 14.41 L/d for CON, DG, CO, and CaS, respectively). Digestible energy was greater for DG and CO treatments compared to CON and CaS treatments (57.7, 62.1, 62.0, and 59.0 ± 1.38 for CON, DG, CO, and CaS, respectively). Metabolizable energy was greater in treatments containing DDGS compared to CON

(50.5 vs. 54.0 ± 1.08 for CON vs. DDGS, respectively). Net energy of lactation per unit of DMI was greater in CO than CON (1.55 vs. 1.35 ± 0.06 Mcal/kg for CO vs. CON, respectively). Tissue energy was greater in DG and CO compared to CON (6.08, 7.04, and 3.16 ± 0.99 for DG, CO, and CON, respectively. Nitrogen balance was greater in DG than CO (91.1 vs. 56.6 g/d for DG and CO, respectively). Addition of oil and calcium sulfate to diets containing DDGS may be a viable option to reduce methane production without affecting energy balance in lactating dairy cows.

Key words: dairy cow, dried distillers grains with solubles, energy, methane

INTRODUCTION

Lactating dairy cattle produce approximately 500-600 L/d of methane (**CH_4**) (Beauchemin et al., 2008; Chase, 2014). According to the Environmental Protection Agency (2010), compared to carbon dioxide (**CO_2**), CH_4 is 21-25 times more potent as a greenhouse gas. Lactating dairy cattle contribute 1.9 – 2.2 % to the total GHG emissions in the U.S. (Thoma et al., 2013; Chase, 2014). Ruminants produce approximately 25 % of the total CH_4 production of which dairy cattle contribute approximately 24.8 % of enteric CH_4 production or 0.54 % of GHG total (Chase, 2014). In 2009, the Innovation Center for U.S. Dairy, set a goal to lower total greenhouse gas emissions by dairy operations by 25 % by the year 2020 (Innovation Center, 2009). Given the large contribution of ruminants to total CH_4 production, ample opportunities exist to reduce CH_4 production.

Many strategies have been devised to mitigate CH_4 production and they can be broadly categorized into three main methods: nutritional or feed management, modification the rumen environment to directly inhibit methanogenesis, and management practices that improve productive efficiencies (Knapp et al., 2014). Dietary strategies include the addition of ionophores, fats, altering the forage-to-concentrate ratio, and using alternative hydrogen sinks in the rumen (Johnson and Johnson, 1995; Knapp et al., 2014). The feeding of distillers grains and solubles (**DDGS**) has increased in dairy cattle and has reduced CH_4 production. Benchaar et al. (2013) replaced corn and soybean meal with DDGS and observed a 9 % reduction in CH_4 per unit of energy corrected milk. Similarly, Foth et al. (2015) fed reduced-fat DDGS to lactating dairy cows and observed a 7 % decrease. These studies would suggest that feeding DDGS may be an effective way to reduce CH_4 production. Knapp et al. (2014) observed that by-products such as DDGS

have highly digestible NDF and produce one-half to one-third the CH_4 than forages with similar dry matter digestibility. Lipid supplementation is an additional method that may be used to reduce CH_4 production. Hales et al. (2017) fed increasing concentrations of corn oil in diets fed to growing beef steers and observed a linear decrease in CH_4 production, and CH_4 energy by approximately 30 % when 6 % of the diet dry matter was corn oil. Utilization of sulfate has reduced CH_4 production. When fed to sheep, supplemental sulfate reduced CH_4 production by 16 % (Van Zijderveld et al., 2010) and likely has similar effects if fed to lactating dairy cattle. Feeding different combinations of DDGS, fat, and sulfate may serve as practical methods to consistently reduce CH_4 production in lactating dairy cattle.

Environmental concerns are not the only reason CH_4 production is important in the dairy industry. Methane production may have a negative impact on metabolizable energy available for production and reduce overall efficiency (Gill et al., 2010; Hynes et al., 2016). Energetic losses from CH_4 production are believed to range from 2 to 12 % (Johnson and Johnson, 1995). It has also been suggested that a 25 % reduction in CH_4 production in cattle could translate into an increase in milk production of approximately 1 L/d in dairy cattle (Bruinenberg et al., 2002) or 75 g/d BW gain in beef cattle (Nkrumah et al., 2006). Overall, because CH_4 production represents an energetic loss for cattle, reducing CH_4 production could result in the repartition of more energy towards production processes. However, there is limited research showing how these mitigation techniques affect whole-animal energy and nitrogen balance and the digestibility of the diet in lactating dairy cattle. Therefore, the overall objective of this study was to determine the effects of manipulating the diet with proposed CH_4 reduction techniques

specifically DDGS, corn oil, and calcium sulfate. Specific objectives were to determine CH_4 production and determine the effects of these CH_4 reduction techniques on whole-body energy and nitrogen utilization in dairy cows. It was hypothesized that the additions of DDGS, corn oil, and sulfate would reduce CH_4 production and increase energy balance without negatively affecting production in lactating dairy cows.

MATERIALS AND METHODS

Sixteen multiparous (8 Holstein and 8 Jersey;78 ± 15 DIM; mean ± SD) lactating dairy cows with a BW averaging 593.8 ± 15.7 and 428.3 ± 15.7 kg at the beginning of the experiment were used. The objective of this study was not to determine breed difference. All cows were housed in a temperature-controlled barn at the Dairy Metabolism Facility at the Animal Science Complex at the University of Nebraska – Lincoln (Lincoln, NE) and milked at 0700 and 1800 hr in individual tie stalls equipped with rubber mats. All animal care and experimental procedures were approved by the University of Nebraska – Lincoln Animal Care and Use Committee. At the conclusion of the last experimental period, all cows were less than 90 d pregnant; this allowed energy balances to be calculated because energy committed to fetus development is very minimal less than 90 d pregnant.

The experimental design was a quadruple-replicated 4 × 4 Latin square. Cows were randomly assigned to 1 of the 4 dietary treatments according to Kononoff and Hanford (2006). Treatments were: control (**CON**) diet, which did not contain reduced-fat distillers grain and solubles (**DDGS**), and treatment diets containing 20 % (DM basis) DDGS (**DG**), 20 % DDGS with 1.38 % (DM basis) added corn oil (**CO**), and 20 % DDGS with 0.93 % (DM basis) added calcium sulfate (**CaS**), according to Kononoff and

Hanford (2006). Animals were blocked into each square by milk production. Treatments alternated over 4 experimental periods and measurements were collected on each animal consuming each dietary treatment. The study was conducted with a total of 4 experimental periods, each being 35-d in duration. Each period included 28-d for ab libitum diet adaptation, targeting about 5 % refusals during that time, followed by 4-d of collection with 95 % ad libitum feeding to reduce the amount of refusals.

Diets containing DDGS replaced all soybean meal and a portion of ground corn with DDGS (Table 3.1). The proportion of forage remained constant among all diets with only the concentrate different in composition. Soybean meal was completely replaced by DDGS as well as a portion of the ground corn in the diets containing DDGS. Additional corn was removed from the diet when corn oil or calcium sulfate were added to the diets. All other ingredients were formulated to have similar inclusion rates (Table 3.1). The Cornell-Penn-Miner Dairy model (Boston et al., 2000) was used to balance diets. The study was conducted over 9 mo and forages varied only by year to reduce variability. All dietary treatments contained corn silage, alfalfa hay, brome hay, and a concentrate mixture that was combined as a total mixed ration (**TMR**). The TMR was mixed in a Calan Data Ranger (American Calan, Inc., Northwood, NH) and fed once daily at 0900 hr to the cows.

Laboratory Analysis

Individual feed ingredients were sampled (500 g) on the first day of each collection period and frozen at -20°C. A subsample was sent to Cumberland Valley Analytical Services Inc. (Hagerstown, MD) for complete nutrient analysis of DM (AOAC International, 2000), N (Leco FP-528 N Combustion Analyzer, Leco Corp., St. Joseph,

MO), NDF with sodium sulfite (Van Soest et al., 1991), ADF (method 973.18; AOAC International 2000), lignin (Goering and Van Soest, 1970), NFC (100 – (% NDF + % CP + % Fat + % Ash)), sugar (DuBois et al., 1956), starch (Hall, 2009), crude fat (2003.05; AOAC International 2006), ash (943.05; AOAC International 2000), and minerals (985.01; AOAC International 2000). Total mixed rations were sampled (500 g) on each day of each collection period and were frozen at -20°C. The samples were then composited by period and treatment. A subsample was sent to Cumberland Valley Analytical Services Inc. (Hagerstown, MD) for complete nutrient analysis with the same lab processes as the individual feed ingredients. Particle size of the TMR was determined according to Heinrichs and Kononoff (2002) using the Penn State Particle Separator. Each day of the collection period, refusals were sampled and frozen at -20°C. The samples were composited by period and individual cow. A subsample was sent to Cumberland Valley Analytical Services Inc. (Hagerstown, MD) for nutrient analysis of DM, N, NDF with sodium sulfite, starch, and ash, using previously discussed methods. Water samples were taken on the first day of collections and sent to Midwest Laboratories Inc. for direct metals analysis [livestock suitability water analysis; EPA method 200.7 (EPA, 1994)].

Total fecal and urine output was collected from each individual cow during the collection period for 4 consecutive days. A 137 × 76 cm rubber mat (Snake River Supply, Idaho Falls, ID) was placed behind the cow to collect feces. The feces were deposited multiple times a day from the rubber mats into a large garbage container (Rubbermaid, Wooster, OH) with a black garbage bag covering the top to reduce nitrogen losses prior to subsampling. The feces were subsampled (4 % wet basis) every day for 4 consecutive

days and dried at 60°C in a forced-air oven for 48 hr and then composited by cow and period prior to being ground to pass through a 1-mm screen (Wiley mill, Arthur H. Thomas Co., Philadelphia, PA). The ground feces samples were sent to Cumberland Valley Analytical Services Inc. (Hagerstown, MD) for nutrient analysis of DM, N, NDF with sodium sulfide, starch, and ash, using previously described methods. Total urine was collected by inserting a 30 French foley catheter into each cow's bladder with a stylus (Tamura et al., 2014). The balloon was inflated to 50-mL with physiological saline and urine drained using tygon tubing into a plastic carboy (15 quart) behind the cow. Using the funnel spout of the plastic carboy, urine was deposited into a 55-L plastic container 4 times a day and was acidified with 50-mL of HCl prior to subsampling (2 % wet basis) and frozen at -20°C every day of the collection period. Prior to analysis, urine was thawed and boiled to remove the water content. To boil the urine, two thawed 250-mL bottles of urine were poured into a 600-mL beaker. Twelve urine-filled beakers were placed into a boiling water bath (Ankom Technology, Macedon, NY) underneath a hood. The water bath was turned on in the morning and off in the afternoon, for approximately 6 hr each day, to reduce the chance of the sample being overheated and burned. After water was boiled away, the remaining dark brown paste was then composited by cow and period. The brown paste was then lyophilized (VirTis Freezemobile 25ES, SP Scientific, Gardiner, NY) and analyzed. Once lyophilized, sample size was reduced using mortar and pestle for analysis. Urine samples were analyzed at the University of Nebraska – Lincoln laboratory for corrected DM (100°C oven for 24 hr), N (Leco FP-528, Leco Corp.) and gross energy (GE) (Parr 6400 Calorimeter, Moline, IL).

Milk production was measured daily and milk samples were collected during both the AM and PM milking times for 4 consecutive days or d 29 to 32 of the entire period. Three tubes were collected each milking (150-mL); two 50-mL conical tubes were frozen at -20 °C and one tube was sent off to DHIA, preserved using 2-bromo-2-nitropropane-1,3 diol. Samples were sent to Heart of America DHIA (Kansas City, MO) and were analyzed for fat, protein, lactose, SNF, MUN and SCC using a Bentley FTS/FCM Infrared Analyzer (Bentley Instruments, Chaska, MN). One of the two conical tubes was lyophilized and then composited by cow and period for nutrient analysis. Milk samples were analyzed at the University of Nebraska – Lincoln laboratory for corrected DM, N and GE. To determine the DM content of individual feed ingredients, TMRs, refusals, feces and urine samples were dried at 60 °C in a forced-air oven for 48 hr and then composited by treatment or cow and period. Milk samples were lyophilized to determine DM. Feed ingredients, refusals and feces were ground and analyzed as previously described (with the feces) for lab corrected DM and GE.

Heat production was determined through the headbox-type indirect calorimeters described by Foth et al. (2015) and Freetly et al. (2006) that were built at the University of Nebraska - Lincoln. Prior to collections, 5 headboxes were used to test the rate of recovery of gas by burning 100% ethyl alchohol in the sealed headbox and comparing this measure to calculated gas concentrations. These calculations were based on weight of alcohol burned and a measured volume of gas sample. Five lamp runs were conducted. Recovery rates of oxygen ($\mathbf{O_2}$) and CO_2 averaged 101.0 ± 0.04 and 100.8 ± 0.04 %, respectively. For each cow, a collection period of 2 consecutive 23-hr intervals measured O_2 consumption, and CO_2 and CH_4 production. The design of the headboxes allowed for

feed to be placed in the bottom of the box and ad libitum access to water was available for the cows from a water bowl placed inside the headbox. Within the headbox, temperature and dew point were recorded every minute for a 23 hr interval using a probe (Model TRH-100, Pace Scientific Inc., Moorseville, NC) that was connected to a data logger (Model XR440, Pace Scientific Inc., Moorseville, NC). Fifteen min before the start of the collection, the doors were closed and the motor was turned on, to allow for several air turnovers before gases were collected. Line pressure was measured using a manometer (Item # 1221-8, United Instruments, Westbury, NY). Barometric pressure of the room was also recorded using a barometer (Chaney Instruments Co., Lake Geneva, WI) and uncorrected for sea level. Total volume of gas that passed through the headbox during each run was measured using a dry gas meter (Model AL425, American Meter, Horsham, PA). From the headbox, continuous amounts of outgoing and incoming air were diverted to 2 different collection bags (61 × 61 cm LAM-JAPCON-NSE, 44 L; PMC, Oak Park, IL) using glass tube rotameters (Model 1350E Sho-Rate "50", Brooks Instruments, Hatfield, PA). Collection bags with gas samples inside were analyzed (Emerson X-stream 3-channel analyzer, Solon, OH) at the U.S. Meat Animal Research Center (MARC) according to Nienaber and Maddy (1985). Measurements collected from the 2 d were averaged to obtain one combined value. Heat production was estimated through calculation of O_2 consumption, and CO_2 and CH_4 production with correction for urinary N loss according to Brouwer (1965; Equation 1; Table 3.10). The gaseous products were reported in liters and the mass of urinary N in grams. Respiratory quotient was calculated using the ratio of CO_2 produced to the O_2 consumed and was not corrected for nitrogen. Volume of CH_4 produced was multiplied by a constant of 9.45 kcal/L to

estimate the amount of energy formed from the gaseous products. Energy balance was calculated for each cow and adjusted for excess N intake according to Freetly et al. (2006) using the following equations:

$$HP\ (Mcal/d) = 3.866 \times O_2\ L + 1.200 \times CO_2\ L - 0.518 \times CH_4\ L - 1.431 \times N\ g \quad [1]$$

Metabolizable energy (**ME**) (Mcal/d) = gross energy intake Mcal/d – fecal energy Mcal/d – urinary energy Mcal/d – methane energy Mcal/d [2]

Recovered energy (**RE**) (Mcal/d) = ME – HP [3]

Tissue energy (**TE**) (Mcal/d) = RE – milk energy Mcal/d [4]

Tissue energy in protein (g/d) = (N balance g/d) × (5.88 kg of protein/kg of N) × (5.7 Mcal/kg of protein)/1000 [5]

Metabolizable energy for maintenance was found by regression of RE on ME and is the ME at zero RE as illustrated in Figure 3.1. Tissue energy in protein describes the energy used for tissue protein synthesis (Equation 5).

Statistical Analysis

Data were analyzed using the mixed procedure of SAS (SAS Institute Inc., Cary, NC). Treatment and period were modeled as fixed effects while cow within square was modeled as a random effect. There were no breed × treatment interaction for any measureable item and as such, treatment means contain both Holstein and Jersey cattle data. The LSMEANS option was used to generate least-squares means of treatments listed in this study. Significance was declared at $P \leq 0.05$ and trends at $0.05 < P \leq 0.10$.

RESULTS AND DISCUSSION

Diet Composition

Chemical composition of dietary treatments and feed ingredients are presented in Tables 3.1 and 3.2. Based upon the formulations, the control treatment had a slightly greater estimated energy content (1.70, 1.62, 1.67, and 1.61 NE_L (Mcal/kg) for CON, DG, CO, and CaS, respectively) and protein content (18.0, 17.2, 16.9, and 17.3 % for CON, DG, CO, and CaS, respectively) compared to treatments containing DDGS (Table 3.1). Concentrations of crude fat were higher in treatments containing DDGS and as expected, the corn oil treatment contained the greatest concentration of fat (2.65, 3.38, 4.76, and 3.55 % dietary dry matter for CON, DG, CO, and CaS, respectively). Although fat content varied, all treatments contained fat at less than the recommended maximum inclusion of 7 % (NRC, 2001). Sulfur was greater in treatments containing DDGS and as expected, calcium sulfate contained the highest concentration of sulfur (0.23, 0.32, 0.34 and 0.52 % of dietary dry matter for CON, DG, CO, and CaS, respectively). The sulfur concentration in the calcium sulfate treatment exceeded the recommended concentrations from the 2001 NRC of 0.4 % of dietary dry matter. However, the recommendation with cattle consuming a diet with at least 40 % forage is 0.5 % (NRC, 2005). In the current study, forage was included at 60 % and, therefore, we believed the sulfur would not be problematic, but also could potentially elicit a reduction in CH_4 production.

Particle size of the TMR was not different for treatments (Table 3.3). For the CON treatment, 4.81, 25.2, 50.9, and 18.9 % remained for the > 19.0-mm, 8.0-mm, 1.18-mm and pan (< 1.18-mm), respectively. For the DG treatment, 5.38, 25.2, 45.5, 23.9 %

remained for the > 19.0-mm, 8.0-mm, 1.18-mm and pan (< 1.18-mm), respectively. General recommendations for particle distribution are 2 to 8 % remaining particles on the > 19.0-mm diameter sieve, 30 – 50 % retained on the 8.0-mm and 1.18 -m sieves and ≤ 20 % retained on the bottom pan (Heinrichs and Kononoff, 2002). In the current study, all treatments were within recommended range for the > 19.0-mm and 1.18-mm sieves. The 8.0-mm sieve had lower than the recommended range at approximately 25 %. The bottom sieve for the control treatment was within the recommendation, however, DG, CO, and CaS treatments had greater material than is recommended. If cattle rations deviate from the recommended values, cattle may not be able to maintain healthy rumens, which may ultimately cause sub-acute ruminal acidosis (Zebeli et al., 2010). With increased particle size of the TMR, there is an increase in chewing and increase the production of saliva that buffers in the rumen (White et al., 2017). Although chewing behavior and rumen pH were not measured in the current study, no negative effects on rumen health were observed in this study. Forage inclusion was approximately 60 % and starch content was relatively low at approximately 21 – 22 % for treatments, which may have decreased the risk of acidosis. It should be noted that most feeding recommendations do not account for diets containing large amounts of byproducts that replace corn. High inclusion of DDGS at inclusions greater than 15 % decreases the requirement for physically effective fiber in dairy cattle (Bradford and Mullins, 2012).

Dry Matter Intake, Milk Production and Composition

Inclusion of DDGS has been reported to be an effective feed ingredient in lactating dairy cattle diets without negatively affecting production performance (Castillo-Lopez et al., 2014). Particularly, DMI has increased by 5 to 12 % when DDGS were

included in the diet (Benchaar et al., 2013; Castillo-Lopez et al., 2014). Similarly, in the current study, compared to the control, DMI was greater ($P \leq 0.050$) with the inclusion DG and corn oil (19.1 vs. 20.1 and 20.0 ± 0.37 for CON vs. DG and CO, respectively; Table 3.4). Dry matter intake of cows consuming calcium sulfate (19.3 ± 0.37 kg/d) was not different ($P = 0.250$) than either control or DG. Similar to the current study, Benchaar et al. (2013) and Janicek et al. (2008) observed DMI to increase with inclusion of DDGS in diets fed to lactating dairy cows. Positive effects of feeding DDGS are not always observed. For example, Mjoun et al. (2010) increased DDGS in lactating dairy cow diets and observed no difference in DMI. Overall, the increased DMI from cattle consuming DDGS was expected, as it provided a highly degradable carbohydrate source. Castillo-Lopes et al. (2014) suggested that the inclusion of DDGS may increase DMI due to its effects on gut fill. Furthermore, the small particle size could affect rate of passage. In the current study, particle size was reduced which may have allowed for increased DMI until DMI was regulated by rumen fill. In the current study, feeding corn oil with DDGS increased DMI. Ramirez-Ramirez et al. (2016) and Boerman et al. (2014) fed lactating dairy cattle corn oil with diets containing reduced fat DDGS and observed a decrease in DMI. However, Ramirez-Ramirez et al. (2015) fed corn oil to lactating dairy cattle consuming DDGS and observed no difference in DMI. In beef steers, Hales et al. (2017) observed no difference on DMI with added corn oil, when corn oil replaced dry-rolled corn and a small proportion of soybean meal. In the current study, forage was included at 60 %; whereas, in previous studies, decreased DMI was reported with lactating dairy cows with corn oil supplementation and forage inclusion at approximately 50 % of dry matter. Thus, the effect of corn oil on DMI may be partially determined by the basal

dietary ingredient that corn oil replaces and if the diet is primarily forage or concentrate-based.

Similar to the increased DMI observed with feeding DDGS, milk yield has also been reported to increase (Benchaar et al., 2013). However, a concern with feeding DDGS is the increased fat concentration in the diet and the potential effects on milk production and milk fat yield (Ramirez-Ramirez et al., 2015). Abdelqader et al. (2009) fed diets containing either 30 % DDGS or 2.5 % corn oil and observed a lower milk fat percentage compared to a control diet. However, Janicek et al. (2008) fed up to 30 % DDGS without any negative effects on milk yield or milk composition. In the current study, compared to the control, milk yield was greater ($P \leq 0.017$; Table 3.4) in all 3 treatments containing DDGS (26.3 vs. 27.8 ± 0.47 kg/d for CON vs. DDGS, respectively). The addition of corn oil tended ($P = 0.097$) to increase milk yield compared to DG (28.3 vs. 27.5 ± 0.48 kg/d for CO vs. DG, respectively). Similarly, compared to the control, ECM was greater ($P \leq 0.017$) with the inclusion of DG and corn oil treatments (30.1 vs. 31.4 and 31.7 ± 0.52 kg/d for CON vs. DG and CO, respectively) and inclusion of calcium sulfate tended to have greater ($P = 0.088$) ECM than the control treatment (30.1 vs. 31.0 ± 0.52 kg/d for CON vs. CaS, respectively). Treatments containing DDGS did not differ ($P \geq 0.195$) with a mean of 31.4 ± 0.52 kg/d for ECM. Milk fat percentage did not differ ($P = 0.315$) among treatments with a mean of 4.61 ± 0.10 %, however, compared to the control, milk fat yield tended to be greater ($P \leq 0.086$) in DG and CO treatments (1.19 vs. 1.25 and 1.24 ± 0.03 kg/d for CON vs. DG and CO, respectively). Similar to the current study, Benchaar et al. (2013) observed increased milk yield and milk fat yield with DDGS. Previous research conducted at the University of Nebraska in

the same facility noted a tendency for greater milk production with inclusion of DDGS (Foth et al., 2015). In the current study, the increased milk production may in part be caused by greater dry matter intake. Previous research from our lab also indicated that the inclusion of corn oil can induce milk fat depression (Ramirez-Ramirez et al., 2015). Interestingly, the current study did not observe a depression in milk fat, which may be due to low concentrations of crude fat for all treatments. Ramirez-Ramirez et al. (2015) induced milk fat depression with increasing total dietary fat from 5.0 to 6.5 % and in the current study, the corn oil diet did not reach 5 % dietary fat. Compared to the control, milk protein percent was decreased (P = 0.038; Table 3.4) with the inclusion of corn oil (3.28 vs. 3.18 ± 0.04 % for CON vs. CO, respectively) and CaS tended (P = 0.075) to decrease (3.28 vs 3.20 ± 0.04 % for CON vs. CaS, respectively). Treatments fed DG did not differ (P = 0.643) from the control treatment with a mean of 3.27 ± 0.04 %, for milk protein percent. Milk protein percent did not differ among diets containing DDGS with a mean of 3.21 ± 0.04 % although corn oil tended (P = 0.100) to be reduced compared to DG (3.18 vs. 3.26 ± 0.04 %). Compared to the control, milk protein yield was greater (P = 0.023) with the inclusion of corn oil (0.84 vs. 0.88 ± 0.02 kg/d for CON vs. CO). Similarly, Foth et al. (2015) observed reduced milk protein percent (3.56 vs. 3.41 %) for cattle consuming DDGS. Although lysine is generally believed to be a limiting amino acid in corn-based diets fed to dairy cows, the decreased milk fat percent may have resulted from dilution with greater milk yield, as use of DDGS seldom affects milk protein unless dietary protein is limiting (Schingoethe et al., 2009). In the current study, total milk fat yield was unaffected, potentially due to the effect of dilution. Furthermore, diets containing 20 % DDGS have reported sufficient protein and amino acids (Lysine)

supply to maintain milk protein synthesis (Paz et al., 2013). Milk urea nitrogen was greater ($P < 0.01$) for the control compared to all three treatments containing DDGS (17.3 vs. 14.9 ± 0.41 mg/dl for CON vs. DDGS, respectively). Increased MUN have been observed with excess protein in the diet (Roseler et al., 1993). In the current study, greater MUN from the control treatment may have resulted from increased dietary protein compared to the diets containing DDGS. Soybean meal was removed with the inclusion of DDGS, which resulted in lower CP concentrations. In general, feeding DDGS with added corn oil and calcium sulfate did not negatively affect DMI or milk production and milk composition, which is in agreement with our hypothesis. Free water intake was measured using line meters and did not differ ($P = 0.32$) by treatment with an overall mean of 84.8 ± 4.14L/d. Treatments had similar DM percent which likely resulted in similar water intakes. All water constituents were below the caution level (Table 3.5; NRC, 2001).

Gas Consumption and Production

While attempting to reduce CH_4 production, there is potential to alter the metabolism of the animal and affect O_2 and CO_2 production. However, recent work attempting to reduce CH_4 has not resulted in any effects on O_2 consumption (5242 ± 210 L/d) or CO_2 production (5939 ± 243 L/d) in lactating Holstein cattle (Olijhoek et al., 2016). Likewise, in the current study, O_2 consumption did not differ ($P \geq 0.114$) by treatment although the mean of 4972.1 ± 119.8 L/d was lower compared to Olijhoek et al. (2016; Table 3.6). Carbon dioxide production did not differ ($P \geq 0.209$) by treatment with an overall mean of 5277.3 ± 135.1 L/d observed, which is somewhat surprising since CO_2 is a byproduct of fermentation and DMI differed across treatments. Compared to DG, the

addition of corn oil tended (P = 0.078) to reduce CO_2 (5105.2 vs. 5427.4 ± 135.1 L/d). Distillers grains have reduced CH_4 production in lactating dairy cows (Benchaar et al., 2013; Foth et al., 2015). However, in the current study, total CH_4 production did not differ (P = 0.690) between the control and DG with a mean of 425.5 ± 14.4 L/d. However, compared to the control, calcium sulfate reduced (P = 0.020) CH_4 (421.6 vs. 381.4 ± 14.4 L/d for CON vs. CaS, respectively) and corn oil tended to reduce (P = 0.084) CH_4 compared to the control (421.6 vs. 394.7 ± 14.4 L/d for CON vs. CO, respectively). Calcium sulfate reduced (P = 0.020) CH_4 compared to the DG treatment (381.4 vs. 429.5 ± 14.4L/d for CaS vs. DG, respectively). However, CH_4 production was not different (P = 0.177) between corn oil and DG treatments with a mean of 412.1 ± 14.4L/d). As mentioned earlier, we have previously observed a 7 % reduction in CH_4 with feeding reduced-fat DDGS (Foth et al., 2015). Similarly, DDGS have reduced CH_4 in both beef and dairy cattle (McGinn et al., 2009: Benchaar et al., 2013). The disagreement between DDGS and reduced-fat DDGS could be a result of increased fat content of DDGS. With more fat removed from DDGS, the potential of DDGS to reduce CH_4 production may be hindered, as most of the CH_4 reduction effect is likely caused by elevated fat concentrations. Previous research indicates that CH_4 production was reduced with added DDGS was the result of the effect of fat on fermentation by suppressing methanogens and potential biohydrogenation of unsaturated fatty acids (Benchaar et al., 2013). In the current study, added corn oil decreased CH_4 production by 7 %. Similarly, Hales et al. (2017) added corn oil to finishing beef steer diets and observed a linear reduction in CH_4 production as corn oil increased in the diet. It has been suggested that a 2 % increase of dietary fat would result in a 10 % reduction in CH_4 production due to

decreased DMI (Knapp et al., 2014). In the current study, crude fat increased by 2 % in corn oil treatment, resulting in a 7 % reduction in CH_4 production while increasing DMI. This may suggest that either biohydrogenation provided an alternative H_2 source in the rumen or added fat negatively affected certain rumen microbes. By adding fat into diets that include DDGS, a reduction in CH_4 may occur. Compared to the control, the addition of calcium sulfate reduced CH_4 production by approximately 11 %. Similarly, Van Zijderveld et al. (2010) observed a reduction of 16 % with added sulfur in sheep. However, using diallyl disulfide in lactating dairy cows, Van Zijderveld et al. (2011) did not observe a reduction in CH_4 production, which may be a result of too low of sulfur inclusion. The dairy NRC (2001) set the maximum tolerable concentration of dietary sulfur at 0.4%. In the current study, dietary sulfur exceeded this recommendation without negatively affecting DMI, milk production or overall health of the cows. This may indicate that source of sulfur added to the diet may affect methanogens differently and ultimately CH_4 production. The reduction in CH_4 observed by using corn oil and calcium sulfate supports our hypothesis. However, addition of DG did not affect CH_4 production as was hypothesized.

One alternative method to determine the effects of CH_4 mitigation strategies is to consider the effects on efficiency. Hristov et al. (2013) suggested that increasing overall efficiency may be the most effective way to reduce total CH_4. Determining CH_4 per unit of milk produced, and CH_4 per unit of DMI are beneficial ways to assess the effectiveness of a mitigation strategy. Previous research from our lab indicated that CH_4 production can be reduced 10 % per unit of milk production when feeding DDGS (Foth et al., 2015). However, in the current study, CH_4 per unit of ECM did not differ (P =

0.626) between control and DG treatments with an overall mean of 12.4 ± 0.50 L/kg/d. However, compared to the control, CH_4 per unit of ECM was reduced ($P \leq 0.018$) with the inclusion of corn oil and calcium sulfate to DDGS (14.2 vs. 12.5 and 12.4 ± 0.50 L/kg/d for CON vs. CO and CaS, respectively). Similarly, inclusion of corn oil and calcium sulfate reduced ($P \leq 0.053$) CH_4 per unit of ECM compared to DG (12.5 and 12.4 vs. 13.8 ± 0.50 L/kg/d for CO and CaS vs. DG, respectively). Calcium sulfate reduced CH_4 per unit of ECM by 15 % compared to the control diet while added corn oil decreased CH_4 per unit of ECM by 14 %. Similarly, in lactating dairy cows supplemented with fat, Moate et al. (2011) observed a 10 % reduction in CH_4 per unit of ECM. Feeding DDGS and DDGS plus corn oil to beef cattle observed a 20 % and 26 % reductions, respectively, in CH_4 production per unit of DMI. In the current study, CH_4 per unit of DMI did not differ ($P = 0.424$) between the control and DG treatment with a mean of 21.9 ± 0.75 L/kg/d. However, compared to the control, CH_4 per unit of DMI was reduced ($P \leq 0.031$) with the inclusion of corn oil and calcium sulfate to DDGS (22.3 vs. 19.9 and 19.6 ± 0.75 L/kg/d for CON vs. CO and CaS, respectively). Inclusion of calcium sulfate to DDGS, tended to reduce ($P = 0.088$) CH_4 per unit of DMI compared to DG (19.6 vs 21.4 ± 0.75 L/kg/d for CaS vs. DG, respectively), whereas inclusion of corn oil did not differ ($P = 0.159$) from DG with a mean of 20.7 ± 0.75 L/kg/d. Calcium sulfate reduced CH_4 per unit of DMI 14 % while corn oil reduced CH_4 per unit of DMI by 12 % compared to the control treatment. Moate et al. (2011) observed a 6 % reduction with in CH_4 per unit of DMI with supplemental fat. Assessing CH_4 production may best be suited per unit of animal product as it takes into account increased DMI for cattle that produce

more milk (Hristov et al., 2013), doing so also accounts for the improved efficiency of the animal in reducing CH_4 over time.

Heat production (**HP**) is a loss of energy that is calculated based on calorimetry measurements as the heat of combustion and is based on O_2 consumption and CO_2 production from respired air from the animal (Blaxter, 1962). Determination of HP is needed to accurately estimate energy requirements of the animal. In the current study, HP did not differ ($P \geq 0.105$) by treatment with an overall mean of 25.1 ± 0.62 Mcal/d. Similarly, HP per unit of metabolic body weight did not differ ($P \geq 0.167$) by treatment with an overall mean of 251.9 ± 5.64 kcal/$BW^{0.75}$. Typically, fat has reduced HP in heat-stressed dairy cattle (Moallem et al., 2010). However, in the current study, we did not see a similar effect, most likely because our cows were not experiencing heat stress. Similar to the current study, Van Knegsel et al. (2007) fed 5.4 vs. 3.4 % fat to lactating dairy cattle and observed no effects on heat production. As DMI in cattle increases, heat production has also increased (Purwanto et al., 1990). However, cattle in the current study are in a climate-controlled facility, which may affect any response from fat on heat production as the cows were in their thermoneutral zone throughout the study.

The respiratory quotient (**RQ**) or ratio of CO_2 produced to O_2 consumed, will increase or decrease dependent on the substrate being used for fuel in the animal. This may assist in determining the fuel being used by the animal and assure that gas collections are working properly. Typically, when carbohydrates are used as the main substrate, the RQ is near 1.0 (Brody, 1945; Blaxter, 1962; Ketelaars and Tolkamp, 1996). When proteins are used as the main substrate, the RQ is near 0.83 and with fat synthesis, it is near 1.10 to 1.20. When acetate, propionate, and butyrate are used as main fuel

sources, the RQ's are 1.0, 0.86, and 0.80, respectively (Cherepanov and Agaphonov, 2010). However, these values alone cannot be solely used to make conclusions on the metabolism of the animal. In the current study, RQ did not differ ($P = 0.269$) between the control and DG with a mean of 1.06 ± 0.01. However, compared to the control, RQ was reduced ($P = 0.05$) in the inclusion of corn oil (1.07 vs. 1.05 ± 0.01 for CON vs. CO, respectively), yet this reduction is likely not biologically relevant. RQ did not differ ($P = 0.251$) between control and calcium sulfate with a mean of 1.07 ± 0.01. DG tended ($P \leq 0.093$) to be greater than corn oil (1.06 vs. 1.05 ± 0.01), but did not differ ($P = 0.966$) from calcium sulfate. Thus, the lower RQ in the corn oil treatment could result from increased oxidation of protein, less lipid synthesis, or increased propionate production in the rumen.

Nutrient Digestibility

When consuming DDGS, digestibility of nutrients has been reported to decrease with increasing concentrations of DDGS (Benchaar et al., 2013). Previous research from our lab has indicated decreased dry matter digestibility with inclusion of DDGS (Foth et al., 2015). Similar reductions in fiber digestibility have been observed when supplementing fat (Huhtanen et al., 2009). In the current study, dry matter digestibility was calculated with total tract collections and may prevent error associated with using digestibility markers. In the current study, compared to the control, dry matter digestibility was decreased ($P \leq 0.15$) for all three treatments containing DDGS (68.5 vs. 66.7 ± 0.47 % for CON vs. DDGS, respectively; Table 3.7). Benchaar et al. (2013) observed a linear decease ($P < 0.01$) in dry matter digestibility with increasing concentrations of DDGS. However, dry matter digestibility did not differ ($P \geq 0.065$)

among treatments containing DDGS with a mean of 66.7 ± 0.47 %. This is similar to the observations of Hales et al. (2017) who observed no difference on dry matter digestibility with corn oil supplementation. On an organic matter basis, compared to the control, digestibility was decreased ($P \leq 0.011$) for all three treatments containing DDGS (69.8 vs. 68.7 ± 0.47 % for CON vs. DDGS, respectively). Likewise, Benchaar et al. (2013) observed a linear decrease in organic matter digestibility with increasing concentrations of DDGS. Penner et al. (2009) observed decreased chewing activity with DDGS and Janicek et al. (2008) observed an increased rate of passage, which may explain the increase in DMI and decrease in digestibility with diets containing DDGS. Compared to DG, organic matter digestibility decreased ($P = 0.025$) with the inclusion of calcium sulfate (68.4 vs. 67.2 ± 0.47 % for DG vs. CaS, respectively). Digestibility of CP did not differ ($P = 0.110$) between control and DG treatments with a mean of 72.3 ± 0.50 % which is similar to observations by Foth et al. (2015). However, Benchaar et al. (2013) observed a linear increase with increasing concentrations of DDGS in lactating dairy cows. Compared to the control, CP digestibility decreased ($P \leq 0.008$) with the inclusion of corn oil and calcium sulfate to DDGS (72.8 vs. 71.0 and 71.0 ± 0.50 % for CON vs. CO and CaS, respectively). Others have similarly observed increased CP digestibility with the inclusion of fats although the relationship is not understood at this time (Beauchemin et al., 2007; Benchaar et al., 2013). Many believe that the addition of fat and sulfate decrease digestibility of NDF (Beauchemin et al, 2007), because fat can negatively affect cellulolytic microbes. Van Zijereld et al. (2011) observed no difference on NDF digestibility while supplementing diallyl disulfide to lactating dairy cows. Likewise, in the current study, NDF digestibility did not differ ($P = 0.247$) by treatment

with a mean of 53.8 ± 0.72 %. The addition of calcium sulfate did not negatively affect NDF digestibility. Similarly, Hales et al. (2017) observed no difference in NDF digestibility in increasing concentrations of corn oil. Starch digestibility did not differ ($P = 0.155$) among treatments with an overall mean of 92.7 ± 0.51 % which is in agreement with previous research from Foth et al. (2015) who observed no difference in starch digestibility. However, in the current study, compared to the control, starch digestibility decreased ($P = 0.050$) in the calcium sulfate treatments (93.4 vs. 92.1 ± 0.51 % for CON vs. CaS, respectively). In a meta-analysis, Huhtanen et al. (2009) observed decreased digestibility with increased intake and increased fat concentration. Hence, in the current study, decreased digestibility is possibly the result of increased DMI and increased rate of passage.

Energy Partitioning

Total energy intake. Lactating dairy cattle consuming DDGS have increased DMI, which should result in greater total energy intake. Predicted energy values tend to be low when formulating rations containing DDGS; however, observed energy estimates have been observed to be 7 to 11 % greater in DDGS diets (Birkelo et al., 2004). Compared to the control, gross energy intake (**GE**) was greater ($P \leq 0.023$; Table 3.8) in all three treatments containing DDGS (84.0 vs. 90.5 ± 1.97 Mcal/d for CON vs. DDGS, respectively). Dry matter intake was greater for cattle consuming DDGS, which lead to increased GE intake. In comparison, Foth et al. (2015) observed increased GE intake with the inclusion of DDGS to lactating dairy cows without increased DMI. Compared to the control, digestible energy (**DE**) was greater ($P < 0.001$) in DG and corn oil (57.7 vs. 62.1 and 62.0 ± 1.14 Mcal/d for CON vs. DG and CO, respectively). Inclusion of calcium

sulfate reduced ($P = 0.035$) DE compared to DG (59.0 vs. 62.1 ± 1.14 Mcal/d for CaS vs. DG, respectively). Similarly, compared to the control and DG treatments, DE as a percent of GE was reduced ($P \leq 0.017$) with the inclusion of calcium sulfate (68.7 and 68.0 vs. 66.5 ± 0.52 % for CON and DG vs. CaS, respectively). Control and DG treatments did not differ ($P = 0.287$) with a mean of 68.4 ± 0.52. Addition of calcium sulfate decreased organic matter digestibility. This may be due to the increased mineral content compared to DG, which may be the cause of reduced DE. In addition, calcium sulfate reduced CH_4 production, which may have altered the rumen function and DE. When comparing DDGS to a control, Birkelo et al. (2004) and Foth et al. (2015) observed no difference in DE as a percentage of GE, which is similar to what we observed in the current study. Compared to the control, metabolizable energy (**ME**) intake was greater ($P \leq 0.050$) in all three treatments containing DDGS (51.5 vs. 54.6 ± 1.08 Mcal/d for CON vs. DDGS, respectively). Increased milk production and DMI may have increased energy requirements and ultimately ME in diets containing DDGS. However, ME as a percentage of GE, did not differ ($P = 0.186$) by treatment with a mean of 60.3 ± 0.50 %. Compared to the control, net energy for lactation ($\mathbf{NE_L}$) was greater ($P \leq 0.027$) in DG and corn oil (25.9 vs. 29.6 and 31.1 ± 1.08 Mcal/d for CON vs. DG and CO, respectively). Addition of calcium sulfate did not differ ($P = 0.149$) from the control treatment for NE_L with a mean of 26.9 ± 1.08 Mcal/d. Similarly, cattle consuming DG did not differ ($P \leq 0.233$) from corn oil or calcium sulfate in NE_L, with a mean of 29.6 ± 1.08 Mcal/d. These findings support our hypothesis that energy balance would increase with the addition of corn oil. However, addition of calcium sulfate did not increase energy balance by having more energy available for milk production. Inclusion of DDGS

increased ME and NE_L, likely due to the increased energy value of distillers (Schingoethe et al., 2009; Foth et al., 2015). The observed NE_L was greater than the predicted value determined using the ration-formulation program. Inclusion of corn oil increased the energy available for both ME and NE_L. In finishing beef steers, Hales et al. (2017) observed numerically greater ME intake with increased inclusion of corn oil.

Losses of energy. Dairy cattle lose energy from the feces, urine, CH_4, and heat (Coppock et al., 1985). Fecal energy loss accounts for approximately one-third of energy lost for cattle; whereas, urine and methane account for approximately 3 and 5 %, respectively (Coppock et al., 1985). In the current study, compared to the control, fecal energy lost as a percentage of GE did not differ ($P = 0.168$) between the DG treatments with a mean of 31.6 ± 1.19 %. Similarly, Foth et al. (2015) observed no difference in fecal energy lost when using DDGS. However, inclusion of corn oil in the present study increased ($P = 0.016$) fecal energy loss as a percent of GE compared to the control (30.7 vs. 33.7 ± 1.19 % for CON vs. CO, respectively). The increased energy in the feces may be the result of decreased digestibility of the fat that was excreted; however, crude fat digestibility was not measured in this study. Compared to the control, urine energy lost as a percentage of GE was reduced ($P \leq 0.047$) with the inclusion of corn oil (3.20 vs. 2.90 ± 0.10 % for CON vs. CO, respectively). This may, in part, be caused by the increased CP concentration and digestibility in the control treatment allowing for more protein turnover in tissue. Heat energy as a percentage of GE was reduced ($P = 0.007$) for all three treatments containing DDGS compared to the control (30.0 vs. 27.8 ± 0.85 %). Heat production as a percentage of GE may have been reduced in diets containing DDGS due to the decreased digestibility and thus decreased rumen fermentation. Compared to the

control, CH_4 energy as a percentage of GE was reduced ($P < 0.048$) with the inclusion of corn oil and calcium sulfate (4.78 vs. 4.11 and 4.11 ± 0.16 % for CON vs. CO and CaS, respectively). This resulted in a 16 % reduction in CH_4 with added corn oil and calcium sulfate compared to the control and approximately 9 % compared to reduced-fat DDGS. Similarly, Hales et al. (2017) observed that when corn oil is included at 2 % of the diet DM, CH_4 energy as a percentage of GE intake was reduced by 13 % and Beauchemin et al. (2007) observed a 20 % decrease with sunflower oil. Dietary fat may reduce CH_4 by 3 different mechanisms, increasing the propionate concentration with altering of the microbial community, providing an alternative hydrogen sink via biohydrogenation, and providing more fermentable dietary substrates (Nagaraja et al., 1997). In the current study, altering the microbial community and biohydrogenation may be the most likely modes of action, although the measuring the rumen environment done for this study although it was not directly measured.

Energy gains. Energy gains in the animal can be characterized as energy recovered by the animal, which includes energy in tissue, milk, and conceptus if the animal is pregnant (Ferrell and Oltjen, 2008). In the current study, retained energy (**RE)** is the sum of tissue and milk energy. Inclusion of corn oil increased ($P = 0.035$) milk energy compared to the control treatment (22.7 vs. 24.1 ± 0.58 Mcal/d), which was likely the result of more energy available for lactation. Similar to the current study, Van Knegsel et al. (2007) fed fat to lactating Holstein-Friesian cattle and observed an increase in energy partitioned to milk production. The control, DG, and calcium sulfate treatments did not differ ($P \geq 0.202$) for milk energy, with a mean of 23.2 ± 0.58 Mcal/d. Retained energy is the sum of tissue energy gain or loss plus lactation/milk energy.

Compared to the control, DG increased ($P < 0.001$) retained energy (25.9 vs. 29.6 ± 1.08 Mcal/d). Compared to the control, inclusion of corn oil increased ($P < 0.001$) retained energy (25.9 vs. 31.2 ± 1.08 Mcal/d). Retained energy did not differ ($P = 0.149$) between control and calcium sulfate treatment with a mean of 26.9 ± 1.08 Mcal/d. Retained energy also did not differ ($P = 0.233$) between DG and calcium sulfate with a mean of 28.8 ± 1.08 Mcal/d. Compared to the control, tissue energy was greater ($P \leq 0.042$) in DG (3.19 vs. 6.08 ± 0.99 Mcal/d). Variable results have been observed on the effects of including DDGS on tissue energy. Foth et al (2015) observed increased tissue energy with the inclusion of DDGS whereas Birkelo et al. (2004) observed a decrease in tissue energy with the inclusion of wet DGS. The discrepancy could be caused by the decrease in DMI for wet DGS compared to DDGS, which was used in both the study by Foth et al. (2015) and the current study. Compared to the control, tissue energy was greater ($P = 0.008$) with the inclusion of corn oil (3.19 vs. 7.04 ± 0.99 Mcal/d). The control and calcium sulfate treatments did not differ ($P = 0.329$) for tissue energy with a mean of 3.87 ± 0.99 Mcal/d. Treatments containing DDGS did not differ ($P \geq 0.266$) in tissue energy with a mean of 5.89 ± 0.99 Mcal/d.

Energy intake per unit of dry matter. In order to accurately formulate rations, estimates of energy contents are needed for feeds (Weiss, 1993). Typically, feed value is presented as energy available per unit of DMI with GE, ME, DE, and NE_L being used most often. Inclusion of DDGS has observed a 4 to 6 % increase in GE content (Mcal/kg of DM) of TMR's (Birkelo et al., 2004; Foth et al., 2015). Compared to the control, GE content per kg of DM was greater ($P < 0.001$) for DG (4.40 vs. 4.53 ± 0.01 for CON vs. DG, respectively). This resulted in a 3 % increase in GE for the DG diet. Compared to

control and DG, GE content per kg of DM was greater ($P < 0.001$) with the inclusion of corn oil (4.40 and 4.53 vs. 4.58 ± 0.01 Mcal/kg of DM for CON and DG vs. CO, respectively). Digestible energy has also been reported to increase by 5 % with DDGS (Birkelo et al., 2004). However, in the current study, inclusion of DDGS did not differ ($P = 0.287$) from the control with a mean of 68.4 ± 0.52 Mcal/kg of DM. Compared to the control, DE per kg of DM was greater ($P = 0.024$) with the inclusion of corn oil (3.03 vs. 3.10 ± 0.03 Mcal/kg of DM for CON vs. CO, respectively). Digestible energy for DDGS was greater ($P = 0.017$) than calcium sulfate (3.08 vs. 3.01 ± 0.03 Mcal/kg of DM for DG vs. DG). Birkelo et al. (2004) observed a 5 % increase in ME (Mcal/kg of DM) with the inclusion of DDGS. In the current study, DG increased ($P = 0.018$) ME per kg of DM by 3 % compared to the control (2.67 vs. 2.75 ± 0.03 Mcal/kg of DM for CON vs. DG, respectively). Compared to the control, metabolizable energy per kg of DM increased for corn oil (2.67 vs. 2.78 ± 0.03 Mcal/kg of DM for CON vs. CO, respectively). Net energy of lactation increased by 3 – 7 % in previous work done with DDGS, indicating a greater feeding value (Birkelo et al., 2004; Foth et al., 2015). In the current study, we found a 9 % increase ($P = 0.041$) in NE_L per kg of DM compared to the control and a 15 % increase ($P = 0.001$) with the inclusion of corn oil (1.35 vs. 1.47 and 1.55 ± 0.04 Mcal/kg of DM for CON vs. DG and CO, respectively). More energy was available for lactation from the DG and corn oil treatments with a similar NE_L compared to Foth et al. (2015) with a value of 1.47 Mcal/kg of DM. Overall, the inclusion of DDGS, corn oil, and calcium sulfate increased energy available for lactation. Part of the increased availability of energy may be due to decreased CH_4 energy and aligns with the hypothesis that dietary

strategies can be used to reduce methane emissions and increase energy balance in lactating cattle.

Maintenance energy and efficiency of energy use for lactation. Estimated maintenance energy requirement is illustrated in Figure 3.1 and was determined through regression of ME intake and RE and then solving for ME intake when RE equals zero (Foth et al., 2015). Estimated maintenance requirement was calculated to be 189 kcal/MBW with efficiency of ME use for lactation ($\boldsymbol{k_1}$) of 0.85. In the current study, estimated maintenance requirements and efficiencies were greater than previous estimates, which averaged near 143 ± 26 kcal/MBW for maintenance and 0.64 for k_1 (Birkelo et al., 2004; Moe and Tyrrell, 1971; Vermorel et al., 1982; Xue et al., 2011; Foth et al., 2015). However, Yan et al. (1997) reported maintenance requirements between 146 to 179 kcal/MBW and k_1 between 0.61-0.68 in lactating dairy cows indicating a large range of variation. Grainger et al., (1985) observed maintenance energy requirements of 184 kcal/MBW, which is similar to the current study. Coppock et al. (1964) observed efficiencies of converting ME to milk between 67 and 107 % with an overall mean around 75 %. With increased forage in the diet, it is possible that maintenance requirement increased. Dong et al. (2015) and Yan et al. (1997) observed increased maintenance requirements with increasing forage percentage in the diet which was suggested to be caused by increased size of the gastrointestinal tract. In a recent meta-analysis of energy balance data, Moraes et al. (2015) reported an increase in maintenance requirement, which may be correlated to higher genetic merit of cattle. Overall, the maintenance requirements observed in the current study are within the range found in the literature.

Nitrogen Balance

Nitrogen balance is important in the dairy industry due to the potential ramifications of excess nitrogen excretion as well as its potential to indirectly measure retained energy. Nitrogen balance is the N remaining after subtracting the N lost in the feces, urine, and milk from total N intake. Excretion of N is affected by total N intake (Weiss et al., 2009), which has led to highly variable observations in N balance, particularly when DDGS diets increase intake. Hales et al. (2017) observed a linear increase in urinary N with increasing concentrations of dietary corn oil while fecal N decreased linearly with the inclusion of corn oil. In contrast, Benchaar et al. (2013) observed a linear increase in N balance with linear increases in N intake. This led to N output in the feces, urine and milk with increased N retention in the tissue. In the current study, N intakes were not different ($P = 0.767$) among treatments (365.2 ± 8.52 g/d). Increased DMI with the treatment containing DDGS would have, by itself, let to N intake being greater. However, the control treatment had increased CP compared to the diets containing DDGS, which likely lead to similar N intake. Similarly, total N excretion (fecal plus urinary nitrogen) did not differ ($P = 0.290$) by treatment, with a mean of 365.2 ± 8.52 g/d which is likely related to similar N intakes. Nitrogen balance (intake nitrogen minus urinary, fecal and milk N) did not differ ($P \geq 0.118$) among the control, DG, and calcium sulfate treatments with a mean of 82.7 ± 10.7 g/d (Table 3.9). However, compared to DG, inclusion of corn oil reduced ($P = 0.025$) N balance in lactating cows. This was not expected as nitrogen intake and nitrogen excretion are closely related (Weiss et al., 2009). However, the increased N balance in the DG treatment may be due to a decrease in milk nitrogen (149.2 vs. 167.1 2 ± 3.50 g/d for DG vs. CO, respectively).

CONCLUSIONS

Dietary strategies to reduce methane production increased energy balance in lactating cattle. Inclusion of corn oil and calcium sulfate to diets containing DDGS decreased methane production by 7 and 11 % as well as CH_4 per unit of DMI by 9 % and 14 % per unit of milk yield, respectively. Inclusion of DDGS to the diet increased dry matter intake and milk yield were increased by approximately 5 and 6 %, respectively. Energy balance increased in diets containing DDGS likely the result of increased dry matter intake, a 10 % increase in NE_L and the reduction in CH_4. This is in agreement with our hypothesis that methane reduction strategies would increase energy balance. The inclusion of DDGS decreased digestibility, which may have resulted from increased dry matter intake and rate of passage. Nitrogen intake and balance were not affected by the inclusion of DDGS. Overall, dietary strategies to reduce methane production can improve energy balance in lactating dairy cattle.

REFERENCES

Abdelqader, M.M., A.R. HIppen, K.F. Kalscheur, D.J. Schingoethe, and A.D. Garcia. 2009. Isolipidic additions of fat from corn germ, corn distillers grains, or corn oil in dairy cows diets. J. Dairy Sci. 92:5523-5533.

AOAC International. 2000. Official Methods of Analysis. Vol. 1 and 2. 17th ed. AOAC International, Gaithersburg, MD.

AOAC International. 2006. Official Methods of Analysis. 18th ed. AOAC International, Gaithersburg, MD.

Beauchemin, K.A., S.M. McGinn, and H. Petit. 2007. Methane abatement strategies for cattle: lipid supplementation of diets. Can. J. Anim. Sci. 87:431–440.

Beauchemin, K.A., M. Kreuzer, F. O'Mara, and T. A. McAllister. 2008. Nutritional management for enteric methane abatement: A review. Aust. J. Exp. Ag. 48:21-27.

Benchaar, C., F. Hassanat, R. Gervais, P.Y. Chouinard, C. Julien, H. V Petit, and D.I. Massé. 2013. Effects of increasing amounts of corn dried distillers grains with solubles in dairy cow diets on methane production, ruminal fermentation, digestion, N balance, and milk production. J. Dairy Sci. 96:2413–2427.

Birkelo, C.P., M.J. Brouk, and D.J. Schingoethe. 2004. The energy content of wet corn distillers grains for lactating dairy cows. J. Dairy Sci. 87:1815-1819.

Blaxter, K. L. 1962. The Energy Metabolism of Ruminants. 2nd ed. Hutchinsom & Co. Ltd., London, UK.

Boerman, J.P., C.L. Preseault, and A.L. Lock. 2014. Effect of dietary antioxidant and increasing corn oil inclusion on milk fat yield and fatty acid composition in dairy cattle. J. Dairy Sci. 97:7697-7705.

Boston, R.C., D.G. Fox., C.J. Sniffen, R. Janczewski, R. Munsen, and W. Chalupa. 2000. The conversion of a scientific model describing dairy cow nutrition and production to an industry tool: the CPM Dairy project. Pages 361-377 in Modelling Nutrient Utilization in Farm Animals Edited by J.P. McNamara, J. France and D. Beever. Oxford: CABI Publishing.

Bradford, B.J. and C.R. Mullins. 2012. Invited review: strategies for promoting productivity and health of dairy cattle by feeding nonforage fiber sources. J. Dairy Sci. 95:4735-4746.

Brody, S. 1945. Bioenergetics and Growth. Pages 308-312, 914. Reinhold Publishing Corporation, New York, NY.

Brouwer. E. 1965. Report of sub-committee on constants and factors. Pages 441- 443 in Energy Metabolism. K.L. Blaxter, ed. European Association for Animal Production Publication No. 11, Ayr, Scotland.

Castillo-Lopez, E., H.A. Ramirez Ramirez, T.J. Klopfenstein, D. Hostetler, K. Karges, S.C. Fernando, and P.J. Kononoff. 2014. Ration formulations containing reduced-fat dried distillers grains with solubles and their effect on lactation performance, rumen fermentation, and intestinal flow of microbial nitrogen in Holstein cows. J. Dairy Sci. 97:1578-1593.

Chase, L.E. 2014. Carbon footprint and the dairy industry. Cornell Nutrition Conference Animal Science Conference Proceedings. Cornell Univ. Ithaca, NY.

Cherepanov, G.G., and V.I. Agaphonov. 2010. Estimation of substrate-energetic fluxes in lactating cows. J. Anim. Feed Sci. 19:13-23.

Coppock, C.E., W.P. Flatt, and L.A. Moore. 1964. Effect of hay to grain ratio on utilization of metabolizable energy for milk production by dairy cows. J. Dairy Sci. 47:1330-1338.

Coppock, C.E. 1985. Energy nutrition and metabolism of the lactating dairy cow. J. Dairy Sci. 68:3403-3410.

Dong, L.F., C.P. Ferris, D.A. McDowell, and T. Yan. 2015. Effects of diet forage proportion on maintenance energy requirement and the efficiency of metabolizable energy use for lactation by lactating dairy cows. J. Dairy Sci. 98:8846-8855.

DRMS. 2014. DHI Glossary. Dairy Records Management System., Raleigh, N.C.

DuBois, M., K.A. Giles, J.K. Hamiliton, P.A. Rebers, and F. Smith. 1956. Colorimetric method for determination of sugars and related substances. Anal. Chem. 28:350-356.

Environmental Protection Agency. 2010. Methane and nitrous oxide emissions. U.S. Environmental Protection Agency, Washington, DC, USA.

EPA (US Environmental Protection Agency). 1994. Method 200.7. Determination of metals and trace elements in water and wastes by inductively coupled plasma-atomic emission spectrometry. Revision 4.4. Accessed Sep. 29, 2017. http://www.epa.gov/sam/pdfs/EPA-200.7.pdf.

Ferrell., C.L., and J.W. Oltjen. 2008. ASAS Centennial paper: Net energy systems for beef cattle- Concepts, applications, and future models. J. Anim. Sci. 86:2779-2794.

Freetly, H.C., J.A. Nienaber and T. Brown-Brandl. 2006. Partitioning of energy during lactation of primiparous beef cows. J. Anim. Sci. 84:2157-2162.

Foth, A.J, T. Brown-Brandl, K. J. Hanford, P. S. Miller, G. Garcia Gomez, and P. J. Kononoff. 2015. Energy content of reduced-fat dried distillers grains with solubles for lactating dairy cows. J. Dairy Sci. 98:7142–7152.

Georing, H.K. and P.J. Van Soest. 1970. Forage Fiber Analysis. USDA Agricultural Research Service. Handbook number 379. U.S. Dept. of Agriculture. Superintendent of Documents, US Government Printing Office, Washington D.C. 20402.

Grainger, C, C.W. Holmes, and Y.F. Moore. 1985. Performance of Friesian cows with high and low breeding indexes. Anim. Prod. 40:389-400.

Hales, K. E., A. P. Foote, T. M. Brown-Brandl, and H. C. Freetly. 2017. The effects of feeding increasing concentrations of corn oil on energy metabolism and nutrient balance in finishing beef steers. J. Anim. Sci. 95:939-948.

Hall, M.B. 2009. Analysis of starch, including maltooligosaccharides, in animal feeds: a comparison of methods and a recommended method for AOAC collaborative study. JAOACI 92:42-49.

Heinrichs, A.J., and P.J. Kononoff. 2002. Evaluating particle size of forages and TMRs using the New Penn State Forage Particle Separator. Tech. Bul. DAS 02-42. Pennsylvania State Univ., College Agric. Sci., Cooperative Ext., University Park, PA.

Hristov, A.N., J. Oh, J.L. Firkins, J. Dijkstra, E. Kebreab, G. Waghorn, H.P.S. Makkar, A.T. Adesogan, W. Yang, C. Lee, P.J. Gerber, B. Henderson, and J.M. Tricarico. 2013. Special Topics-Mitigation of methane and nitrous oxide emissions from animal operations: I. A review of enteric methane mitigation options. J. Anim. Sci. 91:5045-5069.

Huhtanen, P., M. Rinne, and J. Nousiainen. 2009. A meta-analysis of feed digestion in dairy cows. 2. The effects of feeding level and diet composition on digestibility. J. Dairy Sci. 92:5031-5042.

Hünerberg, M., S.M. McGinn, K.A. Beauchemin, E.K. Okine, O.M. Harstad, and T.A. McAllister. 2013. Effect of dried distillers' grains with solubles on enteric methane emissions and nitrogen excretion from finishing beef cattle. Can. J. Anim. Sci. 93:373-385.

Innovation Center for U.S. Dairy. 2009. Dairy Industry Applauds White House Strategy for Methane Emissions Reduction. News Release, March 38, 2014. http://www.nmpf.org/files/White-House-Methane-Emissions-Reduction-032814.pdf

Janicek, B.N., P.J. Kononoff, A.M. Gehman, and P.H. Doane, 2008. The effect of feeding dried distillers grains plus solubles on milk production and excretion of urinary purine derivatives. J. Dairy Sci. 91:3544-3553.

Johnson, K.A., and D.E. Johnson. 1995. Methane emissions from cattle. J. Anim. Sci. 73:2483-2492.

Ketelaars, J.J., and B.J. Tolkamp. 1996. Oxygen efficiency and the control of energy flow in animals and humans. J. Anim. Sci. 74:3036-3051.

Knapp, J.R., G.L. Laur, P.A. Vadas, W.P. Weiss, and J.M. Tricarico. 2014. Invited review: enteric methane in dairy cattle production: Quantifying the opportunities and impact of reducing emissions. J. Dairy Sci. 97:3221-3261.

Kononoff, P.J. and K.J. Hanford. 2006. Technical note: Estimating statistical power of mixed models used in dairy nutrition experiments. J. Dairy Sci. 89:3968-3971.

Mjoun, K., K.F. Kalscheur, A.R. Hippen, D.J. Schingoethe, and D.E. Little. 2010. Lactation performance and amino acid utilization of cows fed increasing amounts of reduced-fat dried distillers grain with solubles. J. Dairy Sci. 93:288-303.

Moallem, U., G. Altmark, H. Lehrer, and A. Arieli. 2010. Performance of high yielding dairy cows supplemented with fat or concentrate under hot and humid climates. J. Dairy Sci. 93:3192-3202.

Moate, P. J., S. R. O. Williams, C. Grainger, M. C. Hannah, E. N. Ponnampalam, and R. J. Eckard. 2011. Influence of cold-pressed canola, brewers grains and hominy meal as dietary supplements suitable for reducing enteric methane emissions from lactating dairy cows. Anim. Feed Sci. Technol. 166–167:254–264.

Moe, P. W., H. F. Tyrrell, and W. P. Flatt. 1970. Partial efficiency of energy use for maintenance, lactation, body gain and gestation in the dairy cow. Page 65 in Energy Metabolism of Farm Animals, EAAP Publ. No. 13, Vitznau, Switzerland.

Moe, P.W. and H.F. Tyrrell. 1971. Net energy value for lactation of high- and low-protein diets containing corn silage. J. Dairy Sci. 55:288-303.

Moraes, L.E., E. Kebreab, A.B. Strathe, J. Dijkstra, J. France, D.P. Casper, and J.G. Fadel. 2015. Multivariate and univariate analysis of energy balance data from lactating dairy cows. J. Dairy Sci. 98:4012-4029.

Nagaraja, T.G., C.J. Newbold, C.J. Van NEvel, and D.I Demeyer. 1997. Manipulation of ruminal fermentation. Pages 523-632 in The Rumen Microbial Ecosystem. P.N. Hobson and C.S. Stewart, ed. Chapman and Hall, London, UK.

National Research Council. 2001. Nutrient Requirements of Dairy Cattle. 7th rev. ed. Natl. Acad. Press, Washington, D.C.

National Research Council. 2005. Mineral Tolerance of Animals. Vol. 2nd rev. ed. Washington DC: Natl. Acad. Press.

Nienaber, J.A., and A.L. Maddy. 1985. Temperature controlled multiple chamber indirect calorimeter-design and operation. Trans. ASAE. 28:555-560.

Nkrumah, J.D., E.K. Okine, G.W. Mathison, K. Schmid, C. Li, J.A. Basarab, M.A. Price, Z. Wang, and S.S. Moore. 2006. Relationships of feedlot feed efficiency, performance, and feeding behavior with metabolic rate, methane production, and energy partitioning in beef cattle. J. Anim. Sci. 84:145-153.

Olijhoek, D.W., A.L.F. Hellwing, M. Brask, M.R. Weisbjerg, O. Højberg, M.K. Larsen, J. Dijkstra, E.J. Erlandsen, and P. Lund. 2016. Effect of dietary nitrate level on enteric methane production, hydrogen emission, rumen fermentation, and nutrient digestibility in dairy cows. J. Dairy Sci. 99:6191-6205.

Paz, H.A., M.J. de Veth, R.S. Ordway, and P.J. Kononoff. 2013. Evaluation of rumen-protected lysine supplementation to lactating dairy cows consuming increasing amounts of distillers dried grains with solubles. J. Dairy Sci. 96:7210-7222.

Penner, G.B., P. Yu, and D.A. Christensen. 2009. Effect of replacing forage or concentrate with wet or dry distillers' grains on the productivity and chewing activity of dairy cattle. Anim. Feed Sci. and Tech. 153:1-10.

Purwanto, B. P., Y. Abo, R. Sakamoto, F. Furumoto, and S. Yamamoto. 1990. Diurnal patterns of heat production and heart rate under thermoneutral conditions in Holstein Friesian cows differing in milk production. J. Agric Sci. (Camb.) 114:139–142.

Ramirez Ramirez, H.A., E. Castillo Lopez, K.J. Harvatine, and P.J. Kononoff. 2015. Fat and starch as additive risk factors for milk fat depression in dairy diets containing corn dried distillers grains with solubles. J. Dairy Sci. 98:1903–1914.

Ramirez Ramirez, H.A., K.J. Harvatine, and P.J. Kononoff. 2016. Short communication: Forage particle size and fat intake affect rumen passage, the fatty acid profile of milk and milk fat production in dairy cows consuming dried distillers grains with solubles. J. Dairy Sci. 99:392-398.

Roseler, D.K., J.D. Ferguson, C.J. Sniffen, and J. Herrema. 1993. Dietary protein degradability effects on plasma and milk urea nitrogen and milk nonprotein nitrogen in Holstein cows. J. Dairy Sci. 76:525-534.

Schingoethe, D.J., K.F. Kalscheur, A.R. Hippen, and A.D. Garcia. 2009. Invited review: The use of distillers products in dairy cattle diets. J. Dairy Sci. 92:5802-5813.

Tamura, T., H. Nakamura, S. Sato, M. Seki, and H. Nishiki. 2014. A modified catheterization procedure to reduce bladder damage when collecting urine samples from Holstein cows. J. Vet. Med. Sci. 76(6)819-826.

Van Knegsel, A.T.M., H. van den Brand, J. Dijkstra, W.M. van Straalen, M.J.W. Heetkamp, S. Tamminga, and B. Kemp. 2007. Dietary energy source in dairy cows in early lactation: energy partitioning and milk production. J. Dairy Sci. 90:1467-1476.

Van Soest, P.J., J.B. Robertson, and B.A. Lewis. 1991. Methods for dietary fiber, neutral detergent fiber, and nonstarch polysaccharides in relation to animal nutrition. J. Dairy Sci. 74:3583-3597.

Van Zijderveld, S. M., W. J. J. Gerrits, J. A. Apajalahti, J. R. Newbold, J. Dijkstra, R. A. Leng, and H. B. Perdok. 2010. Nitrate and sulfate: Effective alternative hydrogen sinks for mitigation of ruminal methane production in sheep. J. Dairy Sci. 93:5856-5866.

Van Zijderveld, S.M., J. Dijkstra, H.B. Perdok, J.R. Newbold, and W.J.J. Gerrits. 2011. Dietary inclusion of diallyl disulfide, yucca powder, calcium fumarate, an extruded linseed product, or medium-chain fatty acids does not affect methane production in lactating dairy cows. J. Dairy Sci. 94:3094-3104.

Vermorel, M., B. Remond, J.Vernet, and D. Liamadis. 1982. Utilization of body reserves by high-producing cows in early lactation; effects of crude protein and amino-acid supply. Pages 18-21in Energy Metabolism of Farm Animals. A. Ekern and F. Sundstøl, ed. European Association for Animal Production Publication No. 29, Ås, Norway.

Weiss, W.P. 1993. Predicting energy values of feeds. J. Dairy Sci. 76:1802-1811.

Weiss, W.P., L.B. Willett, N.R. St-Pierre, D.C. Borger, T.R. McKelvey, and D.J. Wyatt. 2009. Varying forage type, metabolizable protein concentration, and carbohydrate source affects manure excretion, manure ammonia, and nitrogen metabolism of dairy cows. J. Dairy Sci. 92:5607-5619.

White, R.R., M.B. Hall, J.L. Firkins, and P.J. Kononoff. 2017. Physically adjusted neutral detergent fiber system for lactating dairy cow rations. I:Deriving equations that identify factors that influence effectiveness of fiber. J. Dairy Sci. 100:1-18.

Wildman, E.E. G.M. Jones, P.E. Wagner, R.L. Boman, H.F. Troutt and T.N Lesch. 1982. A dairy cow body condition scoring system and its relationship to selected production characteristics. J. Dairy Sci. 65:495-501.

Xue, B. T. Yan, C.F. Ferris, and C.S. Mayne. 2011. Milk production and energy efficiency of Holstein and Jersey-Holstein crossbred dairy cows offered diets containing grass silage. J. Dairy Sci. 94:1455-1464.

Yan, T., F.J. Gordon, R.E. Agnew, M.G. Porter, and D.C. Patterson. 1997. The metabolisable energy requirement for maintenance and the efficiency of utilization of metabolisable energy for lactation by dairy cows offered grass silage-based diets. Livest. Prod. Sci. 51:141-150.

Zebeli, Q., D. Mansmann, B.N. Ametaj, H. Steingass, and W. Drochner 2010. A model to optimize the requirements of lactating dairy cows for physically effective neutral detergent fiber. Arch Anim Nutr. 64:265-278.

Table 3. 1. Chemical composition and analysis of treatments formulated to reduce methane.

	Treatment[1]			
Item	CON	DG	CO	CaS
Ingredient, % DM				
Corn silage	29.8	29.8	29.8	29.8
Alfalfa hay	26.6	26.6	26.6	26.6
Brome hay	2.57	2.57	2.57	2.56
Ground corn	21.8	12.9	11.5	12.6
Ground soybean hulls	0.55	0.55	0.55	0.55
DDGS	--	20.0	20.0	20.0
Soybean meal	11.0	--	--	--
Bypass soy[2]	4.59	4.59	4.59	4.59
Bloodmeal	0.46	0.46	0.46	0.46
Corn oil	--	--	1.38	--
Calcium carbonate	0.75	0.75	0.75	0.18
Calcium sulfate	--	--	--	0.93
Sodium bicarbonate	0.62	0.62	0.62	0.62
Ca-salts of LCFA[3]	0.55	0.55	0.55	0.55
Magnesium oxide	0.24	0.24	0.24	0.24
Salt	0.18	0.18	0.18	0.18
Trace mineral premix[4]	0.09	0.09	0.09	0.09
Vitamin premix[5]	0.09	0.09	0.09	0.09
Chemical Composition[6]				
DM, %	53.9 (0.49)	54.1 (0.49)	54.2 (0.51)	54.0 (0.48)
CP, % DM	18.0 (0.50)	17.2 (0.24)	16.9 (0.21)	17.3 (0.37)
Crude fat, % DM	2.65 (0.16)	3.38 (0.37)	4.76 (0.21)	3.55 (0.19)
ADF, % DM	22.0 (0.63)	23.2 (0.99)	23.3 (0.81)	23.5 (0.91)
NDF, % DM	31.5 (1.00)	34.7 (1.68)	35.1 (0.75)	35.6 (0.45)
Lignin, % DM	4.20 (0.12)	4.52 (0.20)	4.64 (0.24)	4.52 (0.19)
Ash, % DM	7.79 (0.15)	7.78 (0.24)	7.83 (0.18)	8.16 (0.49)
Starch, % DM	26.9 (1.62)	23.2 (1.41)	21.9 (0.72)	22.4 (0.65)
Sulfur, % DM	0.23 (0.03)	0.32 (0.04)	0.34 (0.01)	0.52 (0.03)
Gross energy, cal/g[7]	4387.9 (58.1)	4500.4 (41.8)	4558.5 (42.8)	4492.2 (51.8)
ME, Mcal/kg[8]	2.64	2.51	2.59	2.50
NE_L, Mcal/kg[8]	1.70	1.62	1.67	1.61

[1]Treatments: CON = control; DG = reduced-fat dried distillers grains with solubles; CO = DG plus corn oil; CaS = DG plus calcium sulfate.

[2] Soypass, LignoTech, Overland Park, KS.

[3]Calcium salts of long-chain fatty acids marketed as Megalac by Church & Dwight Co. Inc. Princeton, NJ.

[4] Formulated to supply approximately 2,300 mg/kg Co, 25,000 mg/kg Cu, 2,600 mg/kg I, 1,000 mg/kg Fe, 150,000 mg/kg Mn, 820 mg/kg Se and 180,000 mg/kg Zn in total rations.

[5] Formulated to supply approximately 148,500 IU/d vitamin A, 38,500 IU/d vitamin D and 902 IU/d vitamin E in total rations.

[6]Values determined by Cumberland Valley Analytical Services, Hagerstown, MD, Mean (SD).

[7]Determined from composite samples from experiment and analyzed at the University of Nebraska-Lincoln, mean (SD).

[8]Values formulated from Cornell-Penn-Miner dairy model (Boston et al., 2000).

Table 3. 2. Feed chemical analysis for alfalfa hay, brome hay, corn silage, and concentrate mixes (DM basis)[1].

	Alfalfa		Brome Hay		Corn Silage		CON Concentrate		DG Concentrate		CO Concentrate		CaS Concentrate	
Chemical	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
DM, %	89.9	1.25	89.6	1.30	36.7	2.71	90.3	1.07	90.4	1.11	90.9	0.94	90.8	0.90
CP, % of DM	17.5	1.68	10.6	2.42	8.20	0.29	26.0	1.49	24.0	0.62	23.2	1.49	24.3	0.42
Soluble Protein, % of DM	6.30	0.47	2.64	0.60	4.06	0.56	3.99	1.08	2.76	1.05	2.14	1.20	3.41	0.87
ADICP[2], % of DM	1.97	0.25	1.86	1.18	0.93	0.14	1.09	0.64	1.48	0.25	1.80	0.53	1.74	0.42
NDICP[2], % of DM	2.59	0.20	3.63	1.10	1.03	0.20	2.34	0.90	2.87	0.26	2.95	0.32	2.91	0.23
ADF, % of DM	42.8	2.34	42.9	3.08	25.1	1.17	5.01	1.45	7.94	1.68	8.08	1.57	8.70	1.75
NDF, % of DM	49.8	3.37	65.9	1.23	38.5	1.61	12.3	4.54	20.2	3.81	21.1	1.21	22.2	2.04
Lignin, % of DM	9.64	0.45	5.97	0.95	3.31	0.32	1.16	0.38	1.95	0.79	2.24	1.18	1.95	0.66
NFC[3], % of DM	24.5	1.40	15.2	1.97	45.6	2.14	52.8	4.23	45.6	4.56	42.2	1.89	42.1	2.50
Starch, % of DM	1.53	0.44	1.46	0.98	36.3	2.15	38.3	6.02	29.1	3.54	26.1	0.96	27.3	1.46
Sugar, % of DM	4.68	0.79	5.29	2.05	1.01	0.52	6.09	1.75	4.70	0.97	4.43	1.00	4.55	1.63
Crude fat, % of DM	1.34	0.29	1.75	0.53	3.45	0.19	2.97	0.76	4.76	1.10	8.13	0.49	5.17	0.40
Ash, % of DM	9.54	0.42	10.3	1.35	5.27	1.03	8.33	0.84	8.29	0.28	8.42	0.61	9.22	0.67
Ca, % of DM	1.12	0.18	0.42	0.14	0.22	0.05	1.22	0.36	1.21	0.19	1.13	0.11	1.00	0.20
P, % of DM	0.33	0.02	0.29	0.05	0.25	0.03	0.54	0.09	0.71	0.09	0.71	0.06	0.69	0.08
Mg, % of DM	0.22	0.03	0.14	0.03	0.12	0.01	0.55	0.08	0.61	0.06	0.59	0.07	0.56	0.09
K, % of DM	2.99	0.11	2.67	0.54	1.10	0.12	1.26	0.07	1.29	0.31	1.22	0.33	1.21	0.36
S, % of DM	0.21	0.03	0.17	0.03	0.13	0.01	0.33	0.10	0.54	0.11	0.59	0.02	1.02	0.09
Na, % of DM	0.03	0.01	0.02	0.01	0.01	0.01	0.73	0.21	0.78	0.09	0.76	0.05	0.73	0.13
Cl, % of DM	0.11	0.03	0.70	0.26	0.19	0.05	0.45	0.21	0.39	0.06	0.38	0.03	0.37	0.08
Fe, mg/kg	304.3	95.8	276.4	176.6	196.5	98.9	278.4	49.6	284.8	35.5	273.1	32.68	224.8	33.7
Zn, mg/kg	23.4	3.20	22.6	4.53	22.9	3.31	337.3	159.5	336.3	128.8	332.4	146.4	299.0	131.1
Cu, mg/kg	8.38	0.52	7.75	1.49	6.25	0.46	62.1	25.4	54.8	14.9	57.3	26.2	79.5	53.7
Mn, mg/kg	33.5	6.48	44.4	6.41	25.8	6.82	189.8	59.2	253.8	105.5	253.1	111.0	223.3	151.6
DCAD[4]	61.6	2.83	38.7	5.48	14.5	2.26	31.0	5.90	22.0	10.4	16.7	9.46	-11.6	12.6

[1]Mean and SD were calculated based on samples of each feedstuff collected during each period and estimated by a commercial feed testing laboratory (Cumberland Valley Analytical Services, Hagerstown, MD) treatments: CON = Control; DG = Reduced-fat dried distillers grains with solubles; CO = DG plus corn oil; and CaS = DG plus calcium sulfate.

[2]ADICP = Acid-detergent-insoluble crude protein; NDICP = Neutral-detergent-insoluble crude protein

[3]NFC = Nonfiber carbohydrate calculated by difference 100-(% NDF + % CP + % Fat + % Ash)

[4]Dietary cation-anion difference (mEq/100g of DM = ((Na + K) – (Cl + S))/100 g of DM)

Table 3. 3. Particle distribution of treatments formulated to reduce methane based on the total mixed ration (as-fed basis)[1]

	CON		DG		CO		CaS	
Particle Size, %[2]	Mean	SD	Mean	SD	Mean	SD	Mean	SD
> 19.0 mm	4.81	1.28	5.69	1.85	5.38	1.50	5.06	1.77
19.0 -- 8.0 mm	25.2	1.87	24.6	1.67	25.9	1.98	25.1	2.28
8.0 -- 1.18 mm	50.9	2.92	45.2	1.56	45.8	1.38	45.5	1.86
< 1.18 mm	18.9	2.32	24.3	1.78	23.0	2.03	24.4	2.06

[1]Treatments: CON = Control; DG = Reduced-fat dried distillers grains with solubles; CO = DG plus corn oil; and CaS = DG plus calcium sulfate.

[2]Determined using the Penn State Particle Separator on wet basis (Heinrichs and Kononoff, 2002).

Table 3. 4. DMI, milk production and composition, body weight and BCS[5], and water intake of treatments formulated to reduce methane.

	Treatment[1]					
Item	CON	DG	CO	CaS	SEM[2]	*P*-value
DMI, kg/d	19.1[b]	20.1[a]	20.0[a]	19.6[ab]	0.37	0.126
Milk yield, kg/d	26.3[b]	27.5[a]	28.3[a]	27.6[a]	0.67	0.002
ECM[3], kg/d	30.1[b]	31.4[a]	31.7[a]	31.0[ab]	0.66	0.024
Fat, %	4.70	4.64	4.53	4.57	0.10	0.315
Fat yield, kg/d	1.19	1.25	1.24	1.22	0.03	0.224
FCM kg/d	30.7[b]	32.1[a]	32.4[a]	31.7[ab]	0.67	0.035
Protein, %	3.28[a]	3.26[ab]	3.18[b]	3.20[ab]	0.04	0.108
Protein yield, kg/d	0.84[b]	0.87[ab]	0.88[a]	0.86[ab]	0.02	0.118
Lactose, %	4.90	4.91	4.92	4.92	0.02	0.769
MUN[4], mg/dl	17.3[a]	15.0[bc]	14.4[c]	15.3[b]	0.59	< 0.001
SCC[5], cells/mL	98.7	111.3	136.7	133.6	39.7	0.740
Free water intake, L/d	82.1	84.3	89.5	83.2	3.61	0.315
Body weight, kg	508.1	513.4	513.2	510.7	11.1	0.497
BCS[6]	3.23[a]	3.13[b]	3.16[ab]	3.20[ab]	0.06	0.063

[1]Treatments: CON = Control; DG = Reduced-fat dried distillers grains with solubles; CO = DG plus corn oil; and CaS = DG plus calcium sulfate.

[2]Lowest standard error of treatment means is listed.

[3]Energy corrected milk = 0.327 × milk yield [kg] + 7.2 × protein [kg] adjusted for 3.5% fat and 3.2% total protein (DHI Glossary, 2014).

[4]MUN = Milk urea nitrogen.

[5]SCC = Somatic cell count.

[6]BCS = Body Condition Score 1-5 scale according to Wildman et al. (1982).

[abc]Means within rows lacking common superscript differ ($P < 0.05$).

Table 3. 5. Water quality constituent analysis of on-site tap water for lactating dairy cows.

Item	Mean	SD	Caution level[1]
Constituent, ppm			
TDS[2]	373.1	14.9	500
Ca	59.4	4.44	80
Cl	23.3	1.98	200
Fe	0.01	0.02	0.3
Fl	0.89	0.06	4
Mg	14.0	1.39	30
Mn	ND[3]	--	0.05
NO_3-N	0.64	0.14	10
Na	36.4	5.17	100
SO_4	92.0	10.00	400
Conductivity, mS/cm	0.57	0.02	0.75
Hardness,	12.0	0.92	20
pH	7.84	0.09	6.5/9
Total Coliform, MPN/100 mL	ND	--	1

[1]Caution levels from Midwest Laboratories Inc. (Omaha, NE).
[2]TDS = total dissolved solids.
[3]ND = not detected.

Table 3. 6. Methane production, methane efficiencies, and heat production for treatments formulated to reduce methane.

	Treatment[1]					
Item	CON	DG	CO	CaS	SEM[2]	*P*-value
O_2 consumption, L/d	4978.2	5107.1	4862.4	4940.7	119.8	0.443
CO_2 production, L/d	5331.4	5427.4	5105.2	5245.3	135.1	0.325
CH_4 production, L/d	421.6^{a}	429.5^{a}	394.7^{ab}	381.4^{b}	14.4	0.065
CH_4/MY, L/kg/d	16.7^{a}	16.2^{a}	14.4^{b}	14.3^{b}	0.60	0.003
CH_4/ECM, L/kg/d	14.2^{a}	13.8^{ab}	12.5^{bc}	12.4^{c}	0.50	0.019
RQ[3], L/L	1.07^{a}	1.06^{ab}	1.05^{b}	1.06^{ab}	0.01	0.058
CH_4/DMI, L/kg/d	22.3^{a}	21.4^{ab}	19.9^{b}	19.6^{b}	0.75	0.049
HP[4], Mcal/d	25.1	25.8	24.4	24.9	0.62	0.426
HP, kcal/$BW^{0.75}$	253.7	256.9	246.5	250.5	5.64	0.541

[1]Treatments: CON = Control; DG = Reduced-fat dried distillers grains with solubles; CO = DG plus corn oil; and CaS = DG plus calcium sulfate.

[2]Lowest standard error of treatment means is listed.

[3]RQ = Respiratory quotient (CO_2 production/O_2 consumption).

[4]HP = Heat production, calculated with Brouwer's (1965) equation from O_2 consumption (L), CO_2 production (L), methane production (L) and urine–N (g) ($HP = 3.866 \times O_2 + 1.200 \times CO_2 – 0.518 \times CH_4 – 1.431 \times N$).

[abc]Means within rows lacking common superscript differ ($P < 0.05$).

Table 3. 7. Apparent DM, OM, CP, NDF, Starch and Ash digestibility of treatments.

	Treatment[1]					
Component	CON	DG	CO	CaS	SEM[2]	*P*-value
DM, %	68.5[a]	67.2[b]	66.7[b]	66.3[b]	0.47	< 0.001
OM, %	69.8[a]	68.4[b]	67.9[bc]	67.2[c]	0.47	< 0.001
CP, %	72.8[a]	71.8[ab]	71.0[b]	71.0[b]	0.50	0.022
NDF, %	52.8	54.3	54.3	53.7	0.72	0.247
Starch, %	93.4[a]	92.9[ab]	92.2[ab]	92.1[b]	0.51	0.155
Ash, %	45.1[ab]	44.9[ab]	45.7[a]	42.8[b]	1.20	0.223

[1]Treatments: CON = Control; DG = Reduced-fat dried distillers grains with solubles; CO = DG plus corn oil; and CaS = DG plus calcium sulfate.

[2]Lowest Standard error of treatment means is listed.

Table 3. 8. Partitioning of energy for treatments formulated to reduce methane.

	Treatment[2]					
Item[1]	CON	DG	CO	CaS	SEM[3]	*P*-value
GE intake, Mcal/d	84.0^{b}	91.2^{a}	91.6^{a}	88.7^{a}	1.67	0.002
DE, Mcal/d	57.7^{b}	62.1^{a}	62.0^{a}	59.0^{b}	1.14	0.006
ME, Mcal/d	50.5^{b}	54.8^{a}	55.0^{a}	52.3^{a}	1.08	0.005
NE_L, Mcal/d	25.9^{c}	29.6^{ab}	31.2^{a}	27.9^{bc}	1.08	0.005
Component, Mcal/d						
Feces	26.4^{b}	29.2^{a}	29.7^{a}	29.7^{a}	0.77	0.001
Methane	3.98^{a}	4.06^{a}	3.73^{ab}	3.61^{b}	0.14	0.065
Urine	2.67	2.66	2.67	2.56	0.10	0.794
Heat	25.1	25.8	24.4	24.9	0.62	0.426
RE[4]	25.9^{c}	29.6^{ab}	31.2^{a}	27.9^{bc}	1.07	0.005
Milk	22.7^{b}	23.5^{ab}	24.1^{a}	23.4^{ab}	0.58	0.199
TE[5]	3.16^{b}	6.08^{a}	7.04^{a}	4.54^{ab}	0.99	0.041
DE, % of GE	68.7^{a}	68.0^{a}	67.6^{ab}	66.5^{b}	0.52	0.009
ME, % of GE	60.7	60.6	60.5	59.5	0.61	0.186
Feces, % of GE	30.7^{b}	32.4^{ab}	33.7^{a}	31.2^{b}	1.19	0.075
Methane, % of GE	4.78^{a}	4.47^{ab}	4.11^{b}	4.11^{b}	0.21	0.010
Urine, % of GE	3.20^{a}	2.93^{ab}	2.91^{b}	2.90^{b}	0.14	0.124
Heat, % of GE	30.0^{a}	28.3^{b}	26.8^{b}	28.3^{b}	0.85	0.007
Milk, % of GE	27.1	25.9	26.4	26.5	0.68	0.528
TE[5], % of GE	3.58^{b}	6.48^{ab}	7.36^{b}	4.72^{ab}	1.06	0.069
GE, Mcal/kg of DM	4.40^{c}	4.53^{b}	4.59^{a}	4.52^{b}	0.01	< 0.001
DE, Mcal/kg of DM	3.03^{bc}	3.09^{ab}	3.10^{a}	3.01^{c}	0.03	0.014
ME, Mcal/kg of DM	2.67^{b}	2.75^{a}	2.78^{a}	2.69^{b}	0.03	0.006
NE_L, Mcal/kg of DM	1.35^{c}	1.47^{ab}	1.55^{a}	1.41^{bc}	0.06	0.009

[1] GE = gross energy; DE = digestible energy; ME = metabolizable energy; NE_L = net energy lactation.

[2]Treatments: CON = Control; DG = Reduced-fat dried distillers grains with solubles; CO = DG plus corn oil; and CaS = DG plus calcium sulfate.

[3]Lowest standard error of treatment means is listed.

[4]RE = retained energy.

[5]TE = tissue energy.

Table 3. 9. Partitioning of nitrogen for treatments formulated to reduce methane.

	Treatment[1]					
Item	CON	DG	CO	CaS	SEM[2]	*P*-value
Mass, g/d						
N intake	606.2	610.3	595.9	599.2	12.70	0.767
Fecal N excretion	165.1	172.1	172.1	173.9	4.60	0.308
Urine N excretion	200.0^{a}	197.8^{ab}	200.1^{a}	179.4^{b}	6.94	0.125
Total N excretion[3]	365.1	370.0	372.2	353.4	10.39	0.290
Milk N concentration	168.0^{a}	149.2^{b}	167.1^{a}	161.8^{a}	3.50	0.001
N balance[4]	73.1^{ab}	91.1^{a}	56.6^{b}	84.1^{ab}	10.67	0.118
TE in protein[5]	2.45^{ab}	3.05^{a}	1.90^{b}	2.82^{ab}	0.49	0.118
N, % of intake						
Fecal N	27.2^{b}	28.2^{ab}	29.0^{a}	29.0^{a}	0.51	0.022
Urine N	33.6	32.7	34.3	30.2	1.46	0.228
Milk N	28.0^{a}	24.7^{b}	28.5^{a}	27.5^{a}	0.64	< 0.001
N balance	11.2^{ab}	14.4^{a}	8.2^{b}	13.3^{a}	1.85	0.085

[1]Treatments: CON = Control; DG = Reduced-fat dried distillers grains with solubles; CO = DG plus corn oil; and CaS = DG plus calcium sulfate.
[2]Lowest standard error of treatment means is listed.
[3]Total N excretion = Fecal N + Urine N.
[4]Nitrogen balance = intake N – Fecal N – urine N – milk N.
[5]TE = Tissue energy.

Table 3. 10. Energy balance equations derived from Brouwer (1965), Moe et al. (1970), and Freetly et al. (2006).

Response	ID	Equation[1]
Heat production (HP)	1	$3.866 \times O_2 + 1.200 \times CO_2 - 0.518 \times CH_4 - 1.431 \times$Urinary N
Metabolizable energy (ME)	2	Intake energy – fecal energy – urinary energy – CH_4 energy
Recovered energy (RE)	3	ME - HP
Tissue energy (TE)	4	RE – milk energy
Tissue energy in protein	5	N balance (tissue N) × (5.88 kg of protein/kg of N) × (5.7 Mcal/kg of protein)
ME_{RE}	6	ME – ME for maintenance
LE_{ME} (negative energy balance)	7	Milk energy + TE ×0.84
ME_{LE} (positive energy balance)	8	ME_{RE} – TE/0.726
N balance (tissue N)	9	Intake N – fecal N – urinary N – milk N

[1]HP, Heat production is Mcal/d where O_2 and CO_2 are L/d and N is g/d; ME, Metabolizable energy is Mcal/d; RE, Recovered energy is Mcal/d; TE, Tissue energy is Mcal/d; Milk energy is energy in milk multiplied by total production (Mcal/d); Tissue energy in protein is kcal/d**;** ME_{RE}, Metabolizable energy for maintenance found by regression of RE on ME and is the value of ME at zero RE based on metabolic body weight (MBW) kcal/MBW; LE_{ME}, Lactation energy received from ME of feed (kcal/d) for cows in a negative energy balance; ME_{LE}, Metabolizable energy available for lactation (kcal/d) for cows in a positive energy balance; N balance, Nitrogen balance is kg/d.

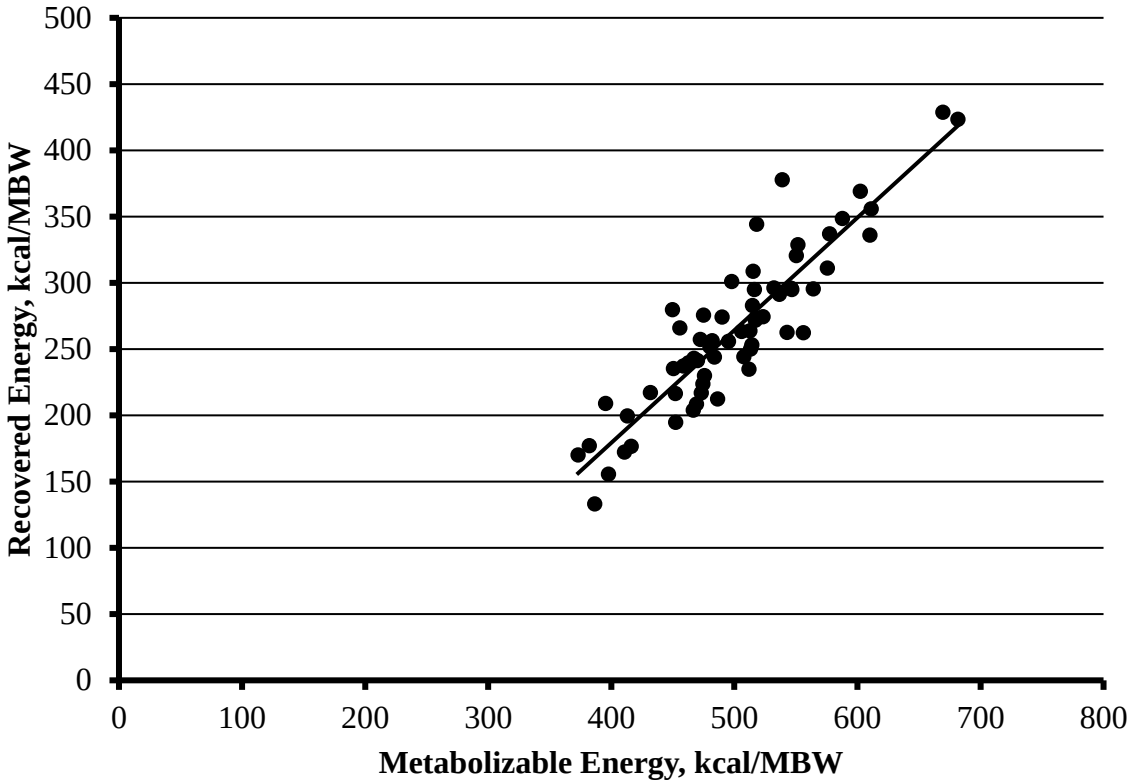

Figure 3. 1. Regression of recovered energy on metabolizable energy intake in kilocalories per metabolic body weight (kcal/MBW; y = 0.8509x – 160.32; R2 = 0.82). Recovered energy = 0 at 189 kcal/MBW and efficiency of converting ME to lactation energy is 85%.

CHAPTER 4

Increasing the concentration of linolenic acid in diets fed to Jersey cows in late lactation does not affect methane production[1]

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ABSTRACT

Although the inclusion of fat has reduced methane production in ruminants, relatively little research has been conducted on comparing the source and profile of fatty acids on methane production in lactating dairy cows. A study using 8 multiparous (325 ± 17 DIM) (mean ± SD) lactating Jersey cows was conducted to determine effects of feeding canola/tallow vs. extruded byproduct containing flaxseed as a fat source on methane production and diet digestibility in late-lactation dairy cows. A crossover design with 35-d periods (28-d adaption and 4-d collections) was used to compare 2 different fat sources. Diets contained approximately 50 % forage mixture of corn silage, alfalfa hay, and brome hay with only the concentrate mixture changing between diets to include either 1) a conventional corn/soybean meal/canola meal with tallow diet (CON), or 2) a conventional corn/soybean meal diet with an extruded byproduct containing flaxseed (EXF) as the fat source. Diets were balanced to decrease corn and canola meal and replace them with EXF to increase linolenic acid supply (21.2 vs 188.8 g/d) to the rumen. Methane production was measured using headbox-style indirect calorimeters. Milk production (17.4 ± 1.04 kg/d) and DMI (15.4 ± 0.71 kg/d) were similar across treatments. Milk fat (5.88 ± 0.25 %) and protein (4.08 ± 0.14 %) were not affected by treatment. For methane production, no difference was observed for total production (352.0 vs. 349.8 ± 16.43 L/d for CON vs. EXF, respectively). Methane production per unit of DMI was not affected and averaged 10.5 ± 0.57 L/kg. Similarly, methane production per unit of energy-corrected milk was not affected by fat source and averaged 7.01 ± 0.68 L/kg. Heat production was similar averaging 21.1 ± 1.02 Mcal/d. Digestibility of NDF, CP, DM, OM, and starch were not affected by diet and averaged 53.6, 73.3, 67.5, 69.9 and 96.1 %

for NDF, CP, DM, OM, and starch, respectively. Results indicate that increasing C18:3 may not affect methane production or digestibility of the diet in lactating dairy cows.

Key Words: linolenic acid, methane, milk

INTRODUCTION

The Innovation Center for the U.S. Dairy (2009) set a goal for the U.S dairy industry to lower the total greenhouse gas production by 25 % by 2020. Methane (**CH_4**) production in lactating dairy cattle contributes to greenhouse gas emissions as they produce approximately 500–600 L/d (Beauchemin et al., 2008; Chase, 2014). Methane is of major interest because its effect on global warming is approximately 21 to 25 times more potent than carbon dioxide (**CO_2**). One strategy believed to reduce CH_4 production in cattle is to add supplemental fat to the diet (Knapp et al., 2014). In support of this, research has demonstrated that the inclusion of fat reduced CH_4 production without adversely affecting milk production or milk components (Beauchemin et al., 2009). Although the reason for this effect has not been clearly determined, it has been suggested that this fat was toxic to CH_4 producing rumen microbes, or the oil provided an alternative hydrogen sink and rather than producing CH_4, rumen microbes acted to hydrogenate fatty acids (Nagaraja et al., 1997).

It is likely that the extent to which fat reduces CH_4 production is affected by the amount of fat included and perhaps even the fatty acid profile of those fats (Martin et al., 2010). One concern of this strategy is that fat supplementation may also inhibit digestibility, the reduced CH_4 production may actually be because of reduced rumen fermentation (Knapp et al., 2014). Indeed research has demonstrated that increasing dietary fat may decrease NDF digestibility (Huhtanen et al., 2009). Similarly when feeding Angus beef heifers, Beauchemin et al. (2007) supplemented three different sources of fat; namely tallow, sunflower seeds, and sunflower oil and observed decreased digestibility with tallow and sunflower seeds, but no effect on digestibility with

sunflower oil was observed. Tallow is comprised of 40 % palmitic acid and 42 % stearic acid as a percentage of total fatty acids (FA; NRC, 2001). Sunflower oil, however, is a fat source rich in oleic (45 % of total FA) and linoleic acid (40% of total FA); and reduced CH_4 without negatively affecting digestibility. Thus, the increase in poly-unsaturated FA may suggest that there is an increasing extent of biohydrogenation and CH_4 production can be reduced. Thus, feeding more linolenic acid may provide a greater hydrogen sink compared with sources of linoleic acid or oleic acid and less CH_4 would be produced. Diets rich in poly-unsaturated FA have reduced CH_4 by 4.8 % per unit of increased dietary fat (Martin et al., 2010). Extruded flaxseed contains approximately 53 % linolenic acid as a percentage of total FA profile (NRC, 2001) and may prove beneficial when aiming to reduce enteric CH_4 production (Martin et al., 2010). In support of this, Benchaar et al. (2015) supplemented flaxseed oil to lactating dairy cows and observed a 26 % reduction in CH_4 production. When flaxseed oil was supplemented to diets containing corn silage, they observed a 15 % reduction in NDF digestibility and a 3 % increase in CP digestibility. However, treatment diets containing flaxseed also had a greater concentration of fat, thus the test did not control for effects of individual fatty acids. Consequently, it is not known if the effect of flaxseed was a result of greater biohydrogenation, because of the greater amounts of linolenic acid consumed, or simply a suppressive effect of fat on digestibility and rumen microbes. Research is needed to compare dietary sources of fat, differing in concentrations of linolenic acid. Research is also needed to determine the effects of these sources of fat on CH_4 production and diet digestibility. Therefore, our objective was to determine the effects of increasing the concentration of linolenic acid in diets fed to lactating dairy cattle. It was hypothesized

that increased supplementation of linolenic acid would reduce enteric CH_4 production in lactating dairy cows without affecting milk production, milk composition, and diet digestibility.

MATERIALS AND METHODS

All animal care and experimental procedures were approved by the University of Nebraska – Lincoln Animal Care and Use Committee. Eight multiparous lactating Jersey cows (325 ± 17 DIM; mean ± SD) with a BW averaging 485.5 ± 19.6 kg were used. All cows were housed in a temperature-controlled barn at the Dairy Metabolism Facility at the Animal Science Complex at the University of Nebraska – Lincoln (Lincoln, NE) and milked at 0700 and 1800 hr in individual tie stalls equipped with rubber mats.

The experimental design was a crossover design. Cows were randomly assigned to 1 of 2 dietary treatments: a conventional corn/soybean meal/canola meal with tallow diet (**CON**), or 2) a conventional corn/soybean meal diet with an extruded byproduct-containing flaxseed (**EXF**) as the fat source. Treatments alternated over 2 experimental periods and measurements were collected on each animal consuming each treatment. The study was conducted with a total of 2 experimental periods, each being 35-d in duration. Each period included 28-d for ab libitum diet adaptation, targeting about 5 % refusals during that time, followed by 4-d of collection with of 95 % ad libitum feeding to reduce the amount of refusals.

The diets contained similar CP and fat concentrations but differed in fatty acid profile (Table 4.1). The fatty acid profile was altered in the EXF diet by replacing porcine

tallow completely and partially replacing canola meal with 10.5 % extruded byproduct-containing flaxseed to provide 188.8 g/d of linolenic acid vs. the 21.1 g/d linolenic acid in the control treatment (Table 4.2). Proportion of forage remained constant among all diets with only the concentrate different in composition. The Cornell-Penn-Miner Dairy model (Boston et al., 2000) was used to balance diets. The study was conducted over 3 mo and forages did not change to reduce variability. All dietary treatments contained corn silage, alfalfa hay, brome hay, and a concentrate mixture that was combined as a total mixed ration (**TMR**). The TMR was mixed in a Calan Data Ranger (American Calan, Inc, Northwood, NH) and fed to the cows once daily at 1000 hr.

Laboratory Analysis

Individual feed ingredients were sampled (500 g) on the first day of each collection period and frozen at -20°C. A subsample was sent to Cumberland Valley Analytical Services Inc. (Hagerstown, MD) for complete nutrient analysis of DM (AOAC International, 2000), N (Leco FP-528 N Combustion Analyzer, Leco Corp., St. Joseph, MO), NDF with sodium sulfite (Van Soest et al., 1991), ADF (method 973.18; AOAC International 2000), lignin (Goering and Van Soest, 1970), NFC (100 – (% NDF + % CP + % Fat + % Ash)), sugar (DuBois et al., 1956), starch (Hall, 2009), crude fat (2003.05; AOAC International 2006), ash (943.05; AOAC International 2000), and minerals (985.01; AOAC International 2000). Total mixed rations were sampled (500 g) on each d of each collection period and were frozen at -20°C. The samples were then composited by period and treatment. A subsample was sent to Cumberland Valley Analytical Services Inc. (Hagerstown, MD) for complete nutrient analysis with the same lab processes as the individual feed ingredients. Particle size of the TMR was determined

size according to Heinrichs and Kononoff (2002) using the Penn State Particle Separator. Each day of the collection period, refusals were sampled and frozen at -20°C. The samples were composited by period and individual cow. A subsample was sent to Cumberland Valley Analytical Services Inc. (Hagerstown, MD) for nutrient analysis of DM, N, NDF with sodium sulfite, starch, and ash, using previously referenced methods.

Total fecal output was collected from each individual cow during the collection period for 4 consecutive d. A 137 × 76 cm rubber mat (Snake River Supply, Idaho Falls, ID) was placed behind the cow to collect feces. The feces were deposited multiple times a day from the rubber mats into a large garbage container (Rubbermaid, Wooster, OH) with a black garbage bag covering the top to reduce nitrogen losses prior to subsampling. The feces were subsampled (4 % wet basis) every day for 4 consecutive days and dried at 60°C in a forced-air oven for 48 hours and then composited by cow and period prior to being ground to pass through a 1 mm screen (Wiley mill, Arthur H. Thomas Co., Philadelphia, PA). The ground feces samples were sent to Cumberland Valley Analytical Services Inc. (Hagerstown, MD) for nutrient analysis of DM, N, NDF with sodium sulfide, starch, and ash, using previously referenced methods.

Milk production was measured daily and milk samples were collected during both the AM and PM milking times for 4 consecutive days or d 29 to 32 of the entire period. Two tubes were collected each milking (100 mL); one 50-mL conical tubes was frozen at -20°C and one tube was sent off to DHIA preserved using 2-bromo-2-nitropropane-1,3 diol. Milk samples were sent to Heart of America DHIA (Kansas City, MO) and were analyzed for fat, protein, lactose, SNF, MUN and SCC using a Bentley FTS/FCM Infrared Analyzer (Bentley Instruments, Chaska, MN). To determine the DM content of

individual feed ingredients, TMRs, refusals, and feces samples were dried at 60°C in a forced-air oven for 48 hr and then composited by treatment or cow and period. Feed ingredients, refusals and feces were ground as previously described with the feces and for laboratory corrected DM.

Heat production was determined through the headbox-style indirect calorimeters described by Foth et al. (2015) and Freetly et al. (2006) that were built at the University of Nebraska - Lincoln. For each cow, a collection period of 2 consecutive 23-hr intervals measured O_2 consumption, and CO_2 and CH_4 production. The design of the headboxes allowed for feed to be placed in the bottom of the box and ad libitum access to water was available for the cows from a water bowl placed inside the headbox. Within the headbox, temperature and dew point were recorded every minute for a 23-hr interval using a probe (Model TRH-100, Pace Scientific Inc., Moorseville, NC) that was connected to a data logger (Model XR440, Pace Scientific Inc., Moorseville, NC). Fifteen min before the start of the collection, the doors were closed and the motor was turned on, to allow for several air turnovers before gases were collected. Line pressure was measured using a manometer (Item # 1221-8, United Instruments, Westbury, NY). Barometric pressure of the room was also recorded using a barometer (Chaney Instruments Co., Lake Geneva, WI) and uncorrected for sea level. Total volume of gas that passed through the headbox during each run was measured using a dry gas meter (Model AL425, American Meter, Horsham, PA). From the headbox, continuous amounts of outgoing and incoming air were diverted to 2 different collection bags (61 × 61 cm LAM-JAPCON-NSE, 44-L; PMC, Oak Park, IL) using glass tube rotameters (Model 1350E Sho-Rate “50”, Brooks Instruments, Hatfield, PA). Collection bags with gas samples inside were analyzed

(Emerson X-stream 3-channel analyzer, Solon, OH) at the U.S. Meat Animal Research Center (MARC) according to Nienaber and Maddy (1985). Measurements collected from the two d were averaged to obtain one combined value. Heat production was estimated through calculation of O_2 consumption, and CO_2 and CH_4 production without correction for urinary N loss according to Nienaber and Maddy (1985; Equation 1). The gaseous products were reported in liters, respiratory quotient was calculated using the ratio of CO_2 produced to the oxygen (**O_2**) consumed and was not corrected for nitrogen.

$$HP\ (Mcal/d) = (16.18 \times O_2\ L + 5.02 \times CO_2\ L - 2.17 \times CH_4\ L)/4.183 \quad [1]$$

Statistical Analysis

Data were analyzed using the mixed procedure of SAS (SAS Institute Inc., Cary, NC). Treatment and period were modeled as fixed effects while cow was modeled as a random effect. The LSMEANS option was used to generate least-squares means of treatments listed in this study. Significance was declared at $P \leq 0.05$ and trends at $0.05 < P \leq 0.10$.

RESULTS AND DISCUSSION

Diet Composition

When unsaturated FA are fed to cattle, biohydrogenation in the rumen occurs at a high rate with increased disappearance of fatty acids with more unsaturated feeds (Beam et al., 2000). In an in vitro study, Beam et al. (2000) observed the losses of unsaturated FA to occur at 78, 83, and 94 % of their intake for oleic, linoleic, and linolenic acid respectively. Therefore, feeding diets high in linolenic acid should utilize more hydrogen during biohydrogenation than other fats that contain saturated FA, which should reduce

CH_4 production. The ingredient composition of diets was manipulated to increase the concentration of linolenic acid and is presented in Table 4.1. In the control treatment, tallow was included at 1.78 % DM, and canola meal was included at 9.17 % DM. In the extruded byproduct-containing flaxseed treatment, EXF was included at 10.5 % DM, replacing all tallow and partially replacing canola meal and ground corn. Soybean meal was also increased in the EXF diet, so dietary CP was similar for both treatments. All other ingredients were formulated to remain constant for inclusion rates. Chemical composition for feed ingredients and TMR's are presented in Tables 4.2 and 4.3. By design, chemical composition was similar between diets except for fatty acid profile. The EXF was formulated to contain 188.8 g/d of linolenic acid compared to the 21.2 g/d for the control. Crude protein content was 18.2 ± 1.11 % (DM basis). Crude fat was 4.68 ± 0.51 % (DM basis).

Dry Matter Intake, Milk Production and Composition

No difference in dry matter intake was expected between treatments, as diet composition was very similar by design. Dry matter intake was not different ($P = 0.262$) between treatments and averaged 15.4 ± 0.71 kg/d (Table 4.5). Similarly, in lactating dairy cows, Martin et al. (2016) observed no difference in DMI with extruded flaxseed. Similar DMI among treatments may be the result of similar concentrations of crude fat in the diet. However, Martin et al. (2008) observed decreased DMI with extruded flaxseed supplementation in lactating dairy cattle. In studies where fat concentration increased compared to a control, DMI is usually reduced when using flaxseed, which likely is the result of improved efficiency of production and energy concentration while feeding fat and not specific fatty acids (Rabiee et al., 2012). Overall, the absence of a treatment

effect on DMI in the current study was expected, as crude fat was similar among treatments.

Similar to DMI, milk yield responses to feeding flaxseed meal have been highly variable. For example, Martin et al. (2016) observed no difference in milk yield with extruded flaxseed meal, whereas; Resende et al. (2015) observed a linear decrease in milk yield with increasing supplementation of flaxseed. With an increased supply of fat (particularly long-chain FA) in the rumen, there is a greater potential to affect the microbial community (Beauchemin et al., 2007). In the current study, milk yield was not different ($P = 0.375$) averaging 17.3 ± 1.04 kg/d. Similarly, Ambrose et al. (2006) fed lactating dairy cows rolled flaxseed and observed no difference in milk yield. In the current study, NE_L was formulated to be similar between treatments, which may have caused the similar milk yields. Typically, inclusion of flaxseed meal has been associated with decreased milk fat and subsequently milk fat depression. Poly-unsaturated FA are rapidly hydrogenated in the rumen but often times there is incomplete biohydrogenation (NRC, 2001). When biohydrogenation is incomplete, intermediates such as CLA isomers are produced, which pass through the rumen and eventually get absorbed into the bloodstream and subsequently the mammary gland (NRC, 2001). In the mammary gland, CLA isomers may inhibit milk fat synthesis, which then results in milk fat depression (Bauman et al., 2008; Benchaar et al., 2015). Beam et al. (2000) observed that fats containing greater amounts of unsaturated FA have an increased rate of biohydrogenation, which may increase the risk of milk fat depression. However, in the current study, neither milk fat percent nor yield were different ($P = 0.864$ and $P = 0.512$) averaging 5.88 ± 0.25 % and 1.02 ± 0.09 kg/d for milk fat percent and yield, respectively.

Beauchemin et al. (2009) tested the effect of including crushed flaxseed in replacement of calcium salts of long-chain FA and beet pulp, and observed no difference in milk fat production. In the current study, inclusion of linolenic acid from extruded flaxseed meal likely was not included at a great enough concentration to induce milk fat depression. Like milk fat, energy corrected milk (**ECM**) was not different ($P = 0.446$) among treatments with an average of 23.9 ± 1.84 kg/d. Milk protein percent and yield were not different ($P = 0.694$ and $P = 0.334$) by treatment averaging of 4.08 ± 0.14 % and 0.70 ± 0.05 kg/d for milk protein percentage and yield, respectively. These data are consistent with previous research in lactating dairy cattle consuming extruded flaxseed, where both Martin et al. (2008) and Beauchemin et al. (2009) observed no difference in milk protein percentage or yield. In the current study, dietary CP was high and thus the supply of metabolizable protein was not expected to limit milk protein for the late-lactation dairy cows.

Gas Consumption and Production

Oxygen consumption is dependent upon animal activity and body size (Brody, 1945). When exercise is performed, O_2 consumption increases, whereas when exercise ceases, O_2 consumption decreases (Brody, 1945). Nutritional factors may also affect O_2 consumption and CO_2 production, as elevated concentrations have been observed when consuming corn silage vs. grass silage (Livingstone et al., 2015). Dry matter intake also increases CO_2 production in cattle (Jentsch et al., 2009) as it is a byproduct of rumen fermentation. Oxygen consumption and CO_2 production were not different ($P = 0.960$ and $P = 0.959$) between treatments averaging 4137.4 ± 205.1 L/d and 4351.4 ± 200.6 L/d for O_2 and CO_2, respectively (Table 4.6). Similarly, Livingstone et al. (2015) fed lactating

dairy cows extruded flaxseed and observed no difference in O_2 consumption and CO_2 production. However, when the forage source changed from corn silage to a grass silage, total consumption of O_2 and production of CO_2 decreased with a similar decrease in DMI, which was likely the result in decreased digestibility of the grass silage vs. corn silage, or the dairy cows eating to a constant energy intake. In the current study, DMI was similar; thus, the similar O_2 consumption and CO_2 production were expected. The respiratory quotient (**RQ**) was not different ($P = 0.413$) between the control and extruded flaxseed meal with a mean of 1.06 ± 0.01, indicating that the cows were in a positive energy balance. Typically, when carbohydrates are used as the main substrate, the RQ is near 1.0 (Brody, 1945; Ketelaars and Tolkamp, 1996). However, these cattle were in late lactation and producing low yields of milk and were likely in a positive energy balance and depositing fat stores in tissue or in the milk. The RQ for fat synthesis is believed to be 1.10 (Blaxter, 1989). With fat synthesis, where the main fuel source is carbohydrate, the RQ observed is possible, however, RQ alone does not always represent the metabolism of the animal due to the large quantity of CO_2 produced in the rumen (Brody, 1945). Heat production and heat production per unit of metabolic body weight were not different ($P = 0.980$ and $P = 0.685$) averaging 21.1 ± 1.02 Mcal/d and 215.1 ± 7.79 kcal/$BW^{0.75}$ for heat production and heat production/$BW^{0.75}$, respectively.

Feeding extruded flaxseed meal has decreased CH_4 production by 38 to 70 % (Martin et al., 2008; Martin et al., 2016). However, Martin et al. (2008) increased crude fat in the diet from 2.6 to about 7.4 % of dietary dry matter. Similarly, Martin et al. (2016) increased concentration of crude fat in the treatments containing extruded flaxseed compared to the control. In the current study, crude fat was formulated for similar

inclusion with the fatty acid profile increasing in linolenic acid for the EXF treatment compared to the control, as we hypothesized that increased concentrations of linolenic acid would decrease CH_4 production. Contrary to our hypothesis, CH_4 production was not different ($P = 0.904$) between the control and extruded flaxseed meal with an average of 350.9 ± 16.4 L/d (Table 5). Likewise, Livingstone et al. (2015) observed no difference in CH_4 production with extruded flaxseed although diets containing flaxseed had a greater crude fat concentration. Additionally, in the current study CH_4 per unit of DMI and ECM were not different ($P = 0.343$ and $P = 0.303$) between the control and extruded flaxseed meal treatments averaging 23.1 ± 0.57 L/kg/d and 15.5 ± 0.68 L/kg/d for CH_4 per unit of DMI and ECM, respectively. Similarly, CH_4 per unit of DMI and NDF digestibility were not different ($P = 0.531$ and $P = 0.397$) between the control and extruded flaxseed meal with an average of 34.3 ± 1.92 L/kg and 44.4 ± 4.23 L/kg for CH_4 per unit of dry matter and NDF, respectively. The disparity between the inclusion of extruded flaxseed meal may be due to varied crude fat concentration in the diet. Martin et al. (2008) observed a decrease in CH_4 production. However, the crude fat as a percentage of dry matter was also increased (7.0 vs. 2.0 % dry matter); thus, it cannot be concluded that the observed effect was a result of increases in linolenic acid per se. In the current study, crude fat was not different between treatments. Fat supplementation usually decreases CH_4 production, and believed to be caused by one or all of three reasons: firstly, increased propionate concentration with altering microbial community; secondly, providing an alternative hydrogen sink via biohydrogenation; and thirdly, providing more fermentable dietary substrates (Hales et al., 2017). In previous research where CH_4 production was reduced with feeding extruded flaxseed meal, the rumen environment may have been altered and

biohydrogenation may have occurred as evidenced by decreased milk fat. However, CH_4 production may not have been affected by the use of hydrogens for biohydrogenation of linolenic acid in the current study.

Nutrient Digestibility

Digestibility of nutrients in diets containing flaxseed has a great deal of variation. Martin et al. (2008) replaced extruded wheat and concentrate with extruded flaxseed meal fed to lactating cattle and observed a 5 % reduction in dry matter and organic matter digestibility and a 25 % reduction in NDF digestibility, whereas starch digestibility was not different. However, Martin et al. (2016) replaced corn grain and wheat bran with extruded flaxseed meal in diets fed to lactating dairy cattle and observed no difference in dry matter, organic matter, NDF, N, and starch digestibility in hay based diets but observed a 25 % reduction in NDF digestibility and a 3 % increase in starch digestibility in corn silage-based diets. In the current study, dry matter and organic matter digestibility were not different ($P = 0.481$ and $P = 0.629$) between the control and extruded flaxseed treatments, averaging 67.5 ± 1.07 % and 69.9 ± 0.95 % for dry matter digestibility and organic matter digestibility, respectively (Table 4.7). Digestibility of CP and NDF were not different ($P = 0.388$ and $P = 0.576$) between control and extruded flaxseed meal with an average of 73.3 ± 1.07 % and 53.6 ± 2.43 % for CP and NDF, respectively. Starch digestibility was not different ($P = 0.221$) between control and extruded flaxseed meal with an average of 96.1 ± 0.64 %. Hammond et al. (2015) replaced cracked wheat with extruded flaxseed and observed similar dry matter and organic matter digestibility. However, CP digestibility increased with the inclusion of extruded flaxseed, which was not observed in this study. Poly-unsaturated FA are known to be toxic to rumen microbes

and may decrease NDF digestibility (Beauchemin et al., 2007). In addition, there is a positive association of degree of unsaturation of FA and ruminal fermentation, which would decrease digestibility with poly-unsaturated FA (NRC, 2001). With the potential negative effects of poly-unsaturated FA on fermentation, digestibility is a concern when feeding linolenic acid. However, in the current study, digestibility was not affected which may have been the result of a lower dietary inclusion of fat. Many of the studies that demonstrated biological effects with the inclusion of flaxseed did so with diets containing 6 or 7 % crude fat, as a percentage of dietary DM. However, in the current study, crude fat was less than 5 % of dietary dry matter. Although the concentration of linolenic acid increased in concentration with extruded flaxseed meal, the concentration may not have been great enough to elicit a large effect on the rumen environment.

CONCLUSIONS

The present study demonstrated that extruded flaxseed meal may be included in the diet as an alternative feed source without negative effects on lactation performace. Inclusion of extruded flaxseed meal to increase linolenic acid did not affect dry matter intake, milk yield, or milk components. Contrary to our hypothesis, methane production was not decreased when the dietary concentration of linolenic was increased. Inclusion of extruded flaxseed meal up to 10 % DM had no negative effect on digestibility in late-lactation dairy cows.

REFERENCES

Ambrose, D.J., J.P. Kastelic, R. Corbett, P.A. Pitney, H.V. Petiti, J.A. Small, and P. Zalkovic. 2006. Lower pregnancy losses in lactating dairy cows fed a diet enriched in α-Linolenic acid. J. Dairy Sci. 89:3066-3074.

AOAC International. 2000. Official Methods of Analysis. Vol. 1 and 2. 17th ed. AOAC International, Gaithersburg, MD.

AOAC International. 2006. Official Methods of Analysis. 18th ed. AOAC International, Gaithersburg, MD.

Bauman, D.E., J.W. Perfield, K.J. Harvatine, and L.H. Baumgard. 2008. Regulation of fat synthesis by conjugated linoleic acid: lactation and the ruminant model. J. Nutr. 138(2):403-409.

Beam, T.M., T.C. Jenkins, P.J. Moate, R.A. Kohn, and D.L. Palmquist. 2000. Effects of amount and source of fat on the rates of lipolysis and biohydrogenation of fatty acids in ruminal contents. J. Dairy Sci. 83:2564-2573.

Beauchemin, K.A., S.M. McGinn, and H. Petit. 2007. Methane abatement strategies for cattle: lipid supplementation of diets. Can. J. Anim. Sci. 87:431–440.

Beauchemin, K.A., M. Kreuzer, F. O'Mara, and T. A. McAllister. 2008. Nutritional management for enteric methane abatement: A review. Aust. J. Exp.Ag. 48:21-27.

Beauchemin, K.A., S.M. McGinn, C. Benchaar, and L. Holtshausen. 2009. Crushed sunflower, flax, or canola seeds in lactating dairy cow diets: Effects on methane production, rumen fermentation, and milk production. J. Dairy Sci. 92:2118-2127.

Benchaar, C., F. Hassanat, R. Martineau, and R. Gervais. 2015. Linseed oil supplementation to dairy cows fed diets based on red clover silage or corn silage: Effects on methane production, rumen fermentation, nutrient digestibility, N balance, and milk production. J. Dairy Sci. 98:7993-8008.

Blaxter, K. 1989. Energy metabolism in animals and man. Pg 42-43. Cambridge University Press, Cambridge, Great Britain.

Boston, R.C., D.G. Fox., C.J. Sniffen, R. Janczewski, R. Munsen, and W. Chalupa. 2000. The conversion of a scientific model describing dairy cow nutrition and production to an industry tool: the CPM Dairy project. Pages 361-377 in Modelling Nutrient Utilization in Farm Animals Edited by J.P. McNamara, J. France and D. Beever. Oxford: CABI Publishing.

Brody, S. 1945. Bioenergetics and Growth. Reinhold Publishing Corporation, New York, NY.

Chase, L.E. 2014. Carbon footprint and the dairy industry. Cornell Nutrition Conference Animal Science Conference Proceedings. Cornell Univ. Ithaca, NY.

DRMS. 2014. DHI Glossary. Dairy Records Management System. Raleigh, N.C.

DuBois, M., K.A. Giles, J.K. Hamiliton, P.A. Rebers, and F. Smith. 1956. Colorimetric method for determination of sugars and related substances. Anal. Chem. 28:350-356.

Freetly, H.C., J.A. Nienaber and T. Brown-Brandl. 2006. Partitioning of energy during lactation of primiparous beef cows. J. Anim. Sci. 84:2157-2162.

Foth, A.J, T. Brown-Brandl, K. J. Hanford, P. S. Miller, G. Garcia Gomez, and P. J. Kononoff. 2015. Energy content of reduced-fat dried distillers grains with solubles for lactating dairy cows. J. Dairy Sci. 98:7142–7152.

Georing, H.K. and P.J. Van Soest. 1970. Forage Fiber Analysis. USDA Agricultural Research Service. Handbook number 379. U.S. Dept. of Agriculture. Superintendent of Documents, US Government Printing Office, Washington D.C. 20402.

Hall, M.B. 2009. Analysis of starch, including maltooligosaccharides, in animal feeds: a comparison of methods and a recommended method for AOAC collaborative study. JAOACI 92:42-49.

Hammond, K.J., D.J. Humphries, L.A. Crompton, P. Kirton, and C.K. Reynolds. 2015. Effects of forage source and extruded linseed supplementation on methane emissions from growing dairy cattle of differing body weights. J. Dairy Sci. 98:8066-8077.

Heinrichs, A.J., and P.J. Kononoff. 2002. Evaluating particle size of forages and TMRs using the New Penn State Forage Particle Separator. Tech. Bul. DAS 02-42. Pennsylvania State Univ., College Agric. Sci., Cooperative Ext., University Park, PA.

Huhtanen, P., M. Rinne, and J. Nousiainen. 2009. A meta-analysis of feed digestion in dairy cows. 2. The effects of feeding level and diet composition on digestibility. J. Dairy Sci. 92:5031-5042.

Innovation Center for U.S. Dairy. 2009. Dairy Industry Applauds White House Strategy for Methane Emissions Reduction. News Release, March 38, 2014. http://www.nmpf.org/files/White-House-Methane-Emissions-Reduction-032814.pdf

Jentsch, W., B. Piatkowski, and M Derno. 2009. Relationship between carbon dioxide production and performance in cattle and pigs. Arch. Tierzucht 52:485-496.

Johnson, K.A., and D.E. Johnson. 1995. Methane emissions from cattle. J. Anim. Sci. 73:2483-2492.

Ketelaars, J.J., and B.J. Tolkamp. 1996. Oxygen efficiency and the control of energy flow in animals and humans. J. Anim. Sci. 74:3036-3051.

Knapp, J.R., G.L. Laur, P.A. Vadas, W.P. Weiss, and J.M. Tricarico. 2014. Invited review: enteric methane in dairy cattle production: Quantifying the opportunities and impact of reducing emissions. J. Dairy Sci. 97:3221-3261.

Livingstone, K.M., D.J. Humphries, P. Kirton, K.E. Kliem, D.I. Givens, and C.K. Reynolds. 2015. Effects of forage type and extruded linseed supplementation on methane production and milk fatty acid composition of lactating dairy cows. J. Dairy Sci. 98:1-12.

Lock, A.L., and D.E. Bauman. 2004. Modifying milk fat composition of dairy cows to enhance fatty acids beneficial to human health. Lipids 39:1197-1206.

Martin, C., J. Rouel, J.P. Jouany, M. Doreau, and Y. Chilliard. 2008. Methane output and diet digestibility in response to feeding dairy cows crude linseed, extruded linseed, or linseed oil. J. Anim. Sci. 86:2642-2650.

Martin, C., D.P., Morgavi, and M. Doreau. 2010. Methane mitigation in ruminants: from microbe to the farm scale. Animal 4:351-365.

Martin, C., A. Ferlay, P. Mosoni, Y. Rochette, Y. Chilliard, and M. Doreau. 2016. Increasing linseed supply in dairy cow diets based on hay or corn silage: Effect on enteric methane emission, rumen microbial fermentation, and digestion. J. Dairy Sci. 99:3445-3456.

Nagaraja, T.G., C.J. Newbold, C.J. Van NEvel, and D.I Demeyer. 1997. Manipulation of ruminal fermentation. Pages 523-632 in The Rumen Microbial Ecosytem. : P.N. Hobson and C.S. Stewart, editors. Chapman and Hall, London, UK.

National Research Council. 2001. Nutrient Requirements of Dairy Cattle. 7th rev. ed. Natl. Acad. Press, Washington, D.C.

Nienaber, J.A., and A.L. Maddy. 1985. Temperature controlled multiple chamber indirect calorimeter-design and operation. Trans. ASAE. 28:555-560.

Rabiee, A.R., K. Breinhild, W. Scott, H.M. Golder, E. Block, and I.J. Lean. 2012. Effect of fat additions to diets of dairy cattle on milk production and components: A meta-analysis and meta-regression. J. Dairy Sci 95:3225-3247.

Resende, T.L., J. Kraft, K.J. Soder, A.B.D. Pereira, D.E. Woitschach, R.B. Reis, and A.F. Brito. 2015. Incremental amounts of ground flaxseed decrease milk yield but increase n-3 fatty acids and conjugated linoleic acids in dairy cows fed high-forage diets. J. Dairy Sci. 98:4785-4799.

Van Soest, P.J., J.B. Robertson, and B.A. Lewis. 1991. Methods for dietary fiber, neutral detergent fiber, and nonstarch polysaccharides in relation to animal nutrition. J. Dairy Sci. 74:3583-3597.

Wildman, E.E. G.M. Jones, P.E. Wagner, R.L. Boman, H.F. Troutt and T.N Lesch. 1982. A dairy cow body condition scoring system and its relationship to selected production characteristics. J. Dairy Sci. 65:495-501.

Table 4. 1. Diet composition of control (CON) and extruded byproduct containing flaxseed (EXF) treatments fed to lactating Jersey cows in late lactation averaging 325 ± 17 days in milk.

	% of DM	
Item	CON	EXF
Corn silage	27.5	27.5
Alfalfa hay	21.0	21.0
Brome hay	1.57	1.57
Ground corn	20.2	17.3
Soybean meal	5.53	6.28
Extruded byproduct containing flaxseed[1]	0.00	10.5
Canola meal	9.17	2.62
Bypass soy[2]	5.24	5.24
Ground soybean hulls	5.24	5.24
Tallow (porcine)	1.78	0.00
Calcium carbonate	0.81	0.81
Sodium bicarbonate	0.67	0.67
Ca-salts of LCFA[3]	0.59	0.59
Bloodmeal	0.26	0.26
Magnesium oxide	0.26	0.26
Salt	0.20	0.20
Vitamin premix[4]	0.04	0.04
Trace mineral premix[5]	0.04	0.04
ME, Mcal/kg[5]	2.69	2.68
NE_L, Mcal/kg[5]	1.74	1.73

[1]Contained about 48% flaxseed, 46% ground peas, 5% alfalfa pellets, 0.1% vitamin E, 0.2% mold inhibitor, and 0.04% ethoxyquin marketed as Linpro-R by O & T farms Regina, SK, Canada.
[2]Soypass, LignoTech, Overland Park, KS.
[3]Calcium salts of long-chain fatty acids marketed as Megalac by Church & Dwight Co. Inc. Princeton, NJ.
[4] Formulated to supply approximately 2,300 mg/kg Co, 25,000 mg/kg Cu, 2,600 mg/kg I, 1,000 mg/kg Fe, 150,000 mg/kg Mn, 820 mg/kg Se and 180,000 mg/kg Zn in total rations.
[5]Values formulated from Cornell-Penn-Miner dairy model (Boston et al., 2000).

Table 4. 2. Chemical composition of diets for control (CON), and extruded byproduct containing flaxseed (EXF) (as-fed basis)[1].

	CON		EXF	
Chemical composition	Mean	SD	Mean	SD
DM, %	62.1	0.21	61.9	0.92
CP, % of DM	18.2	1.72	18.2	0.87
Soluble Protein, % of DM	5.90	0.42	5.63	0.96
ADICP[2], % of DM	1.13	0.19	0.96	0.01
NDICP[2], % of DM	2.39	0.14	2.22	0.11
ADF, % of DM	21.3	1.35	21.8	1.92
NDF, % of DM	32.8	0.20	33.3	2.27
Lignin, % of DM	4.16	0.78	4.18	0.97
NFC, % of DM	39.0	2.44	38.0	0.78
Starch, % of DM	23.5	0.23	23.4	0.69
Sugar, % of DM	3.81	0.39	3.7	0.64
Crude fat, % of DM	4.50	0.63	4.87	0.50
Ash, % of DM	7.92	0.04	7.92	0.03
Ca, % of DM	1.07	0.08	0.89	0.05
P, % of DM	0.42	0.02	0.38	0.02
Mg, % of DM	0.37	0.00	0.33	0.03
K, % of DM	1.60	0.05	1.63	0.06
S, % of DM	0.26	0.01	0.23	0.00
Na, % of DM	0.31	0.02	0.22	0.05
Cl, % of DM	0.20	0.00	0.18	0.05
Fe, mg/kg	249.4	3.86	334.6	84.2
Zn, mg/kg	109.4	5.29	104.9	57.6
Cu, mg/kg	17.9	2.17	16.9	5.00
Mn, mg/kg	82.6	6.98	67.1	8.57
Fatty Acids g/d				
C18:3, intake[2]	21.1	2.25	188.8	31.6
C18:3, % of diet dry matter[2]	0.14	0.02	1.20	0.20
C18:3 Duodenal Flow[3]	2.18		29.8	

[1]Values determined by Cumberland Valley Analytical Services, Hagerstown, MD.
[2]Values determined by Penn State University, University Park, PA.
[3]Values formulated from Cornell-Penn-Miner dairy model (Boston et al., 2000).

Table 4. 3. Chemical composition of alfalfa hay, corn silage, brome hay, control concentrate (CON), and extruded byproduct containing flaxseed (EXF) concentrate used to make the TMR fed to lactating Jersey cows in late lactation averaging 325 ± 17 days in milk.

	Alfalfa		Corn Silage		Brome Hay		CON		EXF	
Chemical composition	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
DM, %	86.8	1.20	32.8	2.40	87.1	1.56	88.9	0.28	89.5	0.64
CP, % of DM	19.6	2.83	7.60	0.42	9.15	0.49	23.8	2.47	23.7	0.78
Soluble Protein, % of DM	12.0	7.28	4.05	0.35	2.35	0.35	4.50	3.68	3.95	0.92
ADICP[2], % of DM	1.81	0.08	0.79	0.05	1.10	0.01	1.04	0.33	0.70	0.05
NDICP[2], % of DM	2.86	0.24	0.79	0.06	3.78	0.23	3.03	0.15	2.68	0.35
ADF, % of DM	35.9	3.11	26.2	0.49	41.0	0.78	11.8	1.70	12.8	2.83
NDF, % of DM	44.7	2.12	40.4	0.42	65.5	0.85	22.5	1.56	23.5	3.39
Lignin, % of DM	7.94	0.49	3.75	0.16	5.69	0.00	2.75	1.44	2.80	1.82
NFC, % of DM	25.9	1.41	43.7	0.21	16.3	0.35	42.7	4.38	40.7	2.05
Starch, % of DM	1.35	0.07	32.4	0.00	0.85	0.07	28.5	0.42	28.4	1.34
Sugar, % of DM	4.80	0.14	0.55	0.21	6.55	0.78	5.10	0.71	4.95	1.20
Crude fat, % of DM	1.87	0.60	3.69	0.78	2.46	0.30	6.11	0.57	6.86	0.30
Ash, % of DM	10.9	0.37	5.43	0.05	10.2	0.59	8.00	0.06	7.99	0.08
Ca, % of DM	1.28	0.11	0.19	0.01	0.46	0.05	1.49	0.20	1.13	0.15
P, % of DM	0.38	0.01	0.23	0.01	0.28	0.01	0.54	0.03	0.48	0.02
Mg, % of DM	0.26	0.02	0.13	0.01	0.14	0.01	0.56	0.00	0.47	0.07
K, % of DM	3.46	0.08	0.95	0.11	2.03	0.05	1.17	0.01	1.23	0.02
S, % of DM	0.24	0.04	0.13	0.00	0.19	0.02	0.34	0.01	0.28	0.01
Na, % of DM	0.03	0.00	0.02	0.01	0.02	0.00	0.59	0.03	0.41	0.10
Cl, % of DM	0.11	0.01	0.08	0.01	0.27	0.03	0.30	0.02	0.26	0.11
Fe, mg/kg	291.0	69.3	164.5	19.1	188.5	51.6	280.5	9.19	451.0	185.3
Zn, mg/kg	25.5	0.71	21.0	4.24	20.5	2.12	196.0	8.49	187.0	113.1
Cu, mg/kg	8.50	0.71	5.50	0.71	7.00	0.00	29.0	4.24	27.0	9.90
Mn, mg/kg	41.5	4.95	32.5	7.78	47.0	2.83	128.5	16.3	97.5	14.8

[1]Values determined by Cumberland Valley Analytical Services, Hagerstown, MD.
[2]Acid detergent insoluble crude protein.
[3]Neutral detergent insoluble crude protein.
[4]NFC = Nonfiber carbohydrate calculated by difference 100-(% NDF + % CP + % Fat + % Ash).

Table 4. 4. Particle size distribution of control (CON), and extruded byproduct containing flaxseed (EXF) diets (as-fed basis)[1].

	CON		EXF	
	Mean	SD	Mean	SD
> 19.0 mm	3.50	0.58	4.00	0.82
19.0 - 8.0 mm	20.5	4.36	20.5	4.44
8.0 - 1.18 mm	52.0	2.16	51.5	2.65
< 1.18 mm	24.0	2.94	23.5	3.51

[1]Determined using the Penn State Particle Separator on wet basis (Heinrichs and Kononoff, 2002).

Table 4. 5. DMI, milk yield and composition, BW and BCS[1] of treatments, which included control (CON) or extruded byproduct containing flaxseed (EXF) fed to lactating Jersey cows in late lactation averaging 325 ± 17 days in milk.

	Treatments			
Item	CON	EXF	SEM[2]	*P*-value
DMI, kg/d	15.0	15.7	0.71	0.262
Milk yield, kg/d	16.8	17.8	1.04	0.375
ECM[3]	23.2	24.6	1.84	0.446
Feed conversion	1.52	1.57	0.08	0.550
Fat, %	5.89	5.86	0.25	0.864
Fat yield, kg/d	0.99	1.04	0.09	0.512
Protein, %	4.09	4.07	0.14	0.694
Protein yield, kg/d	0.68	0.72	0.05	0.334
Lactose, %	4.68	4.72	0.04	0.381
MUN, mg/dl[4]	20.0	19.5	1.00	0.575
Water intake, L/d	73.4	72.1	4.50	0.770
Body weight, kg	484.5	486.5	19.6	0.615
BCS[1]	3.78	3.78	0.07	1.000

[1]BCS = Body condition score 1-5 scale according to Wildman et al. (1982).
[2]Lowest standard error of treatment means is listed.
[3]Energy corrected milk = 0.327 × milk yield [kg] + 7.2 × protein [kg] adjusted for 3.5% fat and 3.2% total protein (DHI Glossary, 2014).
[4]MUN = Milk urea nitrogen.

Table 4. 6. Methane production, and heat production of treatments, which included control (CON) or extruded byproduct containing flaxseed (EXF) fed to lactating Jersey cows in late lactation averaging 325 ± 17 days in milk.

	Treatments			
Item	CON	EXF	SEM[1]	*P*-value
O_2 consumption, L/d	4143.0	4131.7	205.1	0.960
CO_2 production, L/d	4345.5	4357.3	200.6	0.959
CH_4 production, L/d	352.0	349.8	16.4	0.904
RQ[3], L/L	1.05	1.06	0.01	0.413
CH_4/DMI, L/kg/d	23.8	22.4	0.57	0.343
CH_4/milk produced, L/kg/d	22.7	19.8	0.95	0.300
CH_4/ECM, L/kg/D	16.5	14.5	0.68	0.303
CH_4/ DMD, L/kg	35.0	33.5	1.92	0.531
CH_4/ NDF digestibility, L/kg	46.8	41.9	4.23	0.397
Heat production[2], Mcal/d	21.1	21.0	1.02	0.980
Heat production[4], kcal/$MB^{0.75}$	213.1	217.1	7.79	0.685

[1]Lowest standard error of treatment means is listed.
[2]Heat production calculated with Nienaber and Maddy's (1985) equation from O_2 consumption (L), CO_2 production (L), CH_4 production (L), (heat production (Mcal/d) = (16.18 × O_2 L + 5.02 × CO_2 L – 2.17 × CH_4 L)/4.183).
[3]RQ (Respiratory quotient) = CO_2 produced/O_2 consumed.
[4]Heat production, kcal/$MB^{0.75}$ = heat production per unit of metabolic body weight.

Table 4. 7. Apparent digestibility of treatments, which included control (CON), and extruded byproduct-containing flaxseed (EXF) fed to lactating Jersey cows in late lactation averaging 325 ± 17 days in milk.

	Treatments			
Component	CON	EXF	SEM[1]	*P*-value
DM, %	68.0	66.9	1.07	0.481
OM, %	70.2	69.6	0.95	0.629
CP, %	74.0	72.6	1.07	0.388
NDF, %	52.6	54.6	2.43	0.576
Starch, %	96.7	95.4	0.64	0.221
Ash, %	41.7	37.2	3.83	0.444

[1]Lowest standard error of treatment means is listed.

CHAPTER 5

Maintenance energy use and diurnal variation in methane production in late-lactation Jersey cows[1]

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ABSTRACT

Methane production typically increases with increased dry matter intake. However, few studies, have observed the effects of feeding multiple times a day and its effects on diurnal variation in methane production and energy balance in late-lactation dairy cattle. A study using headbox-style indirect calorimetry and 12 multiparous (225 ± 16.2 DIM) (mean ± SD) lactating Jersey cows was conducted to determine the effects of feeding twice daily on diurnal variation in methane production and total energy balance. A crossover design with 14-d periods (10 d adaption and 4 d collections) was used to compare two treatments. Treatments consisted of either once a day feeding (1X), or twice a day feeding (2X) with a common diet fed to both treatments. Dry matter intake was not different between treatments with a mean of 17.3 ± 1.00 kg/d. Once a day feeding tended to have greater milk yield compared to twice a day feeding (21.2 vs. 20.4 ± 1.59 kg/d, respectively). Milk fat and milk protein percent were not different with means of 6.18 ± 0.20 % and 3.98 ± 0.08 % for milk fat and milk protein, respectively. Total methane production did not differ between treatments with a mean of 402.1 ± 20.8 L/d. Similarly, methane per unit of milk yield and DMI were not different between treatments with means of 20.5 ± 1.81 and 23.8 ± 1.21 L/kg/d for milk yield and DMI, respectively. Feeding frequency did not affect diurnal variation of methane production per hr, with a mean of 17.1 ± 0.74 L/hr. A trend was observed for a treatment × hr interaction. Methane production per hr increased after the second feeding for cattle fed twice versus once daily. Gross energy, digestible energy, metabolizable energy, and net energy of lactation per kg of DMI did not differ by feeding frequency, with means of 4.41 ± 0.01, 3.05 ± 0.03, 2.63 ± 0.03, and 1.32 ± 0.08 Mcal/kg of DMI, respectively. Maintenance energy

requirement was 145 kcal per kg of metabolic body weight with an efficiency of converting ME to lactation energy of 76 %. Nitrogen balance did not differ among treatments with a mean balance of 17.3 ± 13.0 g/d. Therefore, methane production is variable throughout the day and caution should be exercised when collecting methane samples at a limited number of time points, as this may under- or overestimate total production.

Key words: dairy cow, diurnal variation, energy, methane

INTRODUCTION

Accurate measurement of methane (**CH_4**) production is needed for energy-balance studies to correctly partition where energy is being used in the animal. Collecting accurate measures of gas production by livestock is a laborious task. In cattle, the release of CH_4 from the rumen is episodic, which may contribute to the challenge of accurate collection (Hegarty, 2013). It is well established that CH_4 production can be altered by manipulation of the diet by including more fat and concentrate grains (Knapp et al., 2014). Feeding practices are also known to affect gas production. This includes time between feedings, and frequency of feedings, but gas production may also be affected by the number of meals consumed, fermentation rate, and fermentation patterns (Brask et al., 2015). Methane production is dependent on feed consumption and digestion and can vary from 0.14 to 0.51 L/min throughout the day (Crompton et al., 2011.)

In a recent study using lactating dairy cows, Brask et al. (2015) described two peaks of CH_4 production over a 24-h period. The first, a minor peak, occurred after morning feeding, while the second, a major peak, occurred after the evening feeding. Similarly, Hollmann et al. (2013) described small peak of CH_4 following the morning feeding followed by a major peak following the afternoon feeding. Other research has described the greater peak for CH_4 production to occur after the morning feeding whereas, after the second feeding, the peak is lower in lactating dairy cattle (Crompton et al., 2011). The increased CH_4 production occurred approximately 120 and 60 minutes post-feeding for morning and afternoon feedings, respectively. In a study using sheep restricted to one meal per day, Van Zijderveld et al. (2010) found a single peak after that feeding. Sheep were fed at 0800 h and the peak CH_4 production occurred around 4 to 6 h

post-feeding, after which it gradually declined until feeding time the next day. Interestingly, peak CH_4 production was reduced in sheep supplemented with nitrate and sheep supplemented with sulfate produced less CH_4, but the observed response was delayed until 10 h post-feeding. In beef cattle, Hales et al. (2017) fed beef steers once daily and observed peak CH_4 production around 4 to 6 hr post-feeding. Therefore, diet type may alter the time to peak CH_4 production.

Over the last several decades, the maintenance energy requirement for dairy cattle has increased (Moraes et al., 2015). Reported estimates of maintenance requirements have ranged from 110 to 208 kcal of Metabolizable Energy/$BW^{0.75}$ (Flatt et al., 1967; Foth et al., 2015). The majority of the studies have used lactating dairy cows at or near peak milk production. The estimation of maintenance in high-producing dairy cows is challenging because they may be in negative energy balance, and as a result, be utilizing body energy stores. Lactation demands a tremendous amount of energy and alters normal function to compensate (Bauman and Currie, 1980). Hence, lactating dairy cattle in a negative energy balance may have an altered metabolic state (Fenwick et al., 2008) and cows may have a greater maintenance energy requirement early in lactation. To our knowledge, little research has measured energy balance in late-lactation dairy cows. Dairy cattle in late lactation are an ideal model for calculating maintenance requirements. Typically, these cattle are past peak milk and no longer in a negative energy balance. During early lactation and negative energy balance, a homeorhetic mechanism controls nutrient partitioning that usually results in body tissues being mobilized to compensate for lack of energy (Bauman and Currie, 1980). This usually results in cattle becoming more efficient and true maintenance may be affected. However, in late lactation, energy

available from DMI meets energy maintenance requirements and might be an ideal model as long as excess body stores are not being accumulated. However, little research is available on maintenance requirements of dairy cattle in late lactation. The objectives of this study were to characterize diurnal CH_4 production and estimate energy maintenance in late lactation dairy cattle being fed either once or twice daily.

MATERIALS AND METHODS

All animal care and experimental procedures were approved by the University of Nebraska – Lincoln Animal Care and Use Committee. Twelve multiparous lactating Jersey cows (225 ± 16.2 DIM; mean ± SD) with a BW averaging 480 ± 12.2 kg/d were used. At the end of the experiment, no cattle were more than 90 d pregnant. All cows were housed in a temperature-controlled barn at the Dairy Metabolism Facility at the Animal Science Complex at the University of Nebraska – Lincoln (Lincoln, NE) and milked at 0700 and 1800 hr in individual tie-stalls equipped with rubber mats.

The experimental design was a crossover design. Cows were randomly assigned to one of two treatments: a conventional one time daily feeding (**1X**), or 2) a twice daily feeding (**2X**). Treatments alternated over 2 experimental periods and measurements were collected on each animal consuming each treatment. The study was conducted with a total of 2 experimental periods each being 14 d in duration. Each period included 10 d for ab libitum treatment adaptation, targeting about 5 % refusals during that time, followed by 4 d of collection of 95 % ad libitum feeding to reduce the amount of refusals. Standing behavior was recorded at 10 min intervals during the collection period for both time standing while inside the headbox as well as outside the headbox in the tie stall, starting

at 1000 hr. Standing behavior was measured to better understand the effects of temporarily modifying the cattle's environment while in the headbox.

The same diet was fed to all cattle with the chemical composition and analysis of the diet and feed ingredients are presented in (Table 5.1 and Table 5.2). The Cornell-Penn-Miner Dairy model (Boston et al., 2000) was used to balance diets. The study was conducted over 1 mo and using the same forage source, reduced variability of the diet. All dietary treatments contained corn silage, alfalfa hay, brome hay, and a concentrate mixture that was combined as a total mixed ration (**TMR**). The TMR was mixed in a Calan Data Ranger (American Calan, Inc, Northwood, NH) and fed either once daily at 1000 hr or twice daily at 1000 hr and 2000 hr. For cattle fed twice daily, 50 % of the feed was delivered during the first feeding, and the second feeding they received the other 50 % of feed.

Laboratory Analysis

Individual feed ingredients were sampled (500 g) on the first day of each collection period and frozen at -20°C. A subsample was sent to Cumberland Valley Analytical Services Inc. (Hagerstown, MD) for complete nutrient analysis of DM (AOAC International, 2000), N (Leco FP-528 N Combustion Analyzer, Leco Corp., St. Joseph, MO), NDF with sodium sulfite (Van Soest et al., 1991), ADF (method 973.18; AOAC International 2000), lignin (Goering and Van Soest, 1970), NFC (100 – (% NDF + % CP + % Fat + % Ash)), sugar (DuBois et al., 1956), starch (Hall, 2009), crude fat (2003.05; AOAC International 2006), ash (943.05; AOAC International 2000), and minerals (985.01; AOAC International 2000). Total mixed rations were sampled (500 g) on each d of each collection period and were frozen at -20°C. The samples were then composited

by period and treatment. Particle size of the TMR was determined according to Heinrichs and Kononoff (2002) using the Penn State Particle Separator. Each day of the collection period, refusals were sampled and frozen at -20°C. The samples were analyzed at the University of Nebraska – Lincoln laboratory for nutrient analysis of DM (AOAC International, 2000), N (FlashSmart N/Protein Analyzer Ce Elantech, Inc. Lakewood, NJ), NDF with sodium sulfite (Van Soest et al., 1991), starch (Hall, 2009) and ash (943.05; AOAC International 2000).

Total fecal and urine output was collected from each individual cow during the collection period for 4 consecutive days. A 137 × 76 cm rubber mat (Snake River Supply, Idaho Falls, ID) was placed behind the cow to collect feces. The feces were deposited multiple times a day from the rubber mats into a large garbage container (Rubbermaid, Wooster, OH) with a black garbage bag covering the top to reduce nitrogen losses prior to subsampling. The feces were subsampled (4 % wet basis) every day for 4 consecutive days and dried at 60°C in a forced-air oven for 48 hr and then composited by cow and period prior to being ground to pass through a 1 mm screen (Wiley mill, Arthur H. Thomas Co., Philadelphia, PA). The ground feces samples were analyzed at the University of Nebraska – Lincoln laboratory for nutrient analysis of DM, N, NDF with sodium sulfide, starch, and ash, using previously discussed methods. Total urine was collected by inserting a 30 cc French foley catheter into each cow's bladder with a stylus (Tamura et al., 2014). The balloon was inflated to 50-mL with physiological saline and urine drained using tygon tubing into a plastic carboy (15 quart) behind the cow. Using the funnel spout of the plastic carboy, urine was deposited into a 55-L plastic container 4 times a day and was acidified with 50-mL of HCl prior to subsampling (2 % wet basis)

and freezing at -20°C every day of the collection period. Prior to analysis, urine was thawed and boiled to remove the water content. To boil the urine, 2 thawed 250-mL bottles of urine were poured into a 600-mL beaker. Fourteen urine-filled beakers were placed into a boiling water bath (Ankom Technology, Macedon, NY) underneath a hood. The water bath was turned on in the morning and off in the afternoon, for approximately 6 hr each d, to reduce the chance of the sample being overheated and burned. After water was boiled away, the remaining paste was then composited by cow and period. The urine paste was then lyophilized (VirTis Freezemobile 25ES, SP Scientific, Gardiner, NY) and analyzed. Once lyophilized, sample size was reduced using mortar and pestle and then used for analysis. Urine samples were analyzed at the University of Nebraska – Lincoln for laboratory corrected DM (100°C oven for 24 hr), N, and gross energy (GE) using a bomb calorimeter (Parr 6400 Calorimeter, Moline, IL).

Milk production was measured daily and milk samples were collected during both milking times for 4 consecutive days or d 11 to 14 of the entire period. Two tubes were collected for each milking (100-mL); one 50-mL conical tube was frozen at -20°C and one tube was sent off to DHIA, preserved using 2-bromo-2-nitropropane-1,3 diol. Samples were sent to Heart of America DHIA (Kansas City, MO) and were analyzed for fat, protein, lactose, SNF, MUN and SCC using a Bentley FTS/FCM Infrared Analyzer (Bentley Instruments, Chaska, MN). The conical tube was lyophilized and then composited by cow and period for nutrient analysis. Milk samples were analyzed at the University of Nebraska – Lincoln for lab corrected DM, N and GE. To determine the DM content of individual feed ingredients, TMRs, refusals, feces and urine samples were dried at 60°C in a forced air oven for 48 hours and then composited by treatment or cow

and period. Milk samples were lyophilized to determine DM. Feed ingredients, refusals and feces were ground as previously described with the feces and for lab corrected DM and GE.

Heat production was determined through the headbox-style indirect calorimeters described by Foth et al. (2015) and Freetly et al. (2006) that were built at the University of Nebraska - Lincoln. Prior to collections, 5 headboxes were used to test the rate of recovery of gas by burning 100% ethyl alcohol in the sealed headbox and comparing this measure to calculated gas concentrations. These calculations were based upon the weight of alcohol burned and a measured volume of gas sample. Prior to the start of the experiment, five gas recoveries were conducted to verify proper function of the system. Recovery rates of oxygen (**O_2**) and carbon dioxide (**CO_2**) averaged 101.0 ± 0.04 and 100.8 ± 0.04 % respectively. For each cow, a collection period of a single 23-hr interval measured O_2 consumption, and CO_2 and CH_4 production. The design of the headboxes allowed for feed to be placed in the bottom of the box and ad libitum access to water was available for the cows from a water bowl placed inside the headbox. Within the headbox, temperature and dew point were recorded every minute for a 23-hr interval using a probe (Model TRH-100, Pace Scientific Inc., Moorseville, NC) that was connected to a data logger (Model XR440, Pace Scientific Inc., Moorseville, NC). Fifteen min before the start of the collection, the doors were closed and the motor was turned on, to allow for several air turnovers before gases were collected. Line pressure was measured using a manometer (Item # 1221-8, United Instruments, Westbury, NY). Barometric pressure of the room was also recorded using a barometer (Chaney Instruments Co., Lake Geneva, WI) and uncorrected for sea level. Total volume of gas that passed through the headbox

during each run was measured using a dry gas meter (Model AL425, American Meter, Horsham, PA). From the headbox, continuous amounts of outgoing and incoming air were diverted to 2 different collection bags (61 × 61 cm LAM-JAPCON-NSE, 44-L; PMC, Oak Park, IL) using glass tube rotameters (Model 1350E Sho-Rate "50", Brooks Instruments, Hatfield, PA). Collection bags with gas samples inside were analyzed at the University of Nebraska – Lincoln laboratory according to Nienaber and Maddy (1985). For diurnal measurements, continuous amounts of outgoing and incoming air were pumped through a sample pump station (Universal Analyzers Inc, Carson City, NV) from the headbox to the gas analyzer and were analyzed once per hr at the University of Nebraska – Lincoln for lab according to Nienaber and Maddy (1985). Heat production was estimated through calculation of O_2 consumption, and CO_2 and CH_4 production, with correction for urinary N loss according to Brouwer (1965; Equation 1; Table 5.9). The gaseous products were reported in liters and the mass of urinary N in grams. Respiratory quotient was calculated using the ratio of CO_2 produced to the O_2 consumed and was not corrected for nitrogen. Volume of CH_4 produced was multiplied by a constant of 9.45 kcal/L to estimate the amount of energy formed from the gaseous products. Energy balance was calculated for each cow and adjusted for excess N intake according to Freetly et al. (2006) using the following equations:

$$\text{HP (Mcal/d)} = 3.866 \times O_2\,\text{L} + 1.200 \times CO_2\,\text{L} - 0.518 \times CH_4\,\text{L} - 1.431 \times \text{N g} \quad [1]$$

Metabolizable energy (**ME**) (Mcal/d) = gross energy intake Mcal/d – fecal energy Mcal/d – urinary energy Mcal/d – methane energy Mcal/d [2]

Recovered energy (**RE**) (Mcal/d) = ME – HP [3]

Tissue energy (**TE**) (Mcal/d) = RE – milk energy Mcal/d [4]

Tissue energy in protein (g/d) = (N balance g/d) × (5.88 kg of protein/kg of N) × (5.7 Mcal/kg of protein)/1000 [5]

Metabolizable energy for maintenance was found by regression of RE on ME and is the ME at zero RE as listed in Figure 1. Tissue energy in protein describes the energy used for tissue protein synthesis (Equation 5). Standing behavior was measured over 4 d encompassing 24hr periods starting on the first day of total collections by visually observing whether cattle were standing every 10-min. It was assumed that the incidence of standing lasted during the entire 10-min interval, thus total minutes of standing was a sum of each observation for the entire day.

Statistical Analysis

Production, energy metabolism, and nitrogen balance data were analyzed using the mixed procedure of SAS (SAS Institute Inc., Cary, NC). Treatment and period were modeled as fixed effects while cow was modeled as a random effect. The LSMEANS option was used to generate least-squares means of treatments listed in this study. Diurnal variation was analyzed as repeated measures by using the autoregressive repeated covariance structure in SAS (SAS Institute Inc., Cary, NC). The effects of period, treatment, hour and treatment × hour interaction were considered as fixed and cow was considered as a random effect. Standing behavior was analyzed using the mixed procedure of SAS (SAS Institute Inc., Cary, NC). Treatment, period, and location were modeled as fixed effects while cow was modeled as a random effect. Significance was declared at $P \leq 0.05$ and trends at $0.05 < P \leq 0.10$.

RESULTS AND DISCUSSION

Diet Composition

The goal of the current study was not to test the effect of dietary treatments but rather to test the effects of feeding frequency on diurnal methane production and energy use in late-lactation cattle. As such, all cows received the same diet and chemical composition of these diets and individual feed ingredients are listed in Table 5.1 and Table 5.2. Crude protein was approximately 18.5 ± 0.25 % while the concentration of gross energy was 4419.1 ± 86.9 cal/g. Particle size distribution was near recommended values (Table 5.3). Specifically, 4.81, 25.2, 50.9, and 18.9 % remained for the > 19.0 mm, 8.0 mm, 1.18 mm and pan (< 1.18 mm), respectively. General recommendations for particle distribution are 2 to 8 % remaining particles on the > 19.0 mm diameter sieve, 30 – 50 % retained on the 8.0 mm, and 1.18 mm sieves and ≤ 20 % retained on the bottom pan (Heinrichs and Kononoff, 2002).

Dry Matter Intake, Milk Production and Composition

Increasing feeding frequency in cattle may stimulate appetite and as a result, increase DMI. Campbell and Merilan (1960) fed lactating Guernsey cattle either 2 or 4 times daily and observed a 1.5 kg/d increase in daily DMI with an accompanying increase in milk yield. Similarly, Crompton et al. (2011) fed lactating Holstein-Friesian cattle either one or two times and observed a 1.2 kg increase in DMI and 1.4 kg increase in milk production. The primary aim of the current study was to test the effects of feeding frequency on CH_4 production and whole-animal energy balance, not to determine if

feeding frequency would affect DMI or milk production. In the current study, DMI did not differ (P = 0.292) by increasing feeding frequency, averaging 17.3 ± 1.00 kg/d (Table 5.4). However, there was a trend for milk yield to decrease (P = 0.097), which decreased with increasing feeding frequency (21.2 vs. 20.4 ± 1.59 kg/d for once vs. twice daily feeding, respectively). Nocek and Braund (1985) fed lactating cattle one, two, four, or eight times daily and observed no difference on DMI or milk yield. However, cattle fed multiple times a day had greater milk fat percentage, which was attributed to the stabilization of rumen pH. In the current study, milk fat percentage did not differ (P = 0.966) by feeding frequency averaging 6.18 ± 0.20 %. In the current study, milk fat yield also tended (P = 0.097) to decrease with increasing feeding frequency (1.30 vs. 1.26 ± 0.10 kg/d for once and twice daily feeding, respectively). The increased milk fat yield is likely due to increased milk yield. However, Macmillan et al. (2017) fed cattle one or three times daily and observed increased milk fat yield, which they suggested may be due to a greater mean pH, which allowed for more cellulolytic activity and acetate production. Russell (1998) observed a decreased acetate-to-propionate ratio when pH decreased in vitro. Milk protein percentage did not differ (P = 0.717) averaging 3.98 ± 0.08 %. In the current study, milk protein yield increased (P = 0.040) when increasing feeding frequency (0.84 and 0.81 ± 0.01 kg/d for once and twice daily feeding, respectively). This increase was likely caused by the increased milk yield.

Gas Consumption and Production

Heat production (**HP**) is a loss of energy that was indirectly measured in the current experiment as the heat of combustion, which was calculated based upon the volume of O_2 consumed and CO_2 and CH_4 produced. Thus, HP was determined from

measuring the concentration of these gases in respired air from the animal (Blaxter, 1962). Determination of HP is needed to accurately estimate total energy production of the animal. Heat production has been demonstrated to increase with increases in DMI (Purwanto et al., 1990). In the current study, O_2 consumption was not affected (P = 0.218) by feeding frequency averaging 4411.3 ± 181.9 L/d. Similarly, CO_2 production was not affected (P = 0.161) by feeding frequency averaging 4673.9 ± 221.0 L/d. Typically, daily CH_4 production ranges from 500–600 L/d for high-producing Holstein cattle (Beauchemin et al., 2008; Chase, 2014). In the current study, total CH_4 production was not affected (P = 0.793) by feeding frequency and, as expected using Jersey cows in late lactation, was lower than for Holstein cows, averaging 402.1 ± 20.8 L/d. Using late lactation Holstein-Friesian cattle, Hatew et al. (2015) observed CH_4 production around 580 L/d. However, it is important to note that CH_4 production and DMI are closely related (Knapp et al., 2014). The current study used Jersey cattle in late lactation. Dry matter intake is determined based on four main factors, which include animal weight, milk yield, energy content of the diet, and stage of lactation (Agricultural Research Council, 1980). As lactation progresses, energy requirements decrease due to the decrease in milk production, which corresponds to a decrease in DMI (NRC, 2001). Jersey cattle are smaller than Holstein cattle and consume less feed and produce less milk. Kristensen et al. (2015) observed a 20 % reduction in DMI for Jersey cattle compared to Holstein cattle. Hence, the low CH_4 production in the current study may be the result of decreased DMI associated with Jersey cattle in late lactation. Methane produced per unit of milk yield were not different (P = 0.233) between feeding frequency averaging 20.5 ± 1.81 L/kg/d. Similar to the current study, Crompton et al. (2011) used

mid-lactation Holstein-Friesian cattle being fed either one or two times daily and observed CH_4 production per unit of milk produced to be 20.9 L/kg. In the current study, CH_4 production per unit of DMI was not different ($P = 0.543$) by increasing feeding frequency with a mean of 23.8 ± 1.21 L/kg/d. Crompton et al. (2011) observed CH_4 production per unit of DMI to be 30.1 L/kg in lactating dairy cattle in mid-lactation. The respiratory quotient (**RQ**), ratio of CO_2 produced to O_2 consumed, is affected by the nature of the substrates being used for fuel in the animal. As a result, this measure may assist in determining the fuel being used by the animal and assure that gas collections are working properly. However, it should be noted that RQ alone cannot be solely used to make unequivocal conclusions on the metabolism of the animal. In the current study, RQ was not affected ($P = 0.238$) by feeding frequency with a mean of 1.05 ± 0.01. Typically, when carbohydrates are used as the main energy substrate, the RQ is near 1.0 (Brody, 1945; Blaxter, 1967; Ketelaars and Tolkamp, 1996). When proteins are used as the main energy substrate, the RQ is near 0.83 and with fat synthesis, the RQ near 1.10 to 1.20. When acetate, propionate, and butyrate are used as main fuel sources, the RQ's are 1.0, 0.86, and 0.80 respectively (Cherepanov and Agaphonov, 2010). With these cattle being in late lactation, and in a positive energy balance, the RQ of near 1.0 was observed was expected. Cattle in the current study were consuming a high carbohydrate diet combined with increased tissue energy, which potentially resulted in the RQ slightly above 1. Heat production was not different ($P = 0.212$) affected by feeding frequency with a mean of 22.1 ± 0.95 Mcal/d (Table 5.5). Results in the current study are lower than previous research using late lactation Holstein-Friesian cattle, which were observed to be approximately 28 Mcal/d (Hatew et al., 2015). Heat production per unit of metabolic

body weight did not differ (P = 0.232) by feeding frequency with a mean of 215.5 ± 8.19 kcal/$BW^{0.75}$. Heat production was also lower than value 227 kcal/$BW^{0.75}$ observed by Hatew et al. (2015). However, Jersey cattle were used in the current study compared to the Holstein-Friesian cattle used by Hatew et al. (2015). Little research is available on Jersey cattle in late lactation, and there is likely a breed difference in HP.

Diurnal Methane Production

In cattle, CH_4 production is episodic (Hegarty, 2013) and can vary by up to five fold throughout the day (Crompton et al., 2011). Feeding practices, such as feeding frequency, have been demonstrated to influence fermentation patterns and resulting gas production in dairy cattle (Brask et al., 2015). In the current study, we fed cattle once in the morning and then again 10 hours later, after the second milking, for the twice-daily feeding. In the current study, the rate of CH_4 production per hr overall was not different (P = 0.445) with a mean of 17.1 ± 0.74 L/h (Figure 5.1). A major objective of this study was to characterize diurnal CH_4 production, and we hypothesized that CH_4 production would increase after each feeding. As hypothesized, CH_4 production was affected (P < 0.001) by time of day. A trend was also observed for the interaction of feeding frequency and time (P = 0.084). Specifically, CH_4 production when feeding twice a day was higher at 2100 to 2300 hr compared with feeding once a day, which corresponds to the second feeding which occurred at 2000 hr (P = 0.014, P < 0.001, and P = 0.004, for hr 21, 22, and 23, respectively). Hence, the increased CH_4 production for cows fed twice daily compared to once daily, corresponded with the second feeding, which occurred 10-hr post feeding. Interestingly, CH_4 increased approximately 2 hr post milking for cattle fed twice daily and may have resulted from activity from milking; however, this effect was

not measured in this study. Previous research has observed increased CH_4 production corresponding to feeding (Crompton et al., 2011). In the current study, peak CH_4 production after the second feeding, was larger than the initial peak following the morning feeding. Similarly, Crompton et al. (2011) observed a larger peak in CH_4 production after the second feeding than the initial feeding. Although the observations of the current study are not new, they support the notion that for CH_4 production to be estimated accurately, spot sampling may be inadequate when trying to accurately estimate daily CH_4 production.

Standing Behavior

Cattle have an inherent need to rest or lie down during the day. Lying down potentially increases milk synthesis by increasing blood flow to the udder and increasing rumination (Grant, 2009). Hence, increased standing time may negatively affect milk yield in lactating dairy cattle. With cattle fed twice daily, DeVries and Von Keyserlingk (2005) observed lactating dairy cattle to stand approximately 11.7 hr/d. In the current study, we tested the effect of feeding frequency on CH_4 production, but also observed and tested standing behavior for cattle either inside or outside the headbox (Figure 5.2, Figure 5.3, Figure 5.4). This test was conducted so we could gain deeper analytical understanding of estimates generated with our apparatus used to indirectly measure HP. Overall, standing behavior was not affected ($P = 0.773$) by feeding frequency averaging 11.5 ± 0.63 hr/d; however, daily standing time was observed to be over 2 hours higher ($P < 0.001$) for cattle placed in the headbox (12.7 vs. 10.1 hr/d for inside vs. outside headbox, respectively). Grant (2009) observed a 1.5 kg increase in milk yield for every additional hr cattle were lying down to rest. It is important to note that increased time

standing may affect energy needs and as a result milk production. Practically, our results may suggest that observations collected in this system may modestly overestimate energy used by the animal to support additional time standing and further research is warranted to determine the extent of this bias. Nonetheless, this observation should not be taken to conclude resting time was inadequate, as lying time was still within the recommended time of 12 to 14 hr/d (Kammel et al., 2017).

Energy Partitioning and Nutrient Digestibility

Total energy intake. The energy content of feed plays a crucial role in the formulation of lactating dairy cattle diets to adequately meet the animals' nutrient requirements (Weiss, 1993). Typically, feed energy is presented as energy available per unit of DMI, which is broken down to gross energy (GE), digestible energy (DE), ME, and net energy for lactation (NE_L) are most frequently used. Gross energy intake did not differ ($P = 0.375$) by feeding frequency averaging 76.1 ± 4.43 Mcal/d (Table 5.6). Previous research has observed GE intake near 86 Mcal/d (Foth et al., 2015); however, that study used Holstein and Jersey cattle compared to Jersey cattle in the current study. Gross energy intake is affected by the energy density of the diet and DMI. However, DE and ME are more beneficial in energy calculations for ruminants. As was established earlier, Jersey cattle consume less feed than Holsteins, so the lower GE was expected for the current study. Similarly, DE and ME did not differ ($P \geq 0.626$) by feeding frequency (52.6 ± 3.02 Mcal/d and 45.5 ± 2.77 Mcal/d, respectively). Net energy for lactation did not differ ($P = 0.702$) by feeding frequency averaging 23.4 ± 2.13 Mcal/d.

Energy loss. In late-lactation dairy cattle, energy lost from feces, heat, urine, and CH_4 is approximately 28, 42, 5 and 5 % of GE, respectively (Flatt et al., 1967). In the

current study, fecal energy as a percentage of GE did not differ ($P = 0.865$) by feeding frequency with a mean of 30.8 ± 0.63 % which is higher than historical data. However, more recent research has observed fecal energy to be approximately 33 % of GE in lactating Holstein and Jersey cattle (Foth et al., 2015). Urine energy in the current study did not differ ($P = 0.722$) by feeding frequency with a mean of 4.44 ± 0.01 %. Methane and heat energy did not differ ($P \geq 0.212$) by feeding frequency with a mean of (5.1 ± 0.26 % and 29.7 ± 1.40 %). Using mid-lactation Holstein and Jersey cattle, Foth et al. (2015) observed CH_4 energy as a percentage of GE to be 5.4 %, which is similar to the results from the current study. Heat production in the current study was lower than previous research, which shows HP to be approximately 33 % of GE (Tine et al., 2001).

Energy gains. Retained energy was determined by subtracting HP from ME. In the current study, RE did not differ ($P = 0.702$) by feeding frequency averaging 23.4 ± 2.13 Mcal/d. This was expected, as HP and ME intake were similar between treatments. A trend was observed for milk energy to be greater ($P = 0.061$) for once a day feeding compared to twice a day feeding (20.9 vs. 19.7 ± 1.53 Mcal/d, respectively). The increased milk energy is the result of greater milk production in cattle fed once daily. Tissue energy did not differ ($P = 0.211$) by feeding frequency averaging 3.03 ± 1.30 Mcal/d. As cattle increase in days in milk, milk production decreases, which in turn decreases the energy needed for lactation (Flatt et al., 1967). As a result, cattle can utilize available energy to build or deposit tissue in late lactation as evidenced by the positive tissue balance.

Energy intake per unit of dry matter. Gross energy intake per kg of DMI did not differ ($P = 0.234$) by feeding frequency averaging 4.41 ± 0.01 Mcal/kg of dry matter.

Similarly, DE and ME intake per kg of DMI did not differ ($P \geq 0.926$) by frequency of feeding averaging 3.05 ± 0.03 and 2.63 ± 0.03 Mcal/kg of dry matter, respectively. Net energy of lactation per kg of DMI did not differ ($P = 0.566$) by frequency of feeding averaging 1.32 ± 0.08 Mcal/kg of dry matter. Published research reports a large range of net energy values. This is expected given the many feed related factors that may influence the energy content of feed. Tine et al. (2001) fed 60 % forage diet consisting of solely brown mid rib corn silage to lactating dairy cattle and observed the net energy of lactation per kg of dietary dry matter to be around 1.60 Mcal/kg. Whereas, Foth et al. (2015) fed lactating dairy cattle 50 % forage diet consisting of corn silage, alfalfa, and brome hay and observed a lower value of 1.45 Mcal/kg of dietary dry matter. The different forage sources and inherent differences in digestibility could explain the increase in NE_L compared to the current study, which used late-lactation Jersey cattle.

Maintenance energy and efficiency of energy use for lactation. Estimated maintenance energy is illustrated in Figure 2.5 and was determined through regression of ME and RE and then solving for ME intake when RE equals zero (Foth et al., 2015). Estimated maintenance requirement was calculated to be 146 kcal/MBW with efficiency of ME use for lactation ($\boldsymbol{k_1}$) of 0.76. In the current study, estimated maintenance requirements and efficiencies were similar to previous estimates, which averaged near 143 ± 26 kcal/MBW for maintenance and 0.64 for k_1 (Birkelo et al., 2004; Moe and Tyrrell, 1971; Vermorel et al., 1982; Xue et al., 2011; Foth et al., 2015;). However, Yan et al. (1997) reported maintenance to range between 146 and 179 kcal/MBW and k_1 to range between 0.61 and 0.68 in lactating dairy cows. In a recent meta-analysis of historical energy balance data collected from the USDA Energy Metabolism Unit

(Beltsville, MD) from 1963 to 1995 with Holstein cows, Moraes et al. (2015) reported an increase in maintenance requirement in more recent decades and this may be a function of increasing genetic merit of cattle. In the current study, late-lactation Jersey cattle were used and similar energy maintenance was observed. Overall, the maintenance energy requirements observed in the current study are within the range observed in the literature. We suggest that, because cows are not mobilizing large amounts of body tissue to support the needs of lactation, it may be easier to estimate maintenance at this stage of lactation.

Nutrient digestibility. Increasing the frequency of feeding from 2 to 4 times in lactating Guernsey cattle has led to an observed increase in dry matter digestibility of approximately 8 % (Campbell and Merilan, 1960). Similarly, Shabi et al. (1999) fed lactating Holstein cattle either two or four times daily and observed an increase in organic matter and crude protein digestibility. Increased digestibility may lead to increased milk yield in lactating dairy cattle (Campbell and Merilan, 1960). However, in the current study, dry matter digestibility was not affected ($P = 0.967$) by increasing feeding frequency averaging 70.2 ± 0.52 % (Table 5.7). Similarly, organic matter, CP, NDF, and starch digestibility were not affected ($P \geq 0.305$) by feeding frequency averaging 73.4 ± 0.56 %, 74.9 ± 0.71 %, 43.8 ± 1.23 %, and 93.5 ± 0.46 %, respectively.

Nitrogen Balance

Nitrogen balance is important in the dairy industry due to the potential negative environmental implications of excess nitrogen excretion and its use when indirectly measuring retained energy. Nitrogen balance is the N remaining after subtracting the N lost in the feces, urine, and milk from total N intake. Total nitrogen intake did not differ ($P = 0.132$) by feeding frequency with a mean of 512.8 ± 29.0 g/d (Table 5.8). Nitrogen

intake has been observed to affect nitrogen excretion (Weiss et al., 2009). In the current study, nitrogen lost in the feces and urine was not affected ($P \geq 0.425$) by feeding frequency averaging 129.6 ± 8.94 g/d and 216.9 ± 11.4 g/d, for feces and urine, respectively. This was expected as total nitrogen intake was similar between treatments. Milk nitrogen was not different ($P = 0.367$) by feeding frequency averaging 149.2 ± 11.8 g/d. Similarly, total nitrogen balance was not different ($P = 0.911$) by feeding frequency averaging 17.3 ± 13.0 g/d. A positive nitrogen balance combined with the positive tissue energy balance observed suggests that the cattle in the current study were depositing body stores. In late lactation, cattle replenish tissue reserves for the subsequent lactation, which likely occurred in the current study (NRC, 2001).

CONCLUSION

The present study demonstrated that increasing feeding frequency does alter the diurnal pattern of methane production. Cattle fed a second time each day have a second larger increase in methane production after this additional feeding. However, total methane production was unaffected by feeding frequency. Milk production and dry matter intake were not affected by feeding frequency. Energy balance was not affected by feeding frequency. The calculated maintenance requirement was 146 kcal/MBW with efficiency of ME use for lactation ($\boldsymbol{k_1}$) of 0.76.

REFERENCES

Agricultural Research Council. 1980. Feed intake. In: The Nutrient Requirements of Ruminant livestock Chapter 2. Pages 59-71. Unwin Brothers, London, UK.

AOAC International. 2000. Official Methods of Analysis. Vol. 1 and 2. 17th ed. AOAC International, Gaithersburg, MD.

AOAC International. 2006. Official Methods of Analysis. 18th ed. AOAC International, Gaithersburg, MD.

Bauman, D.E., and W.B. Currie. 1980. Partitioning of nutrients during pregnancy and lactation: A review of mechanisms involving homeostasis and homeorhesis. J. Dairy Sci. 63:1514-1529.

Beauchemin, K.A., M. Kreuzer, F. O'Mara, and T. A. McAllister. 2008. Nutritional management for enteric methane abatement: A review. Australian Journal of Experimental Ag. 48:21-27.

Birkelo, C.P., M.J. Brouk, and D.J. Schingoethe. 2004. The energy content of wet corn distillers grains for lactating dairy cows. J. Dairy Sci. 87:1815-1819.

Blaxter, K. L. 1962. The energy metabolism of ruminants. Pages 197-200. 1st ed. Charles C. Thomas Publisher, Springfield, IL.

Blaxter, K.L. 1967. The Energy Metabolism of Ruminants. 2nd ed. Hutchison & Co. Ltd., London, UK.

Boston, R.C., D.G. Fox., C.J. Sniffen, R. Janczewski, R. Munsen, and W. Chalupa. 2000. The conversion of a scientific model describing dairy cow nutrition and production to an industry tool: the CPM Dairy project. Pages 361-377 in Modelling Nutrient Utilization in Farm Animals Edited by J.P. McNamara, J. France and D. Beever. Oxford: CABI Publishing.

Brask, M., M. R. Weisbjerg, A. L. F. Hellwing, A. Bannink, and P. Lund. 2015. Methane production and diurnal variation measured in dairy cows and predicted from fermentation pattern and nutrient or carbon flow. Animal 9(11):1795-1806.

Brody, S. 1945. Bioenergetics and Growth. Pages 308-312, 914. Reinhold Publishing Corporation, New York, NY.

Brouwer. E. 1965. Report of sub-committee on constants and factors. Pages 441- 443 in Energy Metabolism. K.L. Blaxter, ed. European Association for Animal Production Publication No. 11, Ayr, Scotland.

Campbell, J.R., and C.P. Merilan. 1960. Effects of frequency of feeding on production characteristics and feed utilization in lactating dairy cows. J. Dairy Sci. 44:664-671.

Chase, L.E. 2014. Carbon footprint and the dairy industry. Cornell Nutrition Conference Animal Science Conference Proceedings. Cornell Univ. Ithaca, NY.

Cherepanov, G.G., and V.I. Agaphonov. 2010. Estimation of substrate-energetic fluxes in lactating cows. J. Anim. Feed Sci. 19:13-23.

Crompton, L.A., J.A.N. Mills, C.K. Reynolds, and J. France. 2011. Fluctuations in methane emission in response to feeding pattern in lactating dairy cows. In: Sauvant, D., J. Van Milgen, P. Faverdin, N. Friggens, (Eds.), Modelling Nutrient Digestion and Utilization in Farm Animals. Wageningen Academic Publishers, Wageningen, the Netherlands, pp. 176-180.

DeVries, T.J., and M.A.G. Von Keyserlingk. 2005. Time of feeding delivery affects the feeding and lying patterns of dairy cows. J. Dairy Sci. 88:625-631.

DRMS. 2014. DHI Glossary. Dairy Records Management System. Raleigh, N.C.

DuBois, M., K.A. Giles, J.K. Hamiliton, P.A. Rebers, and F. Smith. 1956. Colorimetric method for determination of sugars and related substances. Anal. Chem. 28:350-356.

Fenwick, M.A., S. Llewellyn, R. Fitzpatrick, D.A. Kenny, J.J. Murphy, J. Patton, and D.C. Wathes. 2008. Negative energy balance in dairy cows is associated with specific changes in IGF-binding protein expression in the oviduct. Reproduction 135:63-75.

Flatt, W.P., P.W. Moe, and R.R. Oltjen. 1967. Energy metabolism studies with dairy cows receiving purified diets. Pages 109-121 in Energy Metabolism of Farm Animals. ed. By K.L. Blaxter. London: Academic Press. (Proc. 4th symp. Energy Metab. Warsaw, Poland).

Freetly, H.C., J.A. Nienaber and T. Brown-Brandl. 2006. Partitioning of energy during lactation of primiparous beef cows. J. Anim. Sci. 84:2157-2162.

Foth, A.J, T. Brown-Brandl, K. J. Hanford, P. S. Miller, G. Garcia Gomez, and P. J. Kononoff. 2015. Energy content of reduced-fat dried distillers grains with solubles for lactating dairy cows. J. Dairy Sci. 98:7142–7152.

Georing, H.K. and P.J. Van Soest. 1970. Forage Fiber Analysis. USDA Agricultural Research Service. Handbook number 379. U.S. Dept. of Agriculture. Superintendent of Documents, US Government Printing Office, Washington D.C. 20402.

Grant, R. 2009. Stocking density and time budgets. 2009 Proceedings of Western Dairy Management Conference. Pages 7-17.

Hales, K.E., and N.A. Cole. 2017. Hourly methane production in finishing steers fed at different levels of dry matter intake. J. Anim. Sci. 95:2089-2096.

Hall, M.B. 2009. Analysis of starch, including maltooligosaccharides, in animal feeds: a comparison of methods and a recommended method for AOAC collaborative study. JAOACI 92:42-49.

Hatew, B., S.C. Podesta, H. Van Laar, W.F. Pellikaan, J.L. Ellis, J. Dijkstra, and A. Bannink. 2015. Effects of dietary starch content and rate of fermentation on methane production in lactating dairy cows. J. Dairy Sci. 98:486-499.

Hegarty, R.S. 2013. Applicability of short-term emission measurements for on-farm quantification of enteric methane. Animal 7:401-408.

Heinrichs, A.J., and P.J. Kononoff. 2002. Evaluating particle size of forages and TMRs using the New Penn State Forage Particle Separator. Tech. Bul. DAS 02-42. Pennsylvania State Univ., College Agric. Sci., Cooperative Ext., University Park, PA.

Hollmann, M., W.J. Powers, A.C. Fogiel, J.S. Liesman, and D.K. Beede. 2013. Response profiles of enteric methane emissions and lactational performance during habituation to dietary coconut oil in dairy cows. J. Dairy Sci. 96:1769-1781.

Kammel, D.W., J.M. Zulovich, and J.P. Harner. 2017. A systems approach to dairy farmstead design. In Large Dairy Herd Management, 3rd ed. Page 173.

Ketelaars, J.J., and B.J. Tolkamp. 1996. Oxygen efficiency and the control of energy flow in animals and humans. J. Anim. Sci. 74:3036-3051.

Knapp, J.R., G.L. Laur, P.A. Vadas, W.P. Weiss, and J.M. Tricarico. 2014. Invited review: enteric methane in dairy cattle production: Quantifying the opportunities and impact of reducing emissions. J. Dairy Sci. 97:3221-3261.

Kristensen, T., C. Jensen, S. Ostergaard, M.R. Weisberjerg, O. Aaes, and N.I. Nielsen. 2015. Feeding, production, and efficiency of Holstein-Friesian, Jersey, and mixed-breed lactating dairy cows in commercial Danish herds. J. Dairy Sci. 98:263-274.

Macmillan, K., X. Gao, and M. Oba. 2017. Increased feeding frequency increased minlk fat yield and may reduce the severity of subacute ruminal acidosis in higher-risk cows. J. Dairy Sci. 100:1045-1054.

Moe, P. W., H. F. Tyrrell, and W. P. Flatt. 1970. Partial efficiency of energy use for maintenance, lactation, body gain and gestation in the dairy cow. Page 65 in Energy Metabolism of Farm Animals, EAAP Publ. No. 13, Vitznau, Switzerland.

Moe, P.W. and H.F. Tyrrell. 1971. Net energy value for lactation of high- and low-protein diets containing corn silage. J. Dairy Sci. 55:288-303.

Moraes, L.E., E. Kebreab, A.B. Strathe, J. Dijkstra, J. France, D.P. Casper, and J.G. Fadel. 2015. Multivariate and univariate analysis of energy balance data from lactating dairy cows. J. Dairy Sci. 98:4012-4029.

National Research Council. 2001. Nutrient Requirements of Dairy Cattle. 7th rev. ed. Natl. Acad. Press, Washington, D.C.

Nienaber, J.A., and A.L. Maddy. 1985. Temperature controlled multiple chamber indirect calorimeter-design and operation. Trans. ASAE. 28:555-560.

Nocek, J.E., and D.G. Braund. 1985. Effect of feeding frequency on diurnal dry matter and water consumption, liquid dilution rate, and milk yield in first lactation. J. Dairy Sci. 68:2238-2247.

Purwanto, B. P., Y. Abo, R. Sakamoto, F. Furumoto, and S. Yamamoto. 1990. Diurnal patterns of heat production and heart rate under thermoneutral conditions in Holstein Friesian cows differing in milk production. J. Agric Sci. (Camb.) 114:139–142.

Russell, J.B. 1998. The importance of pH in the regulation of ruminal acetate to propionate and methane production in vitro. J. Dairy Sci. 82:3222-3230.

Shabi, Z., I. Bruckental, S. Zamwell, H. Tagari, and A. Arieli. 1999. Effects of extrusion of grain and feeding frequency on rumen fermentation, nutrient digestibility, and milk yield and composition in dairy cows. J. Dairy Sci. 82:1252-1260.

Tamura, T., H. Nakamura, S. Sato, M. Seki, and H. Nishiki. 2014. A modified catheterization procedure to reduce bladder damage when collecting urine samples from Holstein cows. J. Vet. Med. Sci. 76(6)819-826.

Tine, M.A., K.R. Mcleod, R.A. Erdman, and R.L. Baldwin. 2001. Effects of brown midrib corn silage on the energy balance of dairy cattle. J. Dairy Sci. 84:885-895.

Van Soest, P.J., J.B. Robertson, and B.A. Lewis. 1991. Methods for dietary fiber, neutral detergent fiber, and nonstarch polysaccharides in relation to animal nutrition. J. Dairy Sci. 74:3583-3597.

Van Zijderveld, S. M., W. J. J. Gerrits, J. A. Apajalahti, J. R. Newbold, J. Dijkstra, R. A. Leng, and H. B. Perdok. 2010. Nitrate and sulfate: Effective alternative hydrogen

sinks for mitigation of ruminal methane production in sheep. J. Dairy Sci. 93:5856-5866.

Vermorel, M., B. Remond, J.Vernet, and D. Liamadis. 1982. Utilization of body reserves by high-producing cows in early lactation; effects of crude protein and amino-acid supply. Pages 18-21in Energy Metabolism of Farm Animals. A. Ekern and F. Sundstøl, ed. European Association for Animal Production Publication No. 29, Ås, Norway.

Weiss, W.P. 1993. Predicting energy values of feeds. J. Dairy Sci. 76:1802-1811.

Weiss, W.P., L.B. Willett, N.R. St-Pierre, D.C. Borger, T.R. McKelvey, and D.J. Wyatt. 2009. Varying forage type, metabolizable protein concentration, and carbohydrate source affects manure excretion, manure ammonia, and nitrogen metabolism of dairy cows. J. Dairy Sci. 92:5607-5619.

Wildman, E.E. G.M. Jones, P.E. Wagner, R.L. Boman, H.F. Troutt and T.N Lesch. 1982. A dairy cow body condition scoring system and its relationship to selected production characteristics. J. Dairy Sci. 65:495-501.

Xue, B. T. Yan, C.F. Ferris, and C.S. Mayne. 2011. Milk production and energy efficiency of Holstein and Jersey-Holstein crossbred dairy cows offered diets containing grass silage. J. Dairy Sci. 94:1455-1464.

Yan, T., F.J. Gordon, R.E. Agnew, M.G. Porter, and D.C. Patterson. 1997. The metabolisable energy requirement for maintenance and the efficiency of utilization of metabolisable energy for lactation by dairy cows offered grass silage-based diets. Livest. Prod. Sci. 51:141-150.

Table 5. 1. Chemical composition and analysis of diet formulated to measure diurnal variation of methane and measure energy balance of late lactation Jersey cows (225 ± 16.2 DIM) (mean ± SD).

Item	Diet
Ingredient, % DM	
Corn silage	37.7
Alfalfa hay	14.0
Brome hay	2.56
Ground corn	17.1
Soybean meal	14.0
Bypass soy[1]	4.66
Soybean hulls	2.56
Tallow (porcine)	1.98
Bloodmeal	1.56
Calcium carbonate	1.40
Ca-salts of LCFA[2]	0.82
Sodium bicarbonate	0.58
CalciumPhosDi	0.35
Magnesium oxide	0.33
Salt	0.26
Bypass methionine[3]	0.07
Bypass lysine[4]	0.05
Vitamin premix[5]	0.05
Trace mineral premix[6]	0.04
Chemical Composition[7]	
DM, %	61.8 (0.01)
CP, % DM	18.5 (0.25)
Crude fat, % DM	4.22 (0.22)
ADF, % DM	16.6 (0.02)
NDF, % DM	25.6 (0.15)
Lignin, % DM	3.76 (0.20)
Ash, % DM	7.98 (0.02)
Starch, % DM	28.7 (0.57)
Gross energy, cal/g[8]	4419.1 (86.9)
ME, Mcal/kg[9]	2.77
NE_L, Mcal/kg[9]	1.79

[1] Soypass, LignoTech, Overland Park, KS.
[2]Calcium salts of long-chain fatty acids marketed as Megalac by Church & Dwight Co. Inc. Princeton, NJ.
[3]Bypass Methionine marketed as SmartamineM by Adisseo, France.
[4]Bypass Lysine marketed as Ajipro-L by Ajinomoto Heartland, Inc. Chicago, IL.
[5] Formulated to supply approximately 2,300 mg/kg Co, 25,000 mg/kg Cu, 2,600 mg/kg I, 1,000 mg/kg Fe, 150,000 mg/kg Mn, 820 mg/kg Se and 180,000 mg/kg Zn in total rations.
[6]Formulated to supply approximately 148,500 IU/d vitamin A, 38,500 IU/d vitamin D and 902 IU/d vitamin E in total rations.
[7]Values determined by Cumberland Valley Analytical Services, Hagerstown, MD, Mean (SD).
[8]Determined from composite samples from experiment and analyzed at the University of Nebraska-Lincoln, mean (SD).
[9]Values formulated from Cornell-Penn-Miner dairy model (Boston et al., 2000).

Table 5. 2. Feed chemical analysis for alfalfa hay, brome hay, corn silage, and concentrate mix (DM basis)[1].

	Alfalfa		Brome Hay		Corn Silage		Concentrate	
Chemical	Mean	SD	Mean	SD	Mean	SD	Mean	SD
DM, %	88.2	0.07	88.1	0.64	42.3	0.99	88.5	0.42
CP, % of DM	17.7	0.07	8.55	0.21	7.30	0.28	28.5	0.78
Soluble protein, % of DM	6.35	0.07	2.25	0.07	4.20	0.57	7.05	0.07
ADICP[2], % of DM	1.16	1.12	1.03	0.15	0.48	0.01	0.72	0.40
NDICP[2], % of DM	3.70	0.07	3.11	0.71	0.66	0.08	1.74	0.49
ADF, % of DM	40.5	0.14	42.3	0.21	17.5	0.07	7.15	0.07
NDF, % of DM	48.1	0.28	63.7	0.35	30.1	0.14	12.9	0.28
Lignin, % of DM	8.70	0.28	7.17	0.89	2.73	0.05	2.92	0.25
NFC[3], % of DM	26.4	0.14	19.0	1.06	55.9	0.57	44.0	0.35
Starch, % of DM	0.95	0.49	0.25	0.07	42.4	0.57	27.5	1.56
Sugar, % of DM	3.15	0.07	6.20	0.71	1.00	0.00	5.50	0.42
Crude fat, % of DM	1.10	0.28	1.90	0.02	3.14	0.36	6.19	0.09
Ash, % of DM	10.5	0.19	10.0	0.43	4.19	0.28	10.2	0.25
Ca, % of DM	1.17	0.04	0.44	0.06	0.20	0.01	2.35	0.09
P, % of DM	0.37	0.00	0.29	0.01	0.19	0.01	0.61	0.00
Mg, % of DM	0.24	0.01	0.13	0.01	0.13	0.00	0.65	0.02
K, % of DM	3.95	0.01	2.35	0.01	0.94	0.02	1.51	0.01
S, % of DM	0.20	0.01	0.18	0.01	0.11	0.00	0.35	0.02
Na, % of DM	0.04	0.01	0.02	0.00	0.03	0.00	0.75	0.04
Cl, % of DM	0.11	0.01	0.30	0.04	0.15	0.01	0.40	0.02
Fe, mg/kg	279.0	43.8	213.0	21.2	160.5	43.1	474.5	10.6
Zn, mg/kg	24.0	0.00	18.0	0.00	24.5	0.71	230.0	76.4
Cu, mg/kg	9.0	0.00	7.50	0.71	7.00	0.00	38.0	0.00
Mn, mg/kg	34.5	2.12	49.0	0.00	22.0	1.41	139.5	4.95

[1]Mean and SD were calculated based on samples of each feedstuff collected during each period and estimated by a commercial feed testing laboratory (Cumberland Valley Analytical Services, Hagerstown, MD).

[2]ADICP = Acid-detergent-insoluble crude protein; NDICP = Neutral-detergent-insoluble crude protein.

[3]NFC = Nonfiber carbohydrate calculated by difference 100-(% NDF + % CP + % Fat + % Ash).

Table 5. 3. Particle size distribution of experimental diet based on the total mixed ration (as-fed basis).

	Experimental diet	
Particle Size, %[1]	Mean	SD
> 19.0 mm	4.81	1.28
19.0 -- 8.0 mm	25.2	1.87
8.0 -- 1.18 mm	50.9	2.92
< 1.18 mm	18.9	2.32

[1]Determined using the Penn State Particle Separator on wet basis (Heinrichs and Kononoff, 2002).

Table 5. 4. DMI, milk production and composition, body weight and BCS[5], and water intake of late lactation Jersey cows (225 ± 16.2 DIM) (mean ± SD).

	Feeding frequency[1]			
Item	1X	2X	SEM[2]	*P*-value
DMI, kg/d	17.4	17.1	1.00	0.292
Milk yield, kg/d	21.2	20.4	1.59	0.097
ECM[3], kg/d	29.9	28.8	2.21	0.063
Fat, %	6.18	6.18	0.20	0.966
Fat yield, kg/d	1.30	1.26	0.10	0.097
FCM kg/d	30.3	29.3	2.24	0.085
Protein, %	3.98	3.97	0.08	0.717
Protein yield, kg/d	0.84	0.81	0.01	0.040
Lactose, %	4.55	4.53	0.05	0.439
MUN[4], mg/dl	20.9	20.1	0.85	0.056
SCC[5], cells/mL	129.5	106.3	35.1	0.477
Free water intake, L/d	83.8	75.7	5.67	0.026
Body weight, kg	483.0	480.1	12.2	0.223
BCS[6]	3.37	3.43	0.11	0.148

[1]Treatments: 1X = one time a day feeding; 2X = two times a day feeding.

[2]Lowest standard error of treatment means is listed.

[3]Energy corrected milk = 0.327 × milk yield [kg] + 7.2 × protein [kg] adjusted for 3.5% fat and 3.2% total protein (DHI Glossary, 2014).

[4]MUN = Milk urea nitrogen.

[5]SCC = Somatic cell count.

[6]BCS = Body Condition Score 1-5 scale according to Wildman et al. (1982).

Table 5. 5. Methane production, methane efficiencies, and heat production for late lactation Jersey cows (225 ± 16.2 DIM) (mean ± SD).

	Feeding frequency[1]			
Item	1X	2X	SEM[2]	*P*-value
O_2 consumption, L/d	4500.6	4321.9	181.9	0.218
CO_2 production, L/d	4803.3	4544.4	221.0	0.161
CH_4 production, L/d	399.6	404.5	20.8	0.793
CH_4/MY, L/kg/d	19.9	21.0	1.81	0.233
CH_4/ECM, L/kg/d	14.1	14.8	1.20	0.212
RQ[3], L/L	1.06	1.05	0.01	0.238
CH_4/DMI, L/kg/d	23.4	24.1	1.21	0.543
HP[4], Mcal/d	22.6	21.6	0.95	0.212
HP, kcal/$BW^{0.75}$	219.7	211.3	8.19	0.232

[1]Treatments: 1X = one time a day feeding; 2X = two times a day feeding.
[2]Lowest standard error of treatment means is listed.
[3]RQ = Respiratory quotient (CO_2 production/O_2 consumption).
[4]HP = Heat production, calculated with Brouwer's (1965) equation from O_2 consumption (L), CO_2 production (L), methane production (L) and urine–N (g) ($HP = 3.866 \times O_2 + 1.200 \times CO_2 – 0.518 \times CH_4 – 1.431 \times N$).
[abc]Means within rows lacking common superscript differ ($P < 0.05$).

Table 5. 6. Partitioning of energy for treatments for late lactation Jersey cows (225 ± 16.2 DIM) (mean ± SD).

	Feeding frequency[2]			
Item[1]	1X	2X	SEM[3]	*P*-value
GE intake, Mcal/d	76.6	75.6	4.43	0.375
DE, Mcal/d	52.9	52.3	3.02	0.626
ME, Mcal/d	45.8	45.2	2.77	0.634
NE_L, Mcal/d	23.1	23.6	2.13	0.702
Component, Mcal/d				
Feces	23.7	23.3	1.53	0.484
Methane	3.78	3.82	0.20	0.793
Urine	2.67	2.56	0.10	0.794
Heat	22.6	21.6	0.95	0.212
RE[4]	23.1	23.6	2.13	0.702
Milk	20.9	19.7	1.53	0.061
TE[5]	2.22	3.84	1.30	0.211
Feces, % of GE	30.7	30.9	0.63	0.865
Methane, % of GE	5.03	5.16	0.26	0.568
Urine, % of GE	4.46	4.41	0.17	0.722
DE, % of GE	69.3	69.2	0.63	0.865
ME, % of GE	59.8	59.6	0.64	0.794
GE, Mcal/kg of DM	4.40	4.41	0.01	0.234
DE, Mcal/kg of DM	3.05	3.05	0.03	0.977
ME, Mcal/kg of DM	2.63	2.63	0.03	0.926
NE_L, Mcal/kg of DM	1.30	1.34	0.08	0.566

[1]GE = gross energy; DE = digestible energy; ME = metabolizable energy; NE_L = net energy lactation.
[2]Treatments: 1X = one time a day feeding; 2X = two times a day feeding.
[3]Lowest standard error of treatment means is listed.
[4]RE = retained energy.
[5]TE = tissue energy.

Table 5. 7. Apparent digestibility of treatments, which included for late lactation Jersey cows (225 ± 16.2 DIM) (mean ± SD).

	Feeding frequency[1]			
Component	1X	2X	SEM[2]	*P*-value
DM, %	70.2	70.2	0.52	0.967
OM, %	73.6	73.2	0.56	0.630
CP, %	75.0	74.7	0.71	0.676
NDF, %	44.2	43.4	1.23	0.600
Starch, %	93.2	93.8	0.46	0.305
Ash, %	18.5	23.2	4.52	0.313

[1]Treatments: 1X = one time a day feeding; 2X = two times a day feeding.
[2]Lowest standard error of treatment means is listed.

Table 5. 8. Partitioning of nitrogen for treatments for late lactation Jersey cows (225 ± 16.2 DIM) (mean ± SD).

	Feeding frequency[1]			
Item	1X	2X	SEM[2]	*P*-value
Mass, g/d				
N intake	519.5	506.0	29.0	0.132
Fecal N excretion	130.6	128.5	8.94	0.425
Urine N excretion	218.7	215.0	11.4	0.804
Total N excretion[3]	349.3	343.5	17.6	0.709
Milk N concentration	151.9	146.4	11.8	0.367
N balance[4]	18.3	16.2	13.0	0.911
TE in protein[5]	0.61	0.54	0.44	0.911
N, % of intake				
Fecal N	25.0	25.3	0.71	0.676
Urine N	43.1	43.6	2.68	0.848
Milk N	29.1	28.6	1.41	0.615
N balance	2.84	2.56	2.35	0.933

[1]Treatments: 1X = one time a day feeding; 2X = two times a day feeding.
[2]Lowest standard error of treatment means is listed.
[3]Total N excretion = Fecal N + Urine N.
[4]Nitrogen balance = intake N – fecal N – urine N – milk N.
[5]TE = Tissue energy.

Table 5. 9. Energy balance equations derived from Brouwer (1965), Moe et al. (1970), and Freetly et al. (2006).

Response	ID	Equation[1]
Heat Production (HP)	1	$3.866 \times O_2 + 1.200 \times CO_2 - 0.518 \times CH_4 - 1.431 \times$Urinary N
Metabolizable energy (ME)	2	Intake energy – fecal energy – urinary energy – CH_4 energy
Recovered energy (RE)	3	ME - HP
Tissue energy (TE)	4	RE – milk energy
Tissue energy in protein	5	N balance (tissue N) × (5.88 kg of protein/kg of N) × (5.7 Mcal/kg of protein)
ME_{RE}	6	ME – ME for maintenance
LE_{ME} (negative energy balance)	7	Milk energy + TE ×0.84
ME_{LE} (positive energy balance)	8	ME_{RE} – TE/0.726
N balance (tissue N)	9	Intake N – fecal N – urinary N – milk N

[1]HP, Heat production is Mcal/d where O_2 and CO_2 are L/d and N is g/d; ME, Metabolizable energy is Mcal/d; RE, Recovered energy is Mcal/d; TE, Tissue energy is Mcal/d; Milk energy is energy in milk multiplied by total production (Mcal/d); T**issue energy** in protein is kcal/d**;** ME_{RE}, Metabolizable energy for maintenance found by regression of RE on ME and is the value of ME at zero RE based on metabolic body weight (MBW) kcal/MBW; LE_{ME}, Lactation energy received from ME of feed (kcal/d) for cows in a negative energy balance; ME_{LE}, Metabolizable energy available for lactation (kcal/d) for cows in a positive energy balance; N balance, Nitrogen balance is kg/d.

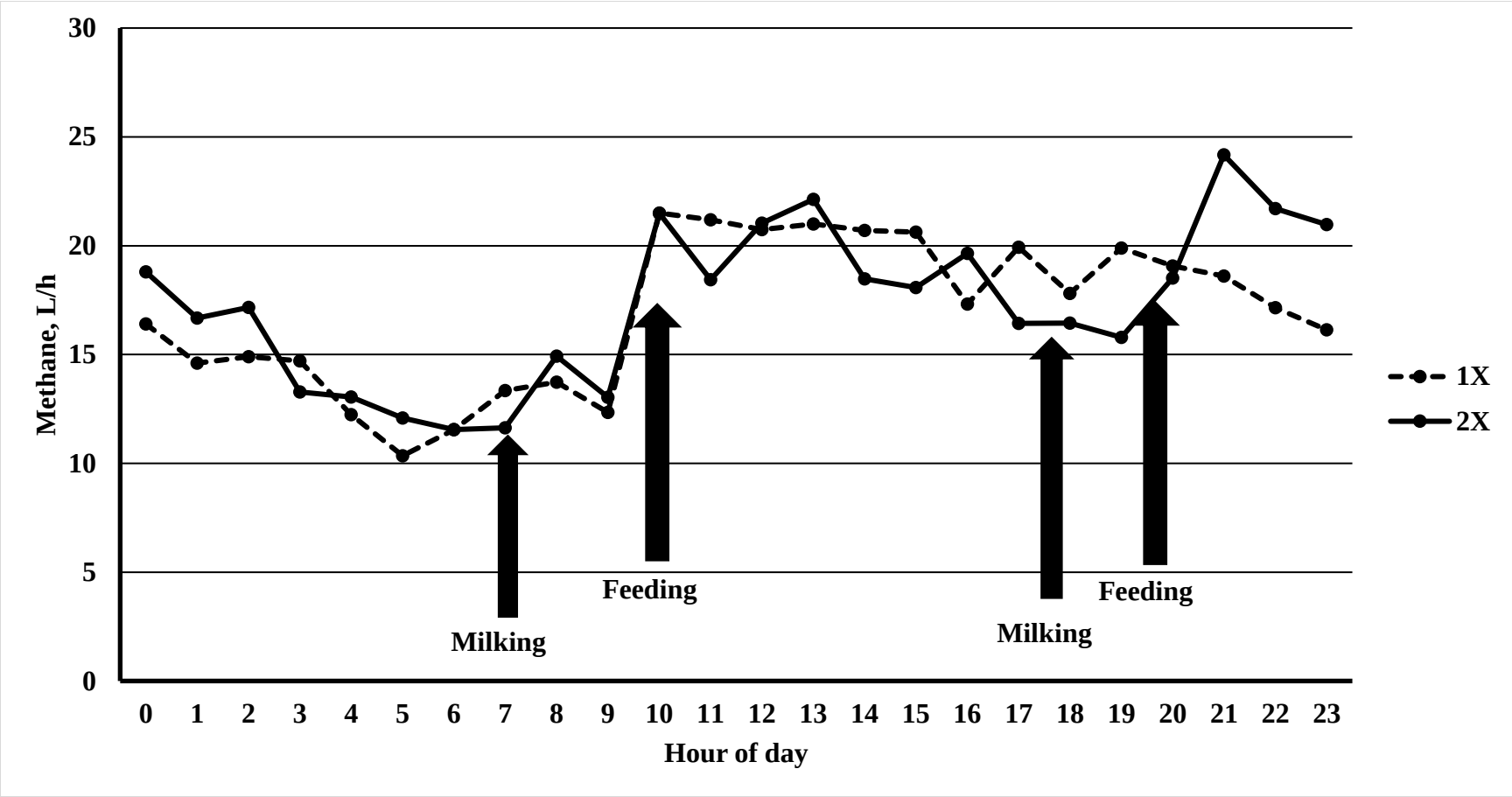

Figure 5. 1. Hourly methane production from late-lactation dairy cows fed once (1X) at 1000 hours or twice daily (2X) at 1000 and 2000 hr daily. Overall methane production per hr was not different ($P = 0.445$) with a mean of 17.1 ± 0.74 L/hr. Hour post-feeding affected ($P < 0.001$) methane production, and there was a trend for trt × hr ($P = 0.084$). Hours 21 to 23, had greater ($P = 0.014$, $P < 0.001$, and $P = 0.004$, for hr 21, 22, and 23, respectively) methane production for cows fed twice daily than once daily corresponding with the second feeding occurring 10 hr post-feeding.

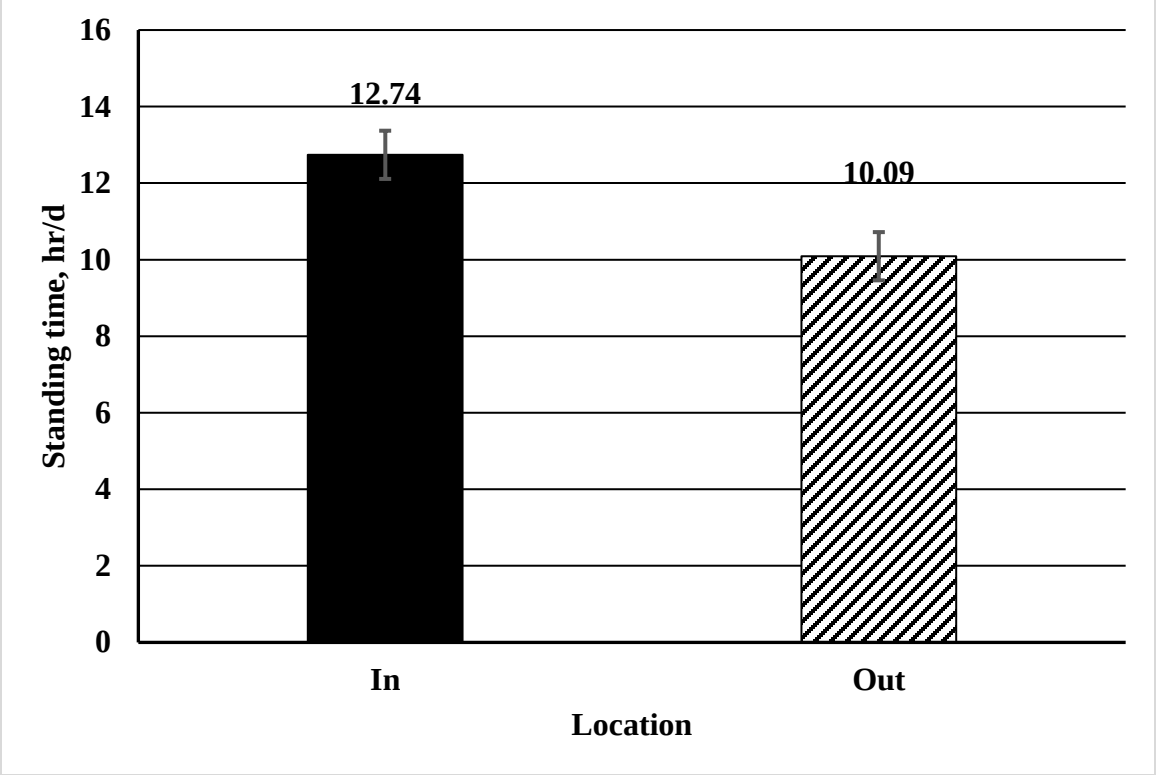

Figure 5. 2. Standing behavior of late-lactation Jersey cows while inside (IN) or outside (OUT) of headbox-style indirect calorimeters. No treatment effect was observed (P = 0.773) for standing behavior. However, cattle inside the headbox, had increased (P < 0.001) standing behavior compared to when they were not in the headbox.

Figure 5. 3. Cattle lying down while inside the headboxes at the University of Nebraska-Lincoln, Dairy Metabolism Unit (Lincoln, NE).

Figure 5. 4. Cattle standing while inside the headboxes at the University of Nebraska-Lincoln, Dairy Metabolism Unit (Lincoln, NE).

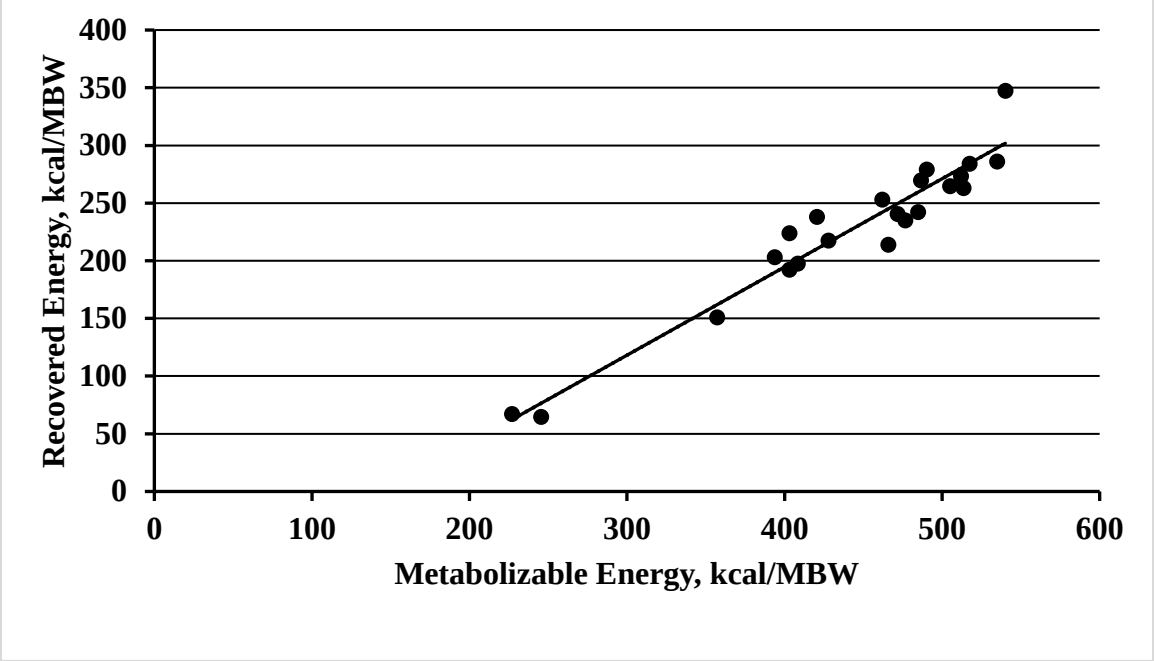

Figure 5. 5. Regression of recovered energy on metabolizable energy intake in kilocalories per metabolic body weight (kcal/MBW; $y = 0.7648x - 111.31$; $R^2 = 0.93$). Recovered energy = 0 at 146 kcal/MBW and efficiency of converting ME to lactation energy is 76 %.

CHAPTER 6

GENERAL CONCLUSIONS AND FUTURE STUDY RECOMMENDATIONS

General Conclusions

With increased concern of global warming and the potency of methane as a greenhouse gas, utilizing dietary strategies to reduce methane production in dairy cattle is needed. However, any method used to reduce methane should not negatively affect productive performance or incorporation by producers would be a major challenge. Distillers grains and solubles are widely used as a feedstuff throughout the industry and have reduced methane production in cattle. Using distillers grains and solubles as well as other dietary manipulations may prove beneficial as producers attempt to reduce methane production by cattle. Fat supplementation is known to reduce methane but it is not well understood if the degree of saturation of fatty acids would affect methane production. Methane is also an energetic loss to the animal, but effects of reducing its production on energy balance are not well described. Analytically speaking, methane is also challenging to measure, this is in part due to the nature of production and eructation. Hence, an understanding the diurnal nature of methane is also need to accurately measure its production while testing strategies designed to reduce production.

The results from this research demonstrated that the addition of fat and calcium sulfate to diets containing reduced-fat dried distillers grains and solubles (**DDGS**) effectively reduced methane production by 7 and 11 %. The addition of corn oil and calcium sulfate to diets containing DDGS also decreased methane production by 9 % per kg of feed intake and by 14 % per unit of milk yield. Additionally, the inclusion of

DDGS increased feed intake and milk yield by approximately 5 and 6 %. Feeding fats containing more poly-unsaturated fatty acids did not reduce methane production compared to saturated fatty acids. Although the addition of fat does reduce methane, we now hypothesize that the degree of saturation and hydrogenation of fats may not play as big of a role in reducing methane. With the addition of fat and DDGS, more energy was partitioned to lactation which increased milk yield resulting in a 10 % increase in the net energy of lactation. Addition of calcium sulfate decreased methane while also increasing feed intake and milk yield. Hence, dietary strategies that include fat and calcium sulfate can potentially be used to reduce methane without negatively affecting production in cattle. This research also demonstrated that feeding cattle multiple times a day alters the diurnal pattern of methane production. Methane production was measured hourly and mean methane production was not different by feeding frequency; however, after cattle being fed twice daily received the second feeding, methane production increased a second time compared to the cattle being fed once daily.

Recommendations for Future Research

An assumption made while feeding fat to reduce methane production is that the microbial community is being altered and biohydrogenation of unsaturated fatty acids takes place in the rumen. This is of concern as production of bioactive fatty acids, which are intermediates in biohydrogenation, can cause milk fat depression. One potential for future research would be to utilize fistulated cattle to measure the extent of biohydrogenation that occurs and measure fatty acid profile in the rumen and then again in the duodenum. This would assist in determining if the reduction in methane is due to ruminal biohydrogenation or some other relationship.

Other potential modifications for future energetic research include measuring the pH of the urine, more frequent gas sampling using the gas analyzer, use of electronic gas meters, and offering animals ad libitum access to fed. In the current work, a fixed volume of HCl was added to collected urine in attempt to acidify the urine thereby preventing the volatilization and loss of nitrogen. By measuring the pH and keeping pH below 5, future investigators may more precisely manage these potential nitrogen loses during collections. More frequent gas sampling may also allow for a more accurate reading of the gas production throughout the day. The system used in our studies is set to sample 3 times per hour, but it has the capability to sample 4 times per hour. Equipping the indirect calorimeters with electronic gas flow meters may reduce the potential human error caused by misreading the numbers, which is associated with the current system. Electronic flow meters may also be more durable, as we experienced multiple failures in the current gas flow meters as parts needed to be replaced. Lastly, it is recommended that in future energy studies, cattle be given ad libitum access to feed during the collection week rather than being offered 95 % as was done in the current studies. The current use of 95 % ab libitum access to feed was used to minimize feed refusals but it comes at the risk of underfeeding the cattle. The underlying rationale for this recommendation is to ensure cattle are not underfed as this would lead to an underestimate of the total gas production and consequently, underestimates of heat production.

Potential research for the future may look at the relationship between heat production and standing behavior. With the continuous gas monitoring system currently in use at the University of Nebraska-Lincoln, heat production could be characterized based upon standing or lying position. Continued research is needed on the use of

linolenic acid and its potential to decrease methane. Use of fistulated cattle to determine the degree of saturation that will occur would be beneficial.

APPENDIX A

Table 3. 11. DMI, milk production and composition, BW and BCS[1] of treatments which included a control which did not contain reduced-fat distillers grain plus solubles (DDGS) (CON), a diet containing 20% DDGS (DDGS), a diet containing 20% DDGS with 1.38% added corn oil (OIL), and a diet containing 20% DDGS with 0.93% added calcium sulfate (CaS)

	Holstein				Jersey							
	Treatments				Treatments					*P*-value		
Item	CON	DDGS	OIL	CaS	CON	DDGS	OIL	CaS	SEM[2]	trt	BRD	trt × BRD
DMI, kg/d	19.66^{bc}	20.79^{ab}	21.04^{a}	21.02^{a}	18.54^{c}	19.45^{bc}	18.91^{c}	18.23^{c}	0.528	0.13	0.001	0.24
Milk Yield, kg/d	29.98^{c}	30.91^{bc}	32.30^{a}	31.43^{ab}	22.60^{e}	24.07^{d}	24.30^{d}	23.74^{de}	0.949	0.0020	< 0.001	0.65
ECM[3]	30.65^{ab}	31.86^{ab}	31.97^{ab}	31.54^{ab}	29.52^{b}	30.96^{ab}	31.42^{a}	30.48^{ab}	0.930	0.024	0.433	0.948
Fat, %	3.85^{b}	3.89^{b}	3.57^{c}	3.71^{bc}	5.54^{a}	5.39^{a}	5.49^{a}	5.43^{a}	0.183	0.32	< 0.001	0.205
Fat Yield, kg/d	1.14^{cd}	1.20^{bcd}	1.15^{cd}	1.16^{cd}	1.24^{bcd}	1.30^{ab}	1.33^{a}	1.28^{abc}	0.04	0.22	0.022	0.45
FCM kg/d	31.49^{ab}	32.75^{a}	32.65^{ab}	32.36^{ab}	29.89^{b}	31.42^{ab}	32.11^{ab}	31.02^{ab}	0.95	0.035	0.304	0.81
Protein, %	2.80^{c}	2.86^{c}	2.80^{c}	2.78^{c}	3.75^{a}	3.65^{ab}	3.57^{b}	3.61^{b}	0.099	0.108	< 0.001	0.164
Protein Yield, kg/d	.84^{b}	.87^{ab}	0.90^{a}	.87^{ab}	.84^{ab}	.88^{ab}	0.86^{ab}	.85^{ab}	0.03	0.118	0.724	0.57
MUN, mg/dl	15.36^{b}	13.11^{c}	12.58^{c}	13.69^{c}	19.21^{a}	16.89^{b}	16.20^{b}	16.88^{b}	0.83	< 0.001	0.002	0.854
CH4 Production, L/d	406.85^{ab}	409.81^{ab}	375.81^{ab}	366.07^{b}	436.38^{a}	449.09^{a}	413.55^{ab}	396.80^{ab}	20.39	0.0649	0.0427	0.99
CH4/FCM	13.09^{ab}	12.75^{ab}	11.53^{b}	11.41^{b}	14.67^{a}	14.45^{a}	13.03^{ab}	12.84^{ab}	0.69	0.016	0.015	0.997
CH4/ECM	13.48^{ab}	13.04^{ab}	11.75^{b}	11.69^{b}	14.85^{a}	14.64^{a}	13.30^{ab}	13.06^{ab}	0.71	0.019	0.021	0.997
CH4/DMI	20.92^{ab}	19.67^{bc}	17.93^{c}	17.43^{c}	23.62^{a}	23.20^{a}	21.95^{ab}	21.80^{ab}	1.06	0.049	< 0.001	0.866
Heat Production, Mcal/d	27.02^{a}	27.59^{a}	25.37^{abc}	26.62^{ab}	23.76^{c}	24.33^{bc}	23.96^{c}	23.52^{c}	0.88	0.42	0.001	0.58
Heat Production/d/metwt	236.77^{b}	238.85^{b}	220.53^{b}	233.19^{b}	270.60^{a}	274.96^{a}	272.53^{a}	267.87^{a}	7.97	0.54	< 0.001	0.565
Water Intake, L/d	84.56^{bc}	88.03^{abc}	97.83^{a}	91.21^{ab}	79.71^{bc}	80.61^{bc}	81.10^{bc}	75.16^{c}	5.10	0.32	0.036	0.38
Body Weight, kg	590^{a}	597^{a}	595^{a}	593^{a}	425^{b}	429^{b}	431^{b}	428^{b}	15.68	0.50	< 0.001	0.96
BCS[1]	3.22^{a}	3.10^{b}	3.10^{b}	3.18^{ab}	3.25^{ab}	3.16^{ab}	3.22^{ab}	3.22^{ab}	0.09	0.06	0.60	0.65

[1]BCS = Body Condition Score 1-5 scale according to Wildman et al. (1982)

[2]Lowest standard error of treatment means is shown

[3]Energy corrected milk = 0.327 × milk yield [kg] + 7.2 × protein [kg] adjusted for 3.5% fat and 3.2% total protein (DHI Glossary, 2014)

[4]MUN = Milk urea nitrogen

[abc]Means within rows lacking common superscript differ ($P < 0.05$)

APPENDIX B: Poster for Joint Annual Meetings 2015

J. Dairy Sci. 98 Suppl 2: 143.

Evaluation of two techniques used to dislodge bacterial from particles contained in rumen digesta

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ABSTRACT

The objective of this study was to estimate the concentration of bacterial crude protein (BCP) in pellets isolated from ruminal digesta using a preparatory step of either blending or shaking to dislodge bacteria from rumen particles. Using a completely randomized design, two multiparous, lactating Holstein cows (DIM 229 ± 7d, DMI 36.1 ± 2.5 kg/d, milk yield 37.7 ± 5.6 kg/d) (mean ± SD), fitted with ruminal cannulas were fed the same diet once daily at 0930 h. Two hours post feeding, approximately 2.5 kg of rumen contents were collected from each cow, then thoroughly mixed and separated into two aliquots (blend or shake) then samples were strained through 4 layers of cheesecloth. Particle associated bacteria were separated from the solid portion of rumen contents by adding and equal amount of McDougal's buffer as was collected in the filtrate and physically shook or blended in a commercial blender for 1 min., followed by straining through 4 layers of cheesecloth. Fluid collected after shaking or blending, as well as fluid retained from the initial straining where combined together. Each sample underwent differential centrifugation which yielded bacterial pellets consisting of fluid associated bacteria and particle associated bacteria. DNA was then extracted from bacterial pellets and from the non-centrifuged samples of rumen content particles. The DNA from the bacterial pellets and samples of rumen content were subjected to real-time PCR using the TaqMan assay. Primers and a probe were designed from the DNA encoding part of the 16s rRNA for bacteria. The concentration of BCP using these two methods to dislodge bacteria did not differ (P = 0.42) (13.9 ± 5.0 and 7.5 ± 1.9 mg BCP/g DM for shake vs. blend, respectively). Results suggest that BCP concentration is not different between shaking or blending to dislodge bacteria, however, further research should examine and attempt to identify the large amount of analytical variation observed in both techniques.

INTRODUCTION

Rumen digesta is often sampled for the purpose of bacterial analysis. Commonly, rumen solids are combined with buffer and shaken in a capped jar (Hristov et al., 2005) in order to dislodge particle associated bacteria (PAB) during isolation. Instead of shaking, other studies have employed the use of a commercial blender to dislodge PAB as it is thought that this method is more efficient at dislodging bacteria.

OBJECTIVE

The objective of this study was to determine the difference between bacterial crude protein concentrations in ruminal digesta when using a shaking or blending method to dislodge bacteria from digesta particles.

MATERIALS AND METHODS

ANIMALS AND SAMPLING

- Two lactating, multiparous Holstein cows (x̄ ± SD, 229 ± 7 d DIM and 37.7 ± 5.6 kg/d milk production) were used.
- Cows were fed the same diet (Table 1) once/d at 0930 and had ad libitum access to food/water.
- Ruminal contents (about 2.5 kg) were sampled 2 h post feeding, then hand mixed and divided into 2 portions for either shaking or blending.
- Digesta was strained through 4 layers of cheesecloth then an equal volume of McDougal's buffer was added to the solid portion and shaken or blended for 1 min.
- Shaking or blending procedures were performed and then portions were analyzed for BCP concentrations.
- The experiment was conducted as a completely randomized design.

USING DNA MARKERS TO ESTIMATE BACTERIAL CRUDE PROTEIN

Bacterial DNA isolation kit

DNA is extracted from samples of isolated bacteria

About 200 mg of isolated bacteria was placed in a 200 µl microcentrifuge tube and had 1.4 ml of ASL buffer added then vortexed for 1 min then heated for 5 min at 70°C and then vortexed for 15 seconds and centrifuged for 1 min to pellet stool. 1.5 ml of supernatant was placed in a new 2 ml tube and had 1 inhibitEX tablet added and vortexed until tablet was dissolved and incubate at room temperature for 1 min and then sample was centrifuged for 3 min. Supernatant was added to a new tube and centrifuged for an additional 3 min. 15 µl Proteinase K added to a new 1.5 ml tube and then 200 µl of supernatant was added then 200 µl of AL buffer was added and vortexed for 15 min and incubated at 70°C for 10 min then 200 µl of ethanol were added to the lysate and vortexed. Then sample was transferred to a spin column and centrifuged for 1 min then the filtrate was discarded. The sample then had 500 µl AW1 buffer added and centrifuged for 1 min and then 500 µl AW2 buffer was added and centrifuged for 3 min with the filtrate being discarded. Then 200 µl AE buffer was added to the spin column membrane and incubated at room temperature for 1 min and then centrifuged for 1 min to elute DNA.

1

Bacterial pellet isolated from centrifugation

DNA concentration is measured with a spectrophotometer

DNA concentrations of each sample were checked with a spectrophotometer (NanoDrop ND-1000 Spectrophotometer, NanoDrop Technologies, Inc. Wilmington, DE).

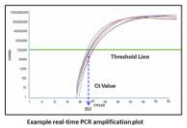
2

mg DNA/g dry bacterial pellet is calculated

ng DNA/g of dry bacterial pellet was calculated by dividing the nanograms of DNA in sample (attained from photospectrometry) by grams of dry matter, and then by dividing by 1,000,00 for unit conversion.

ng DNA = bacterial pellet sample dry weight (g) × 1,000,000 = mg DNA/g DM pellet

3



Example real-time PCR amplification plot

DNA undergoes real-time PCR

Isolated DNA from both methods and cows along with extracted DNA from digesta were prepared for real-time PCR as follows; 10 µL of DNA sample were combined with 1 µL of 10 µM forward primer, 1 µL of 10 µM reverse primer, 0.35 µL of 10 µM TaqMan probe, 12.5 µL of TaqMan Master Mix (Applied Biosystems, Foster City, CA, USA) and 0.15 µL of nanopure water. Two samples with no DNA were included as non-template controls. Each sample was run in duplicate in separate wells of the 384-well real-time PCR plate. DNA samples were subjected to real-time PCR using a 7900HT Fast Real-Time PCR System (Applied Biosystems). Temperature cycling was as described by Minyeri et al. (2009) with some variations, specific conditions were as follows: stage 1: 50 °C for 2 minutes; stage 2: 95 °C for 10 minutes; stage 3: 45 cycles alternating denaturation at 95 °C for 15 seconds, then annealing and polymerization at 60°C for 1 minute.

7900HT Fast Real-Time PCR System

4

Freeze dried sample of ruminal bacteria

CTs used to calculate abundance of target DNA in sample

Abundance of target DNA was calculated using the equation described by Castillo-Lopez (2012):

$(1 + E^{CT}) \div$ ng DNA = 1 Abundance/g DNA * 1,000,000

Where E is the exponential amplification and CT is the number of cycles at threshold.

5

Leco FP-528 Nitrogen Combustion Analyzer

Abundance of target DNA/g of bacterial DM is calculated

Abundance of target bacterial DNA/g of dry bacterial pellet was calculated by multiplying the abundance value by mg of DNA per gram of dry bacterial pellet.

1 Abundance * mg DNA/g DM pellet = 1 Abundance/g DM

6

Straining a sample of rumen digesta through four layers of cheesecloth

Abundance of target DNA/g BCP is calculated using CP % of dry bacterial pellet

Abundance of target DNA per gram of bacterial crude protein was calculated by first attaining the crude protein percentages of dry bacterial samples using a Leco nitrogen analyzer (Leco FP-528 Nitrogen Combustion Analyzer, Leco corp. St. Joseph, MI). Then, the previously calculated abundance/g DM was divided by the crude protein percentage of the dry-bacterial sample and multiplied by 100.

(1 Abundance/g DM ÷ %CP) * 100 = 1 Abundance/g CP

7

Rumen digesta from a cannulated Holstein cow

The ratio of abundance of target DNA/g of BCP is used to estimate BCP concentrations in samples of interest

Samples of ruminal fluid and ruminal particles underwent the same laboratory procedures as described above in order to calculate the amount of target DNA per gram of dry sample. This value was then multiplied by the ratio of abundance to grams of CP (calculated in the above step) and then by 1,000, resulting in mg of BCP per gram of dry sample.

Target DNA/g DM * 1 Abundance/g CP * 1000 = mg BCP/g DM

Table 1. INGREDIENT AND CHEMICAL COMPOSITION OF EXPERIMENTAL TREATMENT

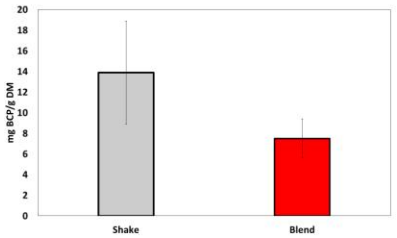
Ingredient, % DM	
Corn silage	28.9
Alfalfa Hay	22.4
Ground Corn	16.8
Reduced Fat DDGS	9.3
Ground Soy Bean Hulls	8.4
Soy Bean Meal	6.5
Soy Pass[1]	2.3
Sodium bicarbonate	1.4
Calcium carbonate	1.0
Blood meal	0.6
Megalac	0.6
Urea	0.5
Tallow	0.5
Dicalcium Phosphate	0.3
Sodium Chloride	0.2
Magnesium Oxide	0.2
Trace Min/Vit Premix[2]	0.2

Chemical, % DM	
CP	18.8
NDF	30.8
Starch	24.6
Ether Extract	3.9

[1]LignoTech, Overland Park, KS.
[2]Formulated to supply approximately 120, 000 IU/d vitamin A, 24, 000 IU/d of vitamin D, and 800 IU/d Vitamin E, 1.0% Ca, 0.50 % P, 0.36% Mg, and 1.3% K in total ration.

RESULTS AND DISCUSSION

Figure 1. Bacterial Crude Protein Concentration in Rumen Digesta for shake vs. blend method to dislodge bacteria (P = 0.42)



CONCLUSIONS

Bacterial crude protein concentrations did not differ between the shake vs. blend method for dislodging bacteria from rumen digesta. However, further investigation should examine and attempt to identify the large amount of analytical variation observed in both techniques. Dislodging bacteria correctly to get a representative sample of bacterial crude protein.

REFERENCES

Castillo-Lopez, E., et al. 2012. Intestinal flow of microbial protein and rumen undegradable protein in cattle fed corn distillers grains and solubles, with an emphasis during lactation. Theses and Dissertations in Animal Science. Paper 60.

Hristov, A.N., et al. 2005. Comparison of purines and nitrogen-15 as microbial flow markers in beef heifers fed barley- or corn-based diets. Can. J. Anim Sci. 85:211–222.

Martin, C., et al. 1994. Isolation and characteristics of the protozoal and bacterial fractions from bovine ruminal contents. J. Anim Sci. 72:2962-2988.

APPENDIX C: Poster for Joint Annual Meetings 2016

J. of Anim. Sci. 94:706. doi:10.2527/jam2016-1454.

Manipulation of lactating dairy cows diet using reduced-fat distillers grains, corn oil and calcium sulfate to reduce methane production measured by indirect calorimetry

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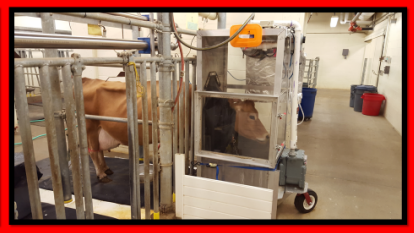
Abstract: 16071

ABSTRACT

A study using 16 multiparous (8 Holstein and 8 Jersey) (78 ± 15 DIM) (mean ± SD) lactating dairy cows, was conducted to determine the effects of dietary manipulation on methane mitigation in dairy cattle. A replicated 4 × 4 Latin square design with 35 d periods (28 d adaption and 7 day collections) used to compare four different dietary treatments. Treatments were composed of a control (CON) diet which did not contain reduced-fat distillers grain plus solubles (RFDDGS), and treatment diets containing 20% (DM basis) RFDDGS (DG), 20% RFDDGS with 1.38% (DM basis) added corn oil (OIL), and 20% RFDDGS with 0.93% (DM basis) added calcium sulfate (CaS). Methane sampling was performed using indirect calorimeters (headboxes). Compared to CON, DMI was greater (P = 0.030) for DG but was not affected (P > 0.05) by either OIL or CaS. Milk production was lowest in CON (P < 0.001) compared to DG, OIL, and CaS (26.3 vs. 27.5, 28.3, and 27.6 ± 0.67 kg/d for CON vs DG, OIL, and CaS respectively). Compared to CON, Energy corrected milk was greater (P = 0.004) in DG and OIL (30.1 vs 31.4, 31.7, and 31.0 ± 0.66 kg/d for the CON, DG, OIL, and CaS respectively). The addition of DG did not affect (P = 0.690) total methane produced compared to CON diet. However, the addition of CaS reduced (P = 0.020) methane production while the addition of OIL tended (P = 0.177) to reduce methane production compared to CON diet (421.6, 429.5, 394.7, and 381.4 ± 14.41 L/d for CON, DG, OIL, and CaS respectively). When expressed as methane per unit of energy corrected milk, cows consuming OIL and CaS produced less methane (P = 0.01) compared with CON and DG (14.2, 13.8, 12.5, and 12.4 ± 0.50 L/kg/d for CON, DG, OIL, and CaS respectively). Similarly, when expressing methane per unit of DMI; cows consuming OIL and CaS produced less methane (P = 0.015) compared to those consuming CON diet (22.3, 21.4, 19.9, and 19.6 ± 0.75 L/kg/d for CON, DG, OIL, and CaS respectively). Results of this study indicate that methane production may be reduced by feeding rations containing RFDDGS with added corn oil or calcium sulfate without adversely affecting milk production.

Key Words: corn oil, dairy cows, dried distillers grains and solubles, methane, sulfate



Metabolism Facility at the University of Nebraska-Lincoln, Lincoln, NE

INTRODUCTION

- There has been an increased interest in the animal industry to reduce the carbon footprint from animal production. Of particular interest for the dairy industry is the mitigation of methane production. Methane has about 21-25(Environmental Protection Agency, 2010) times more effect on global warming than carbon dioxide. Previous research has shown reduced methane production with diets containing reduced-fat dried distillers grains with solubles (Foth et al., 2015), oil, and sulfate (Van Zijderveld et al., 2010). Developing strategies to mitigate methane production that do not adversely affect milk production are crucial for implementation for commercial dairies.

OBJECTIVE

- To test the effects of manipulating lactating dairy cow diets containing reduced-fat dried distillers grains with solubles (RFDDGS), corn oil, or calcium sulfate. Specifically, effects on milk production, milk composition and methane production. Therefore, we hypothesized that addition of reduced fat distillers grains, corn oil, and calcium sulfate would reduce methane production.

MATERIALS AND METHODS

- 16 Multiparous cows (8 Holstein and 8 Jersey) (78 ± 15 DIM) housed in tie stall barn
- Four dietary treatments (detailed in Table 1) formulated with the CPM Model
  - Corn and soybean ration control which contained no RFDDGS (CON)
  - Ration containing 20% reduced-fat dried distillers grains with solubles (DG)
  - 20% RFDDGS plus 1.38% corn oil (Oil)
  - 20% RFDDGS plus 0.93% calcium sulfate (CaS)
- 35-d periods, last 7-d of each period for data collection
- Daily milk production (milked 2x daily)
- Milk composition
- Daily feed intake (fed once daily)
- Methane production (measured 2-d during final week of period) collected using indirect calorimeters
- Data were analyzed using the MIXED procedure of SAS (SAS Institute Inc., Cary, NC)
  - Fixed effects: Period and Treatment
  - Random effect: Cow

Photo of a cow in a headbox in the Metabolism Facility at the University of Nebraska-Lincoln, Lincoln, NE

Manipulation of lactating dairy cows diet using reduced-fat distillers grains, corn oil and calcium sulfate to reduce methane production measured by indirect calorimetry

Abstract: 16071

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MATERIALS AND METHODS

Table 1. Composition and analysis of Total Mixed Rations (TMR) for Control (CON), Reduced-fat dried distillers grains with solubles (DG), RFDDGS plus corn oil (OIL), and RFDDGS plus calcium sulfate (CaS) treatments

Item	CON	DG	OIL	CaS
Ingredient, %DM				
Corn Silage	29.8	29.8	29.8	29.8
Alfalfa hay	26.6	26.6	26.6	26.6
Brome hay	2.57	2.57	2.57	2.56
Ground corn	21.8	12.9	11.5	12.6
Ground soybean hulls	0.55	0.55	0.55	0.55
RFDDGS	--	20.0	20.0	20.0
Soybean meal	11.0	--	--	--
Soypass[2]	4.59	4.59	4.59	4.59
Bloodmeal	0.46	0.46	0.46	0.46
Corn oil	--	--	1.38	--
Calcium carbonate	0.75	0.75	0.75	0.18
Calcium sulfate	--	--	--	0.93
Sodium bicarbonate	0.62	0.62	0.62	0.62
Ca-salts LCFA[3]	0.55	0.55	0.55	0.55
Magnesium oxide	0.24	0.24	0.24	0.24
Salt	0.18	0.18	0.18	0.18
Trace mineral/Vitamin premix[3]	0.18	0.18	0.18	0.18
Chemical Composition, % DM[1]				
CP	17.1	16.6	16.7	16.6
Ether Extract	3.00	4.30	5.30	4.20
NDF	30.6	34.6	34.8	33.3
Ash	7.50	7.50	7.30	7.40
Starch	27.8	24.1	22.2	24.9

[1] Values determined by Cumberland valley Analytical Services, Hagerstown, MD.

[2] Ligno Tech (Overland Park, KS).

[3] Calcium salts of long-chain fatty acids marketed as Megalac by Church & Dwight Co. Inc. Princeton. NJ.

[4]Contained 13.9 % Ca, 0.03 % P, 0.42 % Mg, 0.20 % K, 4.20 % S, 0.08 % Na, 0.03 % Cl, 445 mg/kg Fe, 60,021 mg/kg Zn, 17,375 mg/kg Cu, 43,470 mh/kg Mn, 287 mg/kg Se, 527 mg/kg Co, and 870 mg/kg I, 120,000 IU/d vitamin A, 24,000 IU/d of vitamin D, and 800 IU/d Vitamin E in total ration.

RESULTS

Table 2. Methane production, methane efficiencies, and heat production for Control (CON), Reduced-fat dried distillers grains with solubles (DG), RFDDGS plus corn oil (OIL), and RFDDGS plus Calcium Sulfate (CaS) treatments

	Treatments					
Item	CON	DG	OIL	CaS	SEM[1]	*P*-value
CH_4 Production, L/d	421.6[a]	429.5[a]	394.9[ab]	381.4[b]	14.41	0.02
CH_4/ECM, L/kg/d	14.2[a]	13.8[ab]	12.5[bc]	12.4[c]	0.50	0.01
CH_4/DMI, L/kg/d	22.3[a]	21.4[ab]	19.9[b]	19.6[b]	0.75	0.02
Heat production[2], Mcal/d	25.4	26.0	24.7	25.1	0.62	0.11
Heat production/d/$BW^{0.75}$	253.7	256.9	246.5	250.5	7.32	0.17

[1]Lowest standard error of treatment means is shown

[2] Heat production (HP) calculated with the Nienaber and Maddy (1985) equation from oxygen consumption (L), carbon dioxide production (L), and methane production (L), (HP = (16.18 × O_2 + 5.02 × CO_2 – 2.17 × CH_4)/4.183).

[abc]Means within rows lacking common superscript differ ($P < 0.05$)

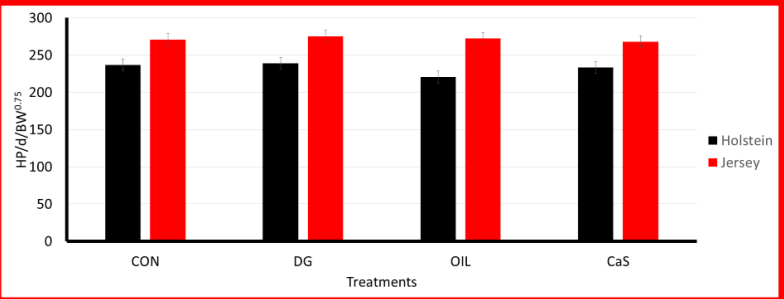

Figure 1. Effects of Control (CON), Reduced-fat dried distillers grains with solubles (DG), Corn Oil (OIL), and Calcium Sulfate (CaS) diets on methane production measured by indirect calorimetry. Heat production in Holsteins was lower ($P < 0.01$) compared to Jerseys (232.3 vs. 271.4 for Holstein vs Jersey, Respectively), however, there was no treatment effect ($P = 0.54$) or treatment by breed interaction ($P = 0.57$).

Manipulation of lactating dairy cows diet using reduced-fat distillers grains, corn oil and calcium sulfate to reduce methane production measured by indirect calorimetry

Abstract: 16071

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RESULTS

Table 3. DMI, milk production and composition, BW and BCS[1] of Control (CON), Reduced-fat dried distillers grains with solubles (DG), RFDDGS plus added corn oil (OIL), and RFDDGS plus added calcium sulfate (CaS) treatments

	Treatments					
Item	CON	DG	OIL	CaS	SEM[2]	*P*-value
DMI, kg/d	19.1^{b}	20.1^{a}	20.0^{ab}	19.6^{ab}	0.37	0.03
Milk yield, kg/d	26.3^{b}	27.5^{a}	28.3^{a}	27.6^{a}	0.67	< 0.01
ECM[3], kg/d	30.1^{b}	31.4^{a}	31.7^{a}	31.0^{ab}	0.66	< 0.01
Fat, %	4.70	4.64	4.53	4.57	0.13	0.09
Fat Yield, kg/d	1.19	1.25	1.24	1.22	0.03	0.07
Protein, %	3.28^{a}	3.26^{ab}	3.18^{b}	3.20^{ab}	0.07	0.38
Protein Yield, kg/d	0.84^{b}	0.87^{ab}	0.88^{a}	0.86^{ab}	0.02	0.02
MUN[4], mg/dl	17.3^{a}	15.0^{bc}	14.4^{c}	15.3^{b}	0.59	< 0.01
Water Intake, L/d	82.1	84.3	89.5	83.2	3.61	0.09
Body Weight, kg	508	513	513	511	11.09	0.18
BCS[1]	3.23^{a}	3.13^{b}	3.16^{ab}	3.20^{ab}	0.06	0.01

[1]BCS = Body Condition Score 1-5 scale according to Wildman et al. (1982)

[2]Lowest standard error of treatment means is shown

[3]Energy corrected milk = 0.327 × milk yield [kg] + 7.2 × protein [kg] adjusted for 3.5% fat and 3.2% total protein (DHI Glossary, 2014)

[4]MUN = Milk urea nitrogen

[abc]Means within rows lacking common superscript differ ($P < 0.05$)

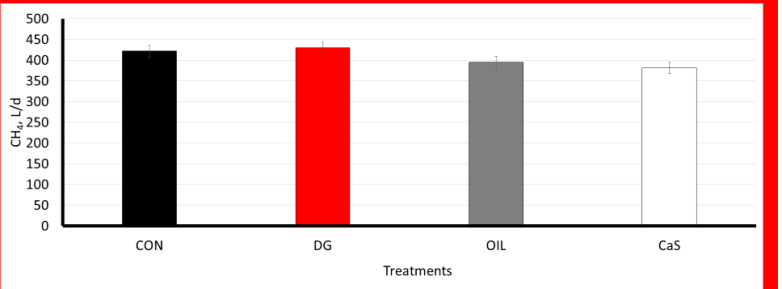

Figure 2. Effects of Control (CON), Reduced-fat dried distillers grains with solubles (DG), RFDDGS plus added corn oil (OIL), and RFDDGS plus added calcium sulfate (CaS) diets on methane production measured by indirect calorimetry. Methane production means were 421.6, 429.5, 394.7, and 381.4 L/d for CON, DDGS, OIL, and CaS, Respectively. The CaS diet decreased ($P = 0.02$) methane production compared to CON and DDGS diets but was not different from OIL. The OIL diet tended ($P = 0.08$) to decrease methane production compared to CON and DDGS diets.

CONCLUSIONS

Results of this study indicate that methane production may be reduced by feeding rations containing RFDDGS with added corn oil or calcium sulfate without adversely affecting milk production and milk components. Addition of fat and corn oil may toxic to the methanogen population thus decreasing total methane production.

AKNOWLEDGEMENTS

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REFERENCES

Environmental Protection Agency. 2010. Methane and nitrous oxide emissions from natural sources. U.S. Environmental Protection Agency, Washington, DC, USA.

Foth, A. J., T. Brown-Brandl, K. J. Hanford, P. S. Miller, G. Garcia Gomez, and P. J. Kononoff. 2015. Energy content of reduced-fat distillers grains with solubles for lactating dairy cows. J. Dairy Sci. 98:7142-7152.

Van Zijderveld, S. M., W. J. J. Gerrits, J.A. Apajalahti, J. R. Newbold, J. Dijkstra, R. A. Leng and H. B. Perdok. 2010. Nitrate and sulfate: Effective alternative hydrogen sinks for mitigation of ruminal methane production in sheep. J. Dairy Sci. 93:5856-5866.

APPENDIX D: Poster for ADSA National Meetings 2017

J. Dairy Sci. 100(Suppl. 2):113.

Abstract M292: Methane mitigation with corn oil and calcium sulfate, responses on whole animal energy and nitrogen balance in dairy cattle consuming reduced-fat dried distillers grains plus solubles

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INTRODUCTION

Lactating dairy cattle produce approximately 500 L/d of methane (Beauchemin et al., 2008). With the goal to lower total greenhouse gas emissions by 25% by the Innovation Center for U.S. Dairy by 2020, many dietary strategies have been devised to reduce methane emissions in ruminants to help meet this goal. These include the addition of fat and sulfate to rations (Van Zijderveld et al., 2010) as well as the inclusion of reduced fat dried distillers grains plus solubles (**RFDDGS**) in the ration (Foth et al., 2015). Methane production also represents an energetic loss for cattle and as such, methane mitigation could repartition energy towards production processes. However, there is limited research showing how these mitigation techniques affect whole-animal energy and nitrogen balance and the digestibility of the diet in lactating dairy cattle.

OBJECTIVE

To determine the effects of methane mitigation techniques including adding corn oil and calcium sulfate to diets containing RFDDGS that are high in forage on energy balance in the lactating dairy cow.

HYPOTHESIS

Rations containing RFDDGS with added corn oil and calcium sulfate will reduce methane production without affecting energy and nitrogen balance.

MATERIALS AND METHODS

- 16 multiparous cows (8 Holstein, 8 Jersey) (78 ± 15 DIM) housed in tie stall barn
- Four dietary treatments formulated with the CPM Model.
  - Corn and soybean ration which contained no RFDDGS (**CON**)
  - Ration containing 20% RFDDGS (**DG**)
  - 20% RFDDGS plus 1.38% corn oil (**CO**)
  - 20% RFDDGS plus 0.93% calcium sulfate (CaS)
- 35-d periods, last 7-d of each period for data collection
  - Daily feed intake (fed once a day, allowing ≈ 10% orts)
  - Daily milk production (2× milking)
  - Milk composition
  - Methane production (measured 2-d during final week of period) collected via the indirect calorimetry method
- Data were analyzed using the MIXED procedure of SAS
  - Fixed effects: Period and Treatment
  - Random effect: Cow

Photo of a cow in a headbox in the Metabolism Facility at the University of Nebraska-Lincoln, Lincoln, NE

Table 1. Composition and analysis of Total Mixed Rations (TMR) for Control (CON), Reduced-fat dried distillers grains with solubles (DG), RFDDGS plus corn oil (CO), and RFDDGS plus calcium sulfate (CaS) treatments

Item	CON	DG	CO	CaS
Ingredient, %DM				
Corn Silage	29.8	29.8	29.8	29.8
Alfalfa hay	26.6	26.6	26.6	26.6
Brome hay	2.57	2.57	2.57	2.56
Ground corn	21.8	12.9	11.5	12.6
Ground soybean hulls	0.55	0.55	0.55	0.55
RFDDGS	--	20.0	20.0	20.0
Soybean meal	11.0	--	--	--
Soypass[2]	4.59	4.59	4.59	4.59
Bloodmeal	0.46	0.46	0.46	0.46
Corn oil	--	--	1.38	--
Calcium carbonate	0.75	0.75	0.75	0.18
Calcium sulfate	--	--	--	0.93
Sodium bicarbonate	0.62	0.62	0.62	0.62
Ca-salts LCFA[3]	0.55	0.55	0.55	0.55
Magnesium oxide	0.24	0.24	0.24	0.24
Salt	0.18	0.18	0.18	0.18
Trace mineral/Vitamin premix[4]	0.18	0.18	0.18	0.18
Chemical Composition, % DM[1]				
CP	17.1	16.6	16.7	16.6
Ether Extract	3.00	4.30	5.30	4.20
NDF	30.6	34.6	34.8	33.3
Ash	7.50	7.50	7.30	7.40
Starch	27.8	24.1	22.2	24.9

[1] Values determined by Cumberland valley Analytical Services, Hagerstown, MD.
[2] Ligno Tech (Overland Park, KS).
[3] Calcium salts of long-chain fatty acids marketed as Megalac by Church & Dwight Co. Inc. Princeton, NJ.
[4] Contained 13.9 % Ca, 0.03 % P, 0.42 % Mg, 0.20 % K, 4.20 % S, 0.08 % Na, 0.03 % Cl, 445 mg/kg Fe, 60,021 mg/kg Zn, 17,375 mg/kg Cu, 43,470 mh/kg Mn, 287 mg/kg Se, 527 mg/kg Co, and 870 mg/kg I, 120,000 IU/d vitamin A, 24,000 IU/d of vitamin D, and 800 IU/d Vitamin E in total ration.

RESULTS

Table 2. DMI, milk production and composition, methane, BW and BCS[1] of control (CON), reduced-fat dried distillers grains with solubles (DG), RFDDGS plus corn oil (CO), and RFDDGS plus calcium sulfate (CaS) treatments

	Diet					
Item	CON	DG	CO	CaS	SEM[2]	*P*-value
DMI, kg/d	19.1[b]	20.1[a]	20.0[ab]	19.6[ab]	0.37	0.03
Milk yield, kg/d	26.3[b]	27.5[a]	28.3[a]	27.6[a]	0.67	< 0.01
ECM[3], kg/d	30.1[b]	31.4[a]	31.7[a]	31.0[ab]	0.66	< 0.01
Fat, %	4.70	4.64	4.53	4.57	0.13	0.09
Protein, %	3.28[a]	3.26[ab]	3.18[b]	3.20[ab]	0.07	0.38
Body Weight, kg	508.1	513.4	513.2	510.7	11.1	0.18
BCS[1]	3.23[a]	3.13[b]	3.16[ab]	3.20[ab]	0.06	0.01
CH_4 production, L/d	421.6[a]	429.5[a]	394.7[b]	381.4[ab]	14.4	0.02
CH_4/DMI, L/kg/d	22.3[a]	21.4[ab]	19.9[b]	19.6[b]	0.75	0.02
CH_4/ECM, L/kg/d	14.2[a]	13.8[ab]	12.5[bc]	12.4[c]	0.50	0.01

[1] BCS = Body Condition Score 1-5 scale according to Wildman et al. (1982)
[2] Lowest standard error of treatment means is shown
[3] Energy corrected milk = 0.327 × milk yield [kg] + 7.2 × protein [kg] adjusted for 3.5% fat and 3.2% total protein (DHI Glossary, 2014
[abc] Means within rows lacking common superscript differ ($P < 0.05$)

Table 3. Partitioning of energy for control (CON), reduced fat dry distillers grains (DG), corn oil (CO), and calcium sulfate (CaS) treatments

	Diet					
Item[1]	CON	DG	CO	CaS	SEM	*P*-value
GE intake, Mcal/d	83.5[b]	90.7[a]	91.1[a]	88.2[a]	1.67	< 0.01
DE, Mcal/d	57.2[b]	61.5[a]	61.4[a]	58.5[b]	1.13	< 0.01
ME, Mcal/d	50.5[b]	54.8[a]	55.0[a]	52.3[a]	1.07	< 0.01
Component, Mcal/d						
Feces	26.4[b]	29.2[a]	29.7[a]	29.7[a]	0.77	< 0.01
Methane	3.98[a]	4.06[a]	3.73[ab]	3.61[b]	0.14	0.07
Urine	2.67	2.66	2.67	2.56	0.10	0.79
Heat	25.1	25.8	24.4	24.9	0.62	0.43
Retained	25.4[b]	29.0[a]	30.6[a]	27.4[b]	1.07	< 0.01
Milk	22.7[b]	23.5[ab]	24.1[a]	23.4[ab]	0.58	0.20
Tissue	2.71[b]	5.51[a]	6.48[a]	3.99[ab]	0.98	0.05
GE, Mcal/kg of DM	4.38[c]	4.51[b]	4.56[a]	4.49[b]	0.01	< 0.01
DE, Mcal/kg of DM	3.00[bc]	3.06[ab]	3.07[a]	2.98[c]	0.03	0.02
ME, Mcal/kg of DM	2.65[c]	2.72[ab]	2.75[a]	2.66[bc]	0.03	< 0.01
NE$_L$, Mcal/kg of DM	1.33[c]	1.44[ab]	1.52[a]	1.38[bc]	0.04	< 0.01

[1] GE = gross energy, DE = digestible energy, ME = metabolizable energy
[2] Lowest standard error of treatment means is shown
[abc] Means within rows lacking common superscript differ ($P < 0.05$)

Table 4. Partitioning of nitrogen and nutrient digestibility for control (CON), reduced fat dry distillers grains (DG), corn oil (CO), and calcium sulfate (CaS) treatments in g/d, percentage of nitrogen intake and percent digestible

	Diet					
Item	CON	DG	CO	CaS	SEM[2]	*P*-value
Mass, g/d						
N intake	606.2	610.3	595.9	599.2	12.70	0.77
Fecal N	165.1	172.1	172.1	173.9	4.60	0.31
Urine N	200.0[a]	197.8[ab]	200.1[a]	179.4[b]	6.94	0.13
Milk N	168.0[a]	149.2[b]	167.1[a]	161.8[a]	3.50	< 0.01
N balance[1]	73.1[ab]	91.1[a]	56.6[b]	84.1[ab]	10.67	0.12
N intake, % of intake						
Fecal N	27.2[b]	28.2[ab]	29.0[a]	29.0[a]	0.51	0.02
Urine N	33.6	32.7	34.3	30.2	1.46	0.23
Milk N	28.0[a]	24.7[b]	28.5[a]	27.5[a]	0.64	< 0.01
N balance	11.2[ab]	14.4[a]	8.2[b]	13.3[a]	1.85	0.09
Digestibility, %						
DM	68.5[a]	67.2[b]	66.7[b]	66.3[b]	0.46	< 0.01
OM	69.8[a]	68.4[b]	67.9[bc]	67.2[c]	0.47	< 0.01
CP	72.6[a]	71.8[ab]	71.0[b]	71.0[b]	0.50	0.02
NDF	52.8	54.3	54.3	53.7	0.72	0.25
Starch	93.4[a]	92.9[ab]	92.2[ab]	92.1[b]	0.57	0.16

[1] Nitrogen balance = intake N – Fecal N – urine N – milk N.
[2] Lowest standard error of treatment means is shown
[abc] Means within rows lacking common superscript differ ($P < 0.05$)

CONCLUSIONS

Added oil and calcium sulfate to diets containing RFDDGS may be a viable option to reduce methane emissions without affecting energy balance in lactating dairy cows.

REFERENCES

*Beauchemin, K.A., M. Kreuzer, F. O'Mara, and T. A. McAllister. 2008. Nutritional management for enteric methane abatement: A review. Australian J. Experimental Ag. 48:21-27.
*Foth, A. J., T. Brown-Brandl, K. J. Hanford, P. S. Miller, G. Garcia Gomez, and P. J. Kononoff. 2015. Energy content of reduced-fat distillers grains with solubles for lactating dairy cows. J. Dairy Sci. In Press.
*Van Zijderveld, S.M., W.J.J. Gerrits, J.A. Apajalahti, J.R. Newbold, J. Dijkstra, R.A. Leng, and H.B. Perdok. 2010. Nitrate and sulfate: Effective alternative hydrogen sinks for mitigation of ruminal methane production in sheep. J. Dairy Sci. 93:5856-5866.

APPENDIX E: Poster for ADSA National Meeting 2017

J. Dairy Sci. 100(Suppl. 2):113.

Abstract M297: Increasing the concentration of linolenic acid in diets fed to Jersey cows in late lactation does not affect methane production

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INTRODUCTION

The Innovation Center for the U.S. Dairy has set the goal to lower the total greenhouse gas production by 25% by 2020. Methane production in lactating dairy cattle contributes to greenhouse gas emissions as they produce approximately 500 L/d (Beauchemin et al., 2008). Methane is of major interest because it has approximately 25 times more potent effect on global warming than CO_2. One strategy believed to reduce methane production by cattle is supplemental fat in the diet (Knapp et al., 2014). Biohydrogenation of fat would utilize hydrogens in the rumen and potentially reduce methane production by competing with the methanogens (Johnson and Johnson, 1995) but others believe that fat supplementation is potent to bacteria and methane is reduced because of decreased digestibility. Beauchemin et al., (2007) found that sunflower oil which is high in C18:2 reduced methane without negatively affecting digestibility compared to tallow. This may suggest that biohydrogenation is occurring and that there is potential for unsaturated FA's to further reduce methane. Therefore, research is needed to compare dietary sources of fat with differing unsaturated fat levels such as C18:3 to determine effects on methane production and diet digestibility.

OBJECTIVE

To determine the effects of feeding canola/tallow vs. extruded byproduct containing flaxseed as a fat source on methane emissions and diet digestibility in late lactation dairy cows.

HYPOTHESIS

Increased supplementation of linolenic acid would reduce methane emissions in lactating dairy cows without affecting milk production, milk composition and diet digestibility

MATERIALS AND METHODS

- 8 multiparous cows (325 ± 17 DIM) housed in tie stall barn
- Two dietary treatments differing in the type of fat in the diet (detailed in Table 2), formulated with the CPM Model.
- 28-d periods, last 7-d of each period for data collection
  - Daily feed intake (fed once a day, allowing ≈ 10% orts)
  - Daily milk production (2× milking)
  - Milk composition
  - Methane production (last 2-d of each period) collected via the indirect calorimetry method
- Data were analyzed using the MIXED procedure of SAS
  - Fixed effects: Period and Treatment
  - Random effect: Cow

Metabolism Facility at the University of Nebraska-Lincoln, Lincoln, NE

Table 1. Composition and analysis of canola meal plus tallow (CM) and extruded byproduct containing flaxseed (EXF) treatments fed to lactating Jersey cows in late lactation averaging 325 ± 17 days in milk.

	% of DM	
Item	CM	EXF
Corn Silage	27.5	27.5
Alfalfa hay	21.0	21.0
Brome hay	1.57	1.57
Ground corn	20.2	17.3
Soybean meal	5.53	6.28
Extruded byproduct containing flaxseed[1]	0.00	10.5
Canola meal	9.17	2.62
Soypass[2]	5.24	5.24
Ground soybean hulls	5.24	5.24
Tallow (Porcine)	1.78	0.00
Calcium carbonate	0.81	0.81
Sodium bicarbonate	0.67	0.67
Ca-salts LCFA[3]	0.59	0.59
Bloodmeal	0.26	0.26
Magnesium oxide	0.26	0.26
Salt	0.20	0.20
Vitamin premix[4]	0.04	0.04
Trace mineral premix[5]	0.04	0.04
Chemical Composition, % DM[6]		
CP	18.3	18.2
Ether Extract	4.70	5.00
ADF	21.1	20.9
NDF	32.2	32.4
Ash	7.73	6.99
Starch	23.0	23.8
NFC	39.6	41.7
Fatty Acids g/d[7]		
C18:3, intake	31.2	201.6
C18:3 Duodenal Flow	2.18	29.8

[1]Contained approximately 48% flaxseed, 46% ground peas, 5% alfalfa pellets, 0.1% vitamin E, 0.2% mold inhibitor, and 0.04% ethoxyquin marketed as Linpro-R by O & T farms Regina, SK, Canada.

[2]LignoTech, Overland Park, KS

[3]Calcium salts of long-chain fatty acids marketed as megalac by Church & Dwight Co. Inc. Princeton, NJ.

[4]Contained 120,000 IU/d vitamin A, 24,000 IU/d of vitamin D, and 800 IU/d Vitamin E in total ration

[5]Contained 13.9 % Ca, 0.03 % P, 0.42 % Mg, 0.20 % K, 4.20 % S, 0.08 % Na, 0.03 % Cl, 445 mg/kg Fe, 60,021 mg/kg Zn, 17,375 mg/kg Cu, 43,470 mg/kg Mn, 287 mg/kg Se, 527 mg/kg Co, and 870 mg/kg I in total ration

[6]Analyzed by Cumberland Valley Analytical Services, Hagerstown, MD

[7]Calculated using the CPM-Dairy Model V 3.0.10

RESULTS

Table 2. DMI, milk yield and composition, and gas production of treatments which included canola meal (CM) or extruded byproduct containing flaxseed (EXF) fed to lactating Jersey cows in late lactation averaging 325 ± 17 days in milk.

	Treatments			
Item	CM	EXF	SEM[1]	*P*-value
DMI, kg/d	15.0	15.7	0.71	0.26
Milk yield, kg/d	16.8	17.8	1.04	0.38
ECM[2]	23.2	24.6	1.84	0.45
Feed conversion	1.52	1.57	0.08	0.55
Fat, %	5.89	5.86	0.25	0.86
Fat yield, kg/d	0.99	1.04	0.09	0.51
Protein, %	4.09	4.07	0.12	0.69
Protein yield, kg/d	0.68	0.72	0.05	0.33
Lactose, %	4.68	4.72	0.04	0.38
MUN, mg/dl[3]	20.0	19.5	1.00	0.58
Water intake, L/d	73.4	72.1	4.50	0.77
O_2 Consumption, L/d	4143.0	4131.7	205.1	0.96
CO_2 Production, L/d	4345.2	4357.3	200.6	0.96
CH_4 Production, L/d	352.0	349.8	0.57	0.90
CH_4/NDF digested, L/kg	35.0	33.5	1.92	0.53
CH_4/NDF digested, L/kg	46.8	41.9	4.23	0.40
CH_4/Milk, L/kg	10.31	8.99	0.95	0.30

[1]Lowest standard error of treatment means is shown

[2]Energy corrected milk = 0.327 × milk yield [kg] + 7.2 × protein [kg] adjusted for 3.5% fat and 3.2% total protein (DHI Glossary, 2014)

[3]MUN = Milk urea nitrogen

Table 3. Apparent digestibility of treatments which included canola meal (CM), and extruded byproduct containing flaxseed (EXF) fed to lactating Jersey cows in late lactation averaging 325 ± 17 days in milk.

	Treatments			
	CM	EXF	SEM[1]	*P*-value
Component, %				
DM	68.0	66.9	1.07	0.48
OM	70.2	69.6	0.95	0.63
CP	74.0	72.6	1.07	0.39
NDF	52.6	54.6	2.43	0.58
Starch	96.7	95.4	0.64	0.22
Ash	41.7	37.2	3.83	0.44

[1]Lowest standard error of treatment means is shown

CONCLUSIONS

Results indicate that increasing C18:3 did not affect milk production or milk components and may not affect methane emissions or digestibility of the diet.

REFERENCES & ACKNOWLEGEMENTS

•Beauchemin, K.A., S. M. McGinn, and H. V. Petit. 2007. Methane abatement strategies for cattle: Lipid supplementation of diets. Can. J. Anim. Sci. 87:431-440.
•Beauchemin, K.A., M. Kreuzer, F. O'Mara and T. A. McAllister. 2008. Nutritional management for enteric methane abatement: A review. Australian Journal of Experimental Agriculture 48:21-27.
•Johnson, K.A., and D.E. Johnson. 1995. Methane emissions from cattle. J. Anim. Sci. 73:2483-2492.
•Knapp, J. R., G. L. Laur, P. A. Vada, W. P. Weiss, and J. M. Tricarico. 2014. Enteric methane in dairy cattle production: Quantifying the opportunities and impact of reducing emissions. J. Dairy Sci. 97:3231-3261.
•Martin, C., J. Rouel, J.P. Jouany, M. Doreau, and Y. Chilliard. 2008. Methane output and diet digestibility in response to feeding dairy cows crude linseed, extruded linseed, or linseed oil. J. Anim. Sci. 86:2642-2650.
•Partially funded by the Nebraska Environmental Trust, Lincoln, NE.

APPENDIX F

CONTROL, 20% DDGS, CORN OIL, AND CALCIUM SULFATE DIETS FOR CHAPTER 3 TREATMENTS AS CALCULATED USING THE CPM DAIRY RATION ANALYZER (2000)

CPM Diet Analysis of Control Treatment (Holstein)

CPM-Dairy — CNCPS Evaluation — 9/10/2015 10:06:52 AM

File: F:\Studies\1504DA\Diet formulations\EXP. 1504DA Control ration-4

Farm: UNL Dairy	BW: 1536 lb	DIM: 140
Ration: 1504-DA Control	BCS: 3.50	Milk: 85.00 lb
Ration By: Paul Kononoff & Jared Judy	Growth: 0.62 lb/d	Fat: 3.50 %
Organization: University of Nebraska	Lact#: 3	TP: 3.10 %

						Ingredient	DM (lb/d)
Cost ($)	0.00	IOF ($)	0.00				
DMI (lb/d)	54.5	Model	52.1	% Model	104.6	Corn Silage	21.250
ME Bal (mCal)	4.5	CP (%)	17.7	NDF (%)	28.5	Alfalfa Hay	9.900
MP Bal (g)	219.3	RUP (% CP)	40.4	ForageNDF (% NDF)	82.5	BrmdHy15Cp55Ndf7LNdf	1.000
NP / MP (%)	58.1	LCFA (%)	2.6	ForageNDF (% DM)	23.5	CornGrainGrndMed	11.900
BactMP (% MP)	51.3	EE (%)	3.2	peNDF (%)	21.5	SoybeanHullsGrnd	0.300
Rumen N Balance				Lignin (%)	3.2	Reduced Fat DDGS	0.000
Pept (g)	54	Pept & NH3 (g)	78	NFC (%)	44.9	SoybeanML47.5Solv	6.000
% rqd	123	% rqd	119	Sil Acids (%)	3.4	Soy Pass	2.500
Amino Acid Balance				Sugar (%)	3.8	BloodMeal	0.250
Met (g)	8.5	Lys (g)	40.4	Starch (%)	30.7	CalciumCarbonate	0.410
Met (% rqd)	117	Lys (% rqd)	125	Sol Fiber (%)	7.0	FatCornOil	0.000
Met (% mp)	1.97	Lys (% mp)	6.82	Lys:Met	3.47:1	SodiumBicarbonate	0.340
Possible production due to ME and MP						Megalac	0.300

	Milk(lb)	Fat (%)	TP (%)	Milk(lb)	Fat (%)	TP (%)	
Trg:	85.0	3.50	3.10	85.0	3.50	3.10	MagOx 0.130
		Yield Constant			Composition Constant		SaltNaCl 0.100
							Trace Premix 0.050
ME:	85.0	n/a	n/a	94.3	3.50	n/a	Vitamin Premix 0.050
MP:	85.0	n/a	3.47	95.1	3.50	3.10	CalciumSulfateDihyd 0.000
Adjustments based on Rulquin AA Ratios:							Total 54.480
	85.0	n/a	-0.04	-1.0	3.50	3.10	

n/a - Equations not available

Ration DM (%) 56.79 — Forage (% DM) 59.01

CPM-Dairy | Diet Summary - Both | 9/10/2015 10:06:52 AM

File: F:\Studies\1504DA\Diet formulations\EXP. 1504DA Control ration-4

Farm: UNL Dairy | BW: 1536 lb | DIM: 140
Ration: 1504-DA Control | BCS: 3.50 | Milk: 85.00 lb
Ration By: Paul Kononoff & Jared Judy | Growth: 0.62 lb/d | Fat: 3.50 %
Organization: University of Nebraska | Lact#: 3 | TP: 3.10 %

Ingredient	Cost $ / T	DM %	AF lb/d	DM lb/d	% AF	% DM
Corn Silage	0.00	36.40	58.38	21.25	60.86	39.01
Alfalfa Hay	0.00	86.50	11.45	9.90	11.93	18.17
BrmdHy15Cp55Ndf7LNdf	0.00	88.10	1.14	1.00	1.18	1.84
CornGrainGrndMed	0.00	88.00	13.52	11.90	14.10	21.84
SoybeanHullsGrnd	0.00	91.00	0.33	0.30	0.34	0.55
Reduced Fat DDGS	0.00	90.19	0.00	0.00	0.00	0.00
SoybeanML47.5Solv	0.00	90.00	6.67	6.00	6.95	11.01
Soy Pass	0.00	90.14	2.77	2.50	2.89	4.59
BloodMeal	0.00	90.00	0.28	0.25	0.29	0.46
CalciumCarbonate	0.00	99.50	0.41	0.41	0.43	0.75
FatCornOil	0.00	99.00	0.00	0.00	0.00	0.00
SodiumBicarbonate	0.00	99.50	0.34	0.34	0.36	0.62
Megalac	0.00	97.00	0.31	0.30	0.32	0.55
MagOx	0.00	99.50	0.13	0.13	0.14	0.24
SaltNaCl	0.00	99.50	0.10	0.10	0.10	0.18
Trace Premix	0.00	95.97	0.05	0.05	0.05	0.09
Vitamin Premix	0.00	95.75	0.05	0.05	0.05	0.09
CalciumSulfateDihyd	0.00	99.50	0.00	0.00	0.00	0.00
Total			95.93	54.48		

Macro Nutrients		
Nutrient	DM	AF
Dry Matter (%)	100.00	56.79
Forage (%)	59.01	33.55
Crude Prot (%)	17.68	10.04
RUP (%CP)	40.44	40.44
RDP (%CP)	59.56	59.56
RDP (%)	10.53	5.98
Sol Prot (%CP)	25.93	25.93
ME (mCal/lb)	1.21	0.69
NEl (mCal/lb)	0.78	0.44
Nem (mCal/lb)	0.78	0.44
NEg (mCal/lb)	0.51	0.29
ADF (%)	19.17	10.89
NDF (%)	28.55	16.21
For NDF (%NDF)	82.48	46.84
Forage NDF (%)	23.55	13.37
peNDF (%)	21.50	12.21
Lignin (%)	3.15	1.79
NFC (%)	44.87	25.49
Sil Acids (%)	3.39	1.93
Sugar (%)	3.79	2.15
Starch (%)	30.73	17.45
Sol Fiber (%)	6.96	3.95
EE Total (%)	3.19	1.81
EE 1 (%)	2.71	1.54
EE 2 (%)	0.01	0.01
EE 3 (%)	0.47	0.26
LCFA Total (%)	2.60	1.48
Ash (%)	8.30	4.71
Cost ($/d)	0.00	0.00
Cost ($T)	0.00	0.00

Minerals and Vitamins		
Nutrient	DM	AF
Dry Matter (%)	100.00	56.79
Calcium (%)	0.77	0.44
Phosphorus (%)	0.36	0.20
Magnesium (%)	0.32	0.18
Potassium (%)	1.56	0.89
Sulfur (%)	0.20	0.12
Sodium (%)	0.27	0.15
Chlorine (%)	0.28	0.16
Iron (ppm)	247.33	140.46
Zinc (ppm)	81.24	46.14
Copper (ppm)	22.30	12.66
Manganese (ppm)	63.13	35.85
Selenium (ppm)	0.29	0.16
Cobalt (ppm)	0.50	0.28
Iodine (ppm)	0.81	0.46
Vitamin A (KIU/lb)	2.40	1.36
Vitamin D (KIU/lb)	0.61	0.35
Vitamin E (IU/lb)	19.31	10.97
DCAD1 (meq/100g)	30.88	17.54
DCAD2 (meq/100g)	35.22	20.00
Cost ($/d)	0.00	0.00
Cost ($T)	0.00	0.00

CPM Diet Analysis of Distillers Grains with Solubles Treatment (Holstein)

CPM-Dairy | CNCPS Evaluation | 5/26/2015 11:36:13 AM

File: F:\1504DA\Diet formulations\EXP. 1504DA 20RFDDGS-3

Farm: UNL Dairy | BW: 1536 lb | DIM: 140
Ration: 1504DA 20RFDDGS | BCS: 3.50 | Milk: 85.00 lb
Ration By: Paul Kononoff & Jared Judy | Growth: 0.62 lb/d | Fat: 3.50 %
Organization: University of Nebraska | Lact#: 3 | TP: 3.10 %

						Ingredient	DM (lb/d)
Cost ($)	0.00	IOF ($)	0.00				
DMI (lb/d)	54.5	Model	52.1	% Model	104.6	Corn Silage	21.250
ME Bal (mCal)	1.5	CP (%)	17.0	NDF (%)	33.1	Alfalfa Hay	9.900
MP Bal (g)	15.2	RUP (% CP)	44.8	ForageNDF (% NDF)	71.1	BrmdHy15Cp55Ndf7LNdf	1.000
NP / MP (%)	64.5	LCFA (%)	3.1	ForageNDF (% DM)	23.5	CornGrainGrndMed	7.000
BactMP (% MP)	49.1	EE (%)	3.9	peNDF (%)	22.0	SoybeanHullsGrnd	0.300
Rumen N Balance				Lignin (%)	3.7	Reduced Fat DDGS	10.900
Pept (g)	24	Pept & NH3 (g)	69	NFC (%)	40.3	SoybeanML47.5Solv	0.000
% rqd	112	% rqd	118	Sil Acids (%)	3.4	Soy Pass	2.500
Amino Acid Balance				Sugar (%)	2.9	BloodMeal	0.250
Met (g)	3.2	Lys (g)	2.2	Starch (%)	25.1	CalciumCarbonate	0.410
Met (% rqd)	106	Lys (% rqd)	101	Sol Fiber (%)	9.0	FatCornOil	0.000
Met (% mp)	1.92	Lys (% mp)	5.96	Lys:Met	3.11:1	SodiumBicarbonate	0.340
Possible production due to ME and MP						Megalac	0.300

	Milk(lb)	Fat (%)	TP (%)	Milk(lb)	Fat (%)	TP (%)	
Trg:	85.0	3.50	3.10	85.0	3.50	3.10	MagOx 0.130
		Yield Constant			Composition Constant		SaltNaCl 0.100
							Trace Premix 0.050
ME:	85.0	n/a	n/a	88.1	3.50	n/a	Vitamin Premix 0.050
MP:	85.0	n/a	3.13	85.7	3.50	3.10	CalciumSulfateDihyd 0.000
Adjustments based on Rulquin AA Ratios:							Total 54.480
	85.0	n/a	-0.16	-4.4	3.50	3.10	

n/a - Equations not available

Ration DM (%) 56.88 | Forage (% DM) 59.01

CPM-Dairy | Diet Summary - Both | 5/26/2015 11:36:13 AM

File: F:\1504DA\Diet formulations\EXP. 1504DA 20RFDDGS-3

Farm: UNL Dairy | BW: 1536 lb | DIM: 140

Ration: 1504DA 20RFDDGS | BCS: 3.50 | Milk: 85.00 lb

Ration By: Paul Kononoff & Jared Judy | Growth: 0.62 lb/d | Fat: 3.50 %

Organization: University of Nebraska | Lact#: 3 | TP: 3.10 %

	Cost		AF	DM		
Ingredient	$ / T	DM %	lb/d	lb/d	% AF	% DM
Corn Silage	0.00	36.40	58.38	21.25	60.95	39.01
Alfalfa Hay	0.00	86.50	11.45	9.90	11.95	18.17
BrmdHy15Cp55Ndf7LNdf	0.00	88.10	1.14	1.00	1.19	1.84
CornGrainGrndMed	0.00	88.00	7.95	7.00	8.31	12.85
SoybeanHullsGrnd	0.00	91.00	0.33	0.30	0.34	0.55
Reduced Fat DDGS	0.00	90.19	12.09	10.90	12.62	20.01
SoybeanML47.5Solv	0.00	90.00	0.00	0.00	0.00	0.00
Soy Pass	0.00	90.14	2.77	2.50	2.90	4.59
BloodMeal	0.00	90.00	0.28	0.25	0.29	0.46
CalciumCarbonate	0.00	99.50	0.41	0.41	0.43	0.75
FatCornOil	0.00	99.00	0.00	0.00	0.00	0.00
SodiumBicarbonate	0.00	99.50	0.34	0.34	0.36	0.62
Megalac	0.00	97.00	0.31	0.30	0.32	0.55
MagOx	0.00	99.50	0.13	0.13	0.14	0.24
SaltNaCl	0.00	99.50	0.10	0.10	0.10	0.18
Trace Premix	0.00	95.97	0.05	0.05	0.05	0.09
Vitamin Premix	0.00	95.75	0.05	0.05	0.05	0.09
CalciumSulfateDihyd	0.00	99.50	0.00	0.00	0.00	0.00
Total			95.78	54.48		

Macro Nutrients		
Nutrient	DM	AF
Dry Matter (%)	100.00	56.88
Forage (%)	59.01	33.60
Crude Prot (%)	16.99	9.66
RUP (%CP)	44.83	44.83
RDP (%CP)	55.17	55.17
RDP (%)	9.37	5.33
Sol Prot (%CP)	27.33	27.33
ME (mCal/lb)	1.15	0.65
NEl (mCal/lb)	0.74	0.42
Nem (mCal/lb)	0.74	0.42
NEg (mCal/lb)	0.47	0.27
ADF (%)	20.50	11.66
NDF (%)	33.10	18.83
For NDF (%NDF)	71.13	40.46
Forage NDF (%)	23.55	13.39
peNDF (%)	22.03	12.53
Lignin (%)	3.74	2.13
NFC (%)	40.32	22.93
Sil Acids (%)	3.39	1.93
Sugar (%)	2.89	1.64
Starch (%)	25.09	14.27
Sol Fiber (%)	8.96	5.09
EE Total (%)	3.93	2.24
EE 1 (%)	3.46	1.97
EE 2 (%)	0.01	0.01
EE 3 (%)	0.47	0.26
LCFA Total (%)	3.11	1.77
Ash (%)	8.70	4.95
Cost ($/d)	0.00	0.00
Cost ($T)	0.00	0.00

Minerals and Vitamins		
Nutrient	DM	AF
Dry Matter (%)	100.00	56.88
Calcium (%)	0.75	0.43
Phosphorus (%)	0.47	0.27
Magnesium (%)	0.34	0.20
Potassium (%)	1.53	0.87
Sulfur (%)	0.34	0.19
Sodium (%)	0.32	0.18
Chlorine (%)	0.31	0.17
Iron (ppm)	243.95	138.76
Zinc (ppm)	78.81	44.83
Copper (ppm)	21.85	12.43
Manganese (ppm)	62.59	35.60
Selenium (ppm)	0.28	0.16
Cobalt (ppm)	0.49	0.28
Iodine (ppm)	0.81	0.46
Vitamin A (KIU/lb)	2.40	1.37
Vitamin D (KIU/lb)	0.61	0.35
Vitamin E (IU/lb)	19.31	10.98
DCAD1 (meq/100g)	23.58	13.41
DCAD2 (meq/100g)	28.21	16.05
Cost ($/d)	0.00	0.00
Cost ($T)	0.00	0.00

CPM Diet Analysis of Corn Oil Treatment (Holstein)

CPM-Dairy | CNCPS Evaluation | 5/26/2015 11:38:41 AM

File: F:\1504DA\Diet formulations\EXP. 1504DA 20RFDDGSplusCornOil-4

Farm: UNL Dairy | BW: 1536 lb | DIM: 140

Ration: 1504-DA 20RFDDGS plus Corn Oil | BCS: 3.50 | Milk: 85.00 lb

Ration By: Paul Kononoff & Jared Judy | Growth: 0.62 lb/d | Fat: 3.50 %

Organization: University of Nebraska | Lact#: 3 | TP: 3.10 %

						Ingredient	DM (lb/d)
Cost ($)	0.00	IOF ($)	0.00				
DMI (lb/d)	54.5	Model	52.1	% Model	104.6	Corn Silage	21.250
ME Bal (mCal)	3.2	CP (%)	16.9	NDF (%)	33.0	Alfalfa Hay	9.900
MP Bal (g)	-23.9	RUP (% CP)	44.8	ForageNDF (% NDF)	71.4	BrmdHy15Cp55Ndf7LNdf	1.000
NP / MP (%)	65.9	LCFA (%)	4.3	ForageNDF (% DM)	23.5	CornGrainGrndMed	6.250
BactMP (% MP)	48.8	EE (%)	5.2	peNDF (%)	22.0	SoybeanHullsGrnd	0.300
Rumen N Balance				Lignin (%)	3.7	Reduced Fat DDGS	10.900
Pept (g)	27	Pept & NH3 (g)	74	NFC (%)	39.3	SoybeanML47.5Solv	0.000
% rqd	114	% rqd	120	Sil Acids (%)	3.4	Soy Pass	2.500
Amino Acid Balance				Sugar (%)	2.9	BloodMeal	0.250
Met (g)	2.3	Lys (g)	-0.2	Starch (%)	24.1	CalciumCarbonate	0.410
Met (% rqd)	104	Lys (% rqd)	100	Sol Fiber (%)	8.9	FatCornOil	0.750
Met (% mp)	1.91	Lys (% mp)	5.96	Lys:Met	3.11:1	SodiumBicarbonate	0.340
Possible production due to ME and MP						Megalac	0.300
	Milk(lb)	Fat (%)	TP (%)	Milk(lb)	Fat (%) TP (%)	MagOx	0.130
Trg:	85.0	3.50	3.10	85.0	3.50 3.10	SaltNaCl	0.100
		Yield Constant			Composition Constant	Trace Premix	0.050
ME:	85.0	n/a	n/a	91.6	3.50 n/a	Vitamin Premix	0.050
MP:	85.0	n/a	3.06	83.9	3.50 3.10	CalciumSulfateDihyd	0.000
Adjustments based on Rulquin AA Ratios:						Total	54.480
	85.0	n/a	-0.16	-4.5	3.50 3.10		
n/a - Equations not available							
Ration DM (%)	56.94		Forage (% DM)		59.01		

CPM-Dairy | Diet Summary - Both | 5/26/2015 11:38:41 AM

File: F:\1504DA\Diet formulations\EXP. 1504DA 20RFDDGSplusCornOil-4

Farm: UNL Dairy	BW: 1536 lb	DIM: 140
Ration: 1504-DA 20RFDDGS plus Corn Oil	BCS: 3.50	Milk: 85.00 lb
Ration By: Paul Kononoff & Jared Judy	Growth: 0.62 lb/d	Fat: 3.50 %
Organization: University of Nebraska	Lact#: 3	TP: 3.10 %

	Cost		AF	DM		
Ingredient	$ / T	DM %	lb/d	lb/d	% AF	% DM
Corn Silage	0.00	36.40	58.38	21.25	61.01	39.01
Alfalfa Hay	0.00	86.50	11.45	9.90	11.96	18.17
BrmdHy15Cp55Ndf7LNdf	0.00	88.10	1.14	1.00	1.19	1.84
CornGrainGrndMed	0.00	88.00	7.10	6.25	7.42	11.47
SoybeanHullsGrnd	0.00	91.00	0.33	0.30	0.34	0.55
Reduced Fat DDGS	0.00	90.19	12.09	10.90	12.63	20.01
SoybeanML47.5Solv	0.00	90.00	0.00	0.00	0.00	0.00
Soy Pass	0.00	90.14	2.77	2.50	2.90	4.59
BloodMeal	0.00	90.00	0.28	0.25	0.29	0.46
CalciumCarbonate	0.00	99.50	0.41	0.41	0.43	0.75
FatCornOil	0.00	99.00	0.76	0.75	0.79	1.38
SodiumBicarbonate	0.00	99.50	0.34	0.34	0.36	0.62
Megalac	0.00	97.00	0.31	0.30	0.32	0.55
MagOx	0.00	99.50	0.13	0.13	0.14	0.24
SaltNaCl	0.00	99.50	0.10	0.10	0.11	0.18
Trace Premix	0.00	95.97	0.05	0.05	0.05	0.09
Vitamin Premix	0.00	95.75	0.05	0.05	0.05	0.09
CalciumSulfateDihyd	0.00	99.50	0.00	0.00	0.00	0.00
Total			95.68	54.48		

Macro Nutrients		
Nutrient	DM	AF
Dry Matter (%)	100.00	56.94
Forage (%)	59.01	33.63
Crude Prot (%)	16.86	9.60
RUP (%CP)	44.83	44.83
RDP (%CP)	55.17	55.17
RDP (%)	9.30	5.30
Sol Prot (%CP)	27.39	27.39
ME (mCal/lb)	1.18	0.67
NEl (mCal/lb)	0.76	0.43
Nem (mCal/lb)	0.76	0.43
NEg (mCal/lb)	0.49	0.28
ADF (%)	20.45	11.64
NDF (%)	32.98	18.78
For NDF (%NDF)	71.40	40.65
Forage NDF (%)	23.55	13.41
peNDF (%)	21.98	12.52
Lignin (%)	3.74	2.13
NFC (%)	39.26	22.35
Sil Acids (%)	3.39	1.93
Sugar (%)	2.86	1.63
Starch (%)	24.06	13.70
Sol Fiber (%)	8.95	5.09
EE Total (%)	5.25	2.99
EE 1 (%)	4.77	2.72
EE 2 (%)	0.01	0.01
EE 3 (%)	0.47	0.26
LCFA Total (%)	4.27	2.43
Ash (%)	8.68	4.94
Cost ($/d)	0.00	0.00
Cost ($T)	0.00	0.00

Minerals and Vitamins		
Nutrient	DM	AF
Dry Matter (%)	100.00	56.94
Calcium (%)	0.75	0.43
Phosphorus (%)	0.46	0.26
Magnesium (%)	0.34	0.19
Potassium (%)	1.53	0.87
Sulfur (%)	0.34	0.19
Sodium (%)	0.32	0.18
Chlorine (%)	0.30	0.17
Iron (ppm)	243.21	138.48
Zinc (ppm)	78.44	44.66
Copper (ppm)	21.78	12.40
Manganese (ppm)	62.51	35.59
Selenium (ppm)	0.28	0.16
Cobalt (ppm)	0.49	0.28
Iodine (ppm)	0.81	0.46
Vitamin A (KIU/lb)	2.40	1.37
Vitamin D (KIU/lb)	0.61	0.35
Vitamin E (IU/lb)	19.31	10.99
DCAD1 (meq/100g)	23.57	13.42
DCAD2 (meq/100g)	28.24	16.08
Cost ($/d)	0.00	0.00
Cost ($T)	0.00	0.00

CPM Diet Analysis of Calcium Sulfate Treatment (Holstein)

CPM-Dairy — CNCPS Evaluation — 5/26/2015 11:40:40 AM

File: F:\1504DA\Diet formulations\EXP. 1504DA 20RFDDGSplusCalciumSulfate-3

Farm: UNL Dairy	BW: 1536 lb	DIM: 140
Ration: 1504-DA Calcium Sulfate Grain Mix 1505	BCS: 3.50	Milk: 85.00 lb
Ration By: Paul Kononoff & Jared Judy	Growth: 0.62 lb/d	Fat: 3.50 %
Organization: University of Nebraska	Lact#: 3	TP: 3.10 %

Cost ($)	0.00	IOF ($)	0.00			Ingredient	DM (lb/d)
DMI (lb/d)	54.6	Model	52.1	% Model	104.8	Corn Silage	21.250
ME Bal (mCal)	1.4	CP (%)	17.0	NDF (%)	33.0	Alfalfa Hay	9.900
MP Bal (g)	8.4	RUP (% CP)	44.9	ForageNDF (% NDF)	71.1	BrmdHy15Cp55Ndf7LNdf	1.000
NP / MP (%)	64.7	LCFA (%)	3.1	ForageNDF (% DM)	23.5	CornGrainGrndMed	6.900
BactMP (% MP)	49.0	EE (%)	3.9	peNDF (%)	22.0	SoybeanHullsGrnd	0.300
Rumen N Balance				Lignin (%)	3.7	Reduced Fat DDGS	10.940
Pept (g)	25	Pept & NH3 (g)	70	NFC (%)	40.1	SoybeanML47.5Solv	0.000
% rqd	112	% rqd	119	Sil Acids (%)	3.4	Soy Pass	2.500
Amino Acid Balance				Sugar (%)	2.9	BloodMeal	0.250
Met (g)	3.0	Lys (g)	1.7	Starch (%)	24.9	CalciumCarbonate	0.100
Met (% rqd)	106	Lys (% rqd)	101	Sol Fiber (%)	8.9	FatCornOil	0.000
Met (% mp)	1.92	Lys (% mp)	5.96	Lys:Met	3.11:1	SodiumBicarbonate	0.340
Possible production due to ME and MP						Megalac	0.300
	Milk(lb)	Fat (%)	TP (%)	Milk(lb)	Fat (%) TP (%)	MagOx	0.130
Trg:	85.0	3.50	3.10	85.0	3.50 3.10	SaltNaCl	0.100
		Yield Constant			Composition Constant	Trace Premix	0.050
ME:	85.0	n/a	n/a	87.8	3.50 n/a	Vitamin Premix	0.050
MP:	85.0	n/a	3.11	85.4	3.50 3.10	CalciumSulfateDihyd	0.510
Adjustments based on Rulquin AA Ratios:						Total	54.620
	85.0	n/a	-0.16	-4.5	3.50 3.10		
n/a - Equations not available							
Ration DM (%)	56.95		Forage (% DM)		58.86		

CPM-Dairy | Diet Summary - Both | 5/26/2015 11:40:40 AM

File: F:\1504DA\Diet formulations\EXP. 1504DA 20RFDDGSplusCalciumSulfate-3

Farm: UNL Dairy | BW: 1536 lb | DIM: 140

Ration: 1504-DA Calcium Sulfate Grain Mix 1505 | BCS: 3.50 | Milk: 85.00 lb

Ration By: Paul Kononoff & Jared Judy | Growth: 0.62 lb/d | Fat: 3.50 %

Organization: University of Nebraska | Lact#: 3 | TP: 3.10 %

	Cost		AF	DM		
Ingredient	$ / T	DM %	lb/d	lb/d	% AF	% DM
Corn Silage	0.00	36.40	58.38	21.25	60.87	38.91
Alfalfa Hay	0.00	86.50	11.45	9.90	11.93	18.13
BrmdHy15Cp55Ndf7LNdf	0.00	88.10	1.14	1.00	1.18	1.83
CornGrainGrndMed	0.00	88.00	7.84	6.90	8.18	12.63
SoybeanHullsGrnd	0.00	91.00	0.33	0.30	0.34	0.55
Reduced Fat DDGS	0.00	90.19	12.13	10.94	12.65	20.03
SoybeanML47.5Solv	0.00	90.00	0.00	0.00	0.00	0.00
Soy Pass	0.00	90.14	2.77	2.50	2.89	4.58
BloodMeal	0.00	90.00	0.28	0.25	0.29	0.46
CalciumCarbonate	0.00	99.50	0.10	0.10	0.10	0.18
FatCornOil	0.00	99.00	0.00	0.00	0.00	0.00
SodiumBicarbonate	0.00	99.50	0.34	0.34	0.36	0.62
Megalac	0.00	97.00	0.31	0.30	0.32	0.55
MagOx	0.00	99.50	0.13	0.13	0.14	0.24
SaltNaCl	0.00	99.50	0.10	0.10	0.10	0.18
Trace Premix	0.00	95.97	0.05	0.05	0.05	0.09
Vitamin Premix	0.00	95.75	0.05	0.05	0.05	0.09
CalciumSulfateDihyd	0.00	99.50	0.51	0.51	0.53	0.93
Total			95.91	54.62		

Macro Nutrients		
Nutrient	DM	AF
Dry Matter (%)	100.00	56.95
Forage (%)	58.86	33.55
Crude Prot (%)	16.95	9.65
RUP (%CP)	44.86	44.86
RDP (%CP)	55.14	55.14
RDP (%)	9.35	5.32
Sol Prot (%CP)	27.33	27.33
ME (mCal/lb)	1.15	0.65
NEl (mCal/lb)	0.74	0.42
Nem (mCal/lb)	0.74	0.42
NEg (mCal/lb)	0.46	0.26
ADF (%)	20.45	11.65
NDF (%)	33.02	18.81
For NDF (%NDF)	71.11	40.50
Forage NDF (%)	23.49	13.37
peNDF (%)	21.97	12.51
Lignin (%)	3.73	2.13
NFC (%)	40.10	22.83
Sil Acids (%)	3.39	1.93
Sugar (%)	2.88	1.64
Starch (%)	24.89	14.17
Sol Fiber (%)	8.95	5.09
EE Total (%)	3.92	2.23
EE 1 (%)	3.44	1.96
EE 2 (%)	0.01	0.01
EE 3 (%)	0.46	0.26
LCFA Total (%)	3.10	1.76
Ash (%)	9.05	5.15
Cost ($/d)	0.00	0.00
Cost ($T)	0.00	0.00

Minerals and Vitamins		
Nutrient	DM	AF
Dry Matter (%)	100.00	56.95
Calcium (%)	0.72	0.41
Phosphorus (%)	0.47	0.27
Magnesium (%)	0.34	0.19
Potassium (%)	1.53	0.87
Sulfur (%)	0.49	0.28
Sodium (%)	0.32	0.18
Chlorine (%)	0.30	0.17
Iron (ppm)	241.59	137.58
Zinc (ppm)	78.56	44.74
Copper (ppm)	21.78	12.41
Manganese (ppm)	60.71	34.58
Selenium (ppm)	0.28	0.16
Cobalt (ppm)	0.49	0.28
Iodine (ppm)	0.81	0.46
Vitamin A (KIU/lb)	2.39	1.36
Vitamin D (KIU/lb)	0.61	0.35
Vitamin E (IU/lb)	19.26	10.97
DCAD1 (meq/100g)	13.93	7.93
DCAD2 (meq/100g)	22.19	12.63
Cost ($/d)	0.00	0.00
Cost ($T)	0.00	0.00

CPM Diet Analysis of Control Treatment (Jersey)

CPM-Dairy | CNCPS Evaluation | 10/2/2015 1:41:47 PM

File: F:\Studies\1504DA\Diet formulations\EXP. 1504DA Control ration-4Jersey

Farm: UNL Dairy	BW: 1536 lb	DIM: 140
Ration: 1504-DA Control	BCS: 3.50	Milk: 85.00 lb
Ration By: Paul Kononoff & Jared Judy	Growth: 0.62 lb/d	Fat: 3.50 %
Organization: University of Nebraska	Lact#: 3	TP: 3.10 %

						Ingredient	DM (lb/d)
Cost ($)	0.00	IOF ($)	0.00				
DMI (lb/d)	54.4	Model	52.1	% Model	104.4	Corn Silage	21.250
ME Bal (mCal)	4.5	CP (%)	17.7	NDF (%)	28.5	Alfalfa Hay	9.900
MP Bal (g)	220.8	RUP (% CP)	40.4	ForageNDF (% NDF)	82.6	BrmdHy15Cp55Ndf7LNdf	1.000
NP / MP (%)	58.0	LCFA (%)	2.6	ForageNDF (% DM)	23.6	CornGrainGrndMed	11.900
BactMP (% MP)	51.3	EE (%)	3.2	peNDF (%)	21.5	SoybeanHullsGrnd	0.300
Rumen N Balance				Lignin (%)	3.2	Reduced Fat DDGS	0.000
Pept (g)	54	Pept & NH3 (g)	78	NFC (%)	44.9	SoybeanML47.5Solv	6.000
% rqd	123	% rqd	119	Sil Acids (%)	3.4	Soy Pass	2.500
Amino Acid Balance				Sugar (%)	3.8	BloodMeal	0.250
Met (g)	8.6	Lys (g)	40.5	Starch (%)	30.8	CalciumCarbonate	0.410
Met (% rqd)	117	Lys (% rqd)	125	Sol Fiber (%)	7.0	FatCornOil	0.000
Met (% mp)	1.97	Lys (% mp)	6.82	Lys:Met	3.47:1	SodiumBicarbonate	0.340
Possible production due to ME and MP						Megalac	0.300
	Milk(lb)	Fat (%)	TP (%)	Milk(lb)	Fat (%) TP (%)	MagOx	0.130
Trg:	85.0	3.50	3.10	85.0	3.50 3.10	SaltNaCl	0.100
		Yield Constant			Composition Constant	Trace Premix	0.020
ME:	85.0	n/a	n/a	94.3	3.50 n/a	Vitamin Premix	0.022
MP:	85.0	n/a	3.47	95.2	3.50 3.10	CalciumSulfateDihyd	0.000
Adjustments based on Rulquin AA Ratios:						Total	54.422
	85.0	n/a	-0.04	-1.0	3.50 3.10		
n/a - Equations not available							
Ration DM (%)	56.77		Forage (% DM)		59.08		

CPM-Dairy | Diet Summary - Both | 10/2/2015 1:41:47 PM

File: F:\Studies\1504DA\Diet formulations\EXP. 1504DA Control ration-4Jersey

Farm: UNL Dairy | BW: 1536 lb | DIM: 140
Ration: 1504-DA Control | BCS: 3.50 | Milk: 85.00 lb
Ration By: Paul Kononoff & Jared Judy | Growth: 0.62 lb/d | Fat: 3.50 %
Organization: University of Nebraska | Lact#: 3 | TP: 3.10 %

	Cost		AF	DM		
Ingredient	$ / T	DM %	lb/d	lb/d	% AF	% DM
Corn Silage	0.00	36.40	58.38	21.25	60.90	39.05
Alfalfa Hay	0.00	86.50	11.45	9.90	11.94	18.19
BrmdHy15Cp55Ndf7LNdf	0.00	88.10	1.14	1.00	1.18	1.84
CornGrainGrndMed	0.00	88.00	13.52	11.90	14.11	21.87
SoybeanHullsGrnd	0.00	91.00	0.33	0.30	0.34	0.55
Reduced Fat DDGS	0.00	90.19	0.00	0.00	0.00	0.00
SoybeanML47.5Solv	0.00	90.00	6.67	6.00	6.95	11.02
Soy Pass	0.00	90.14	2.77	2.50	2.89	4.59
BloodMeal	0.00	90.00	0.28	0.25	0.29	0.46
CalciumCarbonate	0.00	99.50	0.41	0.41	0.43	0.75
FatCornOil	0.00	99.00	0.00	0.00	0.00	0.00
SodiumBicarbonate	0.00	99.50	0.34	0.34	0.36	0.62
Megalac	0.00	97.00	0.31	0.30	0.32	0.55
MagOx	0.00	99.50	0.13	0.13	0.14	0.24
SaltNaCl	0.00	99.50	0.10	0.10	0.10	0.18
Trace Premix	0.00	95.97	0.02	0.02	0.02	0.04
Vitamin Premix	0.00	95.75	0.02	0.02	0.02	0.04
CalciumSulfateDihyd	0.00	99.50	0.00	0.00	0.00	0.00
Total			95.87	54.42		

Macro Nutrients		
Nutrient	DM	AF
Dry Matter (%)	100.00	56.77
Forage (%)	59.08	33.57
Crude Prot (%)	17.70	10.05
RUP (%CP)	40.43	40.43
RDP (%CP)	59.57	59.57
RDP (%)	10.54	5.99
Sol Prot (%CP)	25.93	25.93
ME (mCal/lb)	1.21	0.69
NEl (mCal/lb)	0.78	0.44
Nem (mCal/lb)	0.78	0.44
NEg (mCal/lb)	0.51	0.29
ADF (%)	19.16	10.88
NDF (%)	28.55	16.21
For NDF (%NDF)	82.56	46.87
Forage NDF (%)	23.57	13.38
peNDF (%)	21.52	12.22
Lignin (%)	3.15	1.79
NFC (%)	44.92	25.50
Sil Acids (%)	3.40	1.93
Sugar (%)	3.79	2.15
Starch (%)	30.76	17.46
Sol Fiber (%)	6.97	3.96
EE Total (%)	3.19	1.81
EE 1 (%)	2.72	1.54
EE 2 (%)	0.01	0.01
EE 3 (%)	0.47	0.26
LCFA Total (%)	2.60	1.48
Ash (%)	8.20	4.66
Cost ($/d)	0.00	0.00
Cost ($T)	0.00	0.00

Minerals and Vitamins		
Nutrient	DM	AF
Dry Matter (%)	100.00	56.77
Calcium (%)	0.75	0.43
Phosphorus (%)	0.36	0.20
Magnesium (%)	0.32	0.18
Potassium (%)	1.56	0.89
Sulfur (%)	0.20	0.11
Sodium (%)	0.27	0.15
Chlorine (%)	0.28	0.16
Iron (ppm)	247.17	140.32
Zinc (ppm)	48.24	27.38
Copper (ppm)	12.75	7.24
Manganese (ppm)	39.23	22.27
Selenium (ppm)	0.13	0.07
Cobalt (ppm)	0.21	0.12
Iodine (ppm)	0.33	0.19
Vitamin A (KIU/lb)	1.06	0.60
Vitamin D (KIU/lb)	0.27	0.15
Vitamin E (IU/lb)	8.51	4.83
DCAD1 (meq/100g)	31.05	17.63
DCAD2 (meq/100g)	35.20	19.98
Cost ($/d)	0.00	0.00
Cost ($T)	0.00	0.00

CPM Diet Analysis of Distillers Grain with Solubles Treatment (Jersey)

CPM-Dairy | CNCPS Evaluation | 10/2/2015 1:42:31 PM

File: F:\Studies\1504DA\Diet formulations\EXP. 1504DA 20RFDDGS-4Jersey

Farm: UNL Dairy | BW: 1536 lb | DIM: 140
Ration: 1504DA 20RFDDGS | BCS: 3.50 | Milk: 85.00 lb
Ration By: Paul Kononoff & Jared Judy | Growth: 0.62 lb/d | Fat: 3.50 %
Organization: University of Nebraska | Lact#: 3 | TP: 3.10 %

						Ingredient	DM (lb/d)
Cost ($)	0.00	IOF ($)	0.00				
DMI (lb/d)	54.4	Model	52.1	% Model	104.4	Corn Silage	21.250
ME Bal (mCal)	1.5	CP (%)	17.0	NDF (%)	33.1	Alfalfa Hay	9.900
MP Bal (g)	16.8	RUP (% CP)	44.8	ForageNDF (% NDF)	71.2	BrmdHy15Cp55Ndf7LNdf	1.000
NP / MP (%)	64.4	LCFA (%)	3.1	ForageNDF (% DM)	23.6	CornGrainGrndMed	7.000
BactMP (% MP)	49.1	EE (%)	3.9	peNDF (%)	22.1	SoybeanHullsGrnd	0.300
Rumen N Balance				Lignin (%)	3.7	Reduced Fat DDGS	10.900
Pept (g)	24	Pept & NH3 (g)	68	NFC (%)	40.4	SoybeanML47.5Solv	0.000
% rqd	112	% rqd	118	Sil Acids (%)	3.4	Soy Pass	2.500
Amino Acid Balance				Sugar (%)	2.9	BloodMeal	0.250
Met (g)	3.2	Lys (g)	2.4	Starch (%)	25.1	CalciumCarbonate	0.410
Met (% rqd)	106	Lys (% rqd)	101	Sol Fiber (%)	9.0	FatCornOil	0.000
Met (% mp)	1.92	Lys (% mp)	5.97	Lys:Met	3.11:1	SodiumBicarbonate	0.340
Possible production due to ME and MP						Megalac	0.300
	Milk(lb)	Fat (%)	TP (%)	Milk(lb)	Fat (%) TP (%)	MagOx	0.130
Trg:	85.0	3.50	3.10	85.0	3.50 3.10	SaltNaCl	0.100
		Yield Constant			Composition Constant	Trace Premix	0.020
ME:	85.0	n/a	n/a	88.1	3.50 n/a	Vitamin Premix	0.022
MP:	85.0	n/a	3.13	85.8	3.50 3.10	CalciumSulfateDihyd	0.000
Adjustments based on Rulquin AA Ratios:						Total	54.422
	85.0	n/a	-0.16	-4.4	3.50 3.10		
n/a - Equations not available							
Ration DM (%)	56.86		Forage (% DM)		59.08		

CPM-Dairy | Diet Summary - Both | 10/2/2015 1:42:31 PM

File: F:\Studies\1504DA\Diet formulations\EXP. 1504DA 20RFDDGS-4Jersey

Farm: UNL Dairy | BW: 1536 lb | DIM: 140

Ration: 1504DA 20RFDDGS | BCS: 3.50 | Milk: 85.00 lb

Ration By: Paul Kononoff & Jared Judy | Growth: 0.62 lb/d | Fat: 3.50 %

Organization: University of Nebraska | Lact#: 3 | TP: 3.10 %

	Cost		AF	DM		
Ingredient	$ / T	DM %	lb/d	lb/d	% AF	% DM
Corn Silage	0.00	36.40	58.38	21.25	60.99	39.05
Alfalfa Hay	0.00	86.50	11.45	9.90	11.96	18.19
BrmdHy15Cp55Ndf7LNdf	0.00	88.10	1.14	1.00	1.19	1.84
CornGrainGrndMed	0.00	88.00	7.95	7.00	8.31	12.86
SoybeanHullsGrnd	0.00	91.00	0.33	0.30	0.34	0.55
Reduced Fat DDGS	0.00	90.19	12.09	10.90	12.63	20.03
SoybeanML47.5Solv	0.00	90.00	0.00	0.00	0.00	0.00
Soy Pass	0.00	90.14	2.77	2.50	2.90	4.59
BloodMeal	0.00	90.00	0.28	0.25	0.29	0.46
CalciumCarbonate	0.00	99.50	0.41	0.41	0.43	0.75
FatCornOil	0.00	99.00	0.00	0.00	0.00	0.00
SodiumBicarbonate	0.00	99.50	0.34	0.34	0.36	0.62
Megalac	0.00	97.00	0.31	0.30	0.32	0.55
MagOx	0.00	99.50	0.13	0.13	0.14	0.24
SaltNaCl	0.00	99.50	0.10	0.10	0.10	0.18
Trace Premix	0.00	95.97	0.02	0.02	0.02	0.04
Vitamin Premix	0.00	95.75	0.02	0.02	0.02	0.04
CalciumSulfateDihyd	0.00	99.50	0.00	0.00	0.00	0.00
Total			95.72	54.42		

Macro Nutrients		
Nutrient	DM	AF
Dry Matter (%)	100.00	56.86
Forage (%)	59.08	33.62
Crude Prot (%)	17.01	9.67
RUP (%CP)	44.82	44.82
RDP (%CP)	55.18	55.18
RDP (%)	9.38	5.34
Sol Prot (%CP)	27.33	27.33
ME (mCal/lb)	1.15	0.66
NEl (mCal/lb)	0.74	0.42
Nem (mCal/lb)	0.74	0.42
NEg (mCal/lb)	0.47	0.27
ADF (%)	20.50	11.66
NDF (%)	33.11	18.82
For NDF (%NDF)	71.19	40.48
Forage NDF (%)	23.57	13.40
peNDF (%)	22.06	12.54
Lignin (%)	3.75	2.13
NFC (%)	40.36	22.95
Sil Acids (%)	3.40	1.93
Sugar (%)	2.89	1.64
Starch (%)	25.11	14.28
Sol Fiber (%)	8.97	5.10
EE Total (%)	3.93	2.24
EE 1 (%)	3.46	1.97
EE 2 (%)	0.01	0.01
EE 3 (%)	0.47	0.26
LCFA Total (%)	3.11	1.77
Ash (%)	8.61	4.89
Cost ($/d)	0.00	0.00
Cost ($T)	0.00	0.00

Minerals and Vitamins		
Nutrient	DM	AF
Dry Matter (%)	100.00	56.86
Calcium (%)	0.73	0.42
Phosphorus (%)	0.47	0.27
Magnesium (%)	0.34	0.20
Potassium (%)	1.54	0.87
Sulfur (%)	0.34	0.19
Sodium (%)	0.32	0.18
Chlorine (%)	0.31	0.17
Iron (ppm)	243.79	138.61
Zinc (ppm)	45.81	26.04
Copper (ppm)	12.30	6.99
Manganese (ppm)	38.69	22.00
Selenium (ppm)	0.12	0.07
Cobalt (ppm)	0.20	0.12
Iodine (ppm)	0.33	0.19
Vitamin A (KIU/lb)	1.06	0.60
Vitamin D (KIU/lb)	0.27	0.15
Vitamin E (IU/lb)	8.51	4.84
DCAD1 (meq/100g)	23.75	13.50
DCAD2 (meq/100g)	28.17	16.02
Cost ($/d)	0.00	0.00
Cost ($T)	0.00	0.00

CPM Diet Analysis of Corn Oil Treatment (Jersey)

CPM-Dairy | CNCPS Evaluation | 10/2/2015 1:43:09 PM

File: F:\Studies\1504DA\Diet formulations\EXP. 1504DA 20RFDDGSplusCornOil-4Jersey

Farm: UNL Dairy	BW: 1536 lb	DIM: 140
Ration: 1504-DA 20RFDDGS plus Corn Oil	BCS: 3.50	Milk: 85.00 lb
Ration By: Paul Kononoff & Jared Judy	Growth: 0.62 lb/d	Fat: 3.50 %
Organization: University of Nebraska	Lact#: 3	TP: 3.10 %

Cost ($)	0.00	IOF ($)	0.00			Ingredient	DM (lb/d)
DMI (lb/d)	54.4	Model	52.1	% Model	104.4	Corn Silage	21.250
ME Bal (mCal)	3.2	CP (%)	16.9	NDF (%)	33.0	Alfalfa Hay	9.900
MP Bal (g)	-22.4	RUP (% CP)	44.8	ForageNDF (% NDF)	71.5	BrmdHy15Cp55Ndf7LNdf	1.000
NP / MP (%)	65.8	LCFA (%)	4.3	ForageNDF (% DM)	23.6	CornGrainGrndMed	6.250
BactMP (% MP)	48.8	EE (%)	5.3	peNDF (%)	22.0	SoybeanHullsGrnd	0.300
Rumen N Balance				Lignin (%)	3.7	Reduced Fat DDGS	10.900
Pept (g)	27	Pept & NH3 (g)	74	NFC (%)	39.3	SoybeanML47.5Solv	0.000
% rqd	114	% rqd	120	Sil Acids (%)	3.4	Soy Pass	2.500
Amino Acid Balance				Sugar (%)	2.9	BloodMeal	0.250
Met (g)	2.3	Lys (g)	-0.1	Starch (%)	24.1	CalciumCarbonate	0.410
Met (% rqd)	105	Lys (% rqd)	100	Sol Fiber (%)	9.0	FatCornOil	0.750
Met (% mp)	1.91	Lys (% mp)	5.96	Lys:Met	3.11:1	SodiumBicarbonate	0.340
Possible production due to ME and MP						Megalac	0.300

	Milk(lb)	Fat (%)	TP (%)	Milk(lb)	Fat (%)	TP (%)	
Trg:	85.0	3.50	3.10	85.0	3.50	3.10	MagOx 0.130
		Yield Constant			Composition Constant		SaltNaCl 0.100
ME:	85.0	n/a	n/a	91.6	3.50	n/a	Trace Premix 0.020
MP:	85.0	n/a	3.06	84.0	3.50	3.10	Vitamin Premix 0.022
Adjustments based on Rulquin AA Ratios:							CalciumSulfateDihyd 0.000
	85.0	n/a	-0.16	-4.5	3.50	3.10	Total 54.422

n/a - Equations not available

Ration DM (%) 56.91 Forage (% DM) 59.08

CPM-Dairy | Diet Summary - Both | 10/2/2015 1:43:09 PM

File: F:\Studies\1504DA\Diet formulations\EXP. 1504DA 20RFDDGSplusCornOil-4Jersey

Farm: UNL Dairy | BW: 1536 lb | DIM: 140

Ration: 1504-DA 20RFDDGS plus Corn Oil | BCS: 3.50 | Milk: 85.00 lb

Ration By: Paul Kononoff & Jared Judy | Growth: 0.62 lb/d | Fat: 3.50 %

Organization: University of Nebraska | Lact#: 3 | TP: 3.10 %

Ingredient	Cost $ / T	DM %	AF lb/d	DM lb/d	% AF	% DM
Corn Silage	0.00	36.40	58.38	21.25	61.05	39.05
Alfalfa Hay	0.00	86.50	11.45	9.90	11.97	18.19
BrmdHy15Cp55Ndf7LNdf	0.00	88.10	1.14	1.00	1.19	1.84
CornGrainGrndMed	0.00	88.00	7.10	6.25	7.43	11.48
SoybeanHullsGrnd	0.00	91.00	0.33	0.30	0.34	0.55
Reduced Fat DDGS	0.00	90.19	12.09	10.90	12.64	20.03
SoybeanML47.5Solv	0.00	90.00	0.00	0.00	0.00	0.00
Soy Pass	0.00	90.14	2.77	2.50	2.90	4.59
BloodMeal	0.00	90.00	0.28	0.25	0.29	0.46
CalciumCarbonate	0.00	99.50	0.41	0.41	0.43	0.75
FatCornOil	0.00	99.00	0.76	0.75	0.79	1.38
SodiumBicarbonate	0.00	99.50	0.34	0.34	0.36	0.62
Megalac	0.00	97.00	0.31	0.30	0.32	0.55
MagOx	0.00	99.50	0.13	0.13	0.14	0.24
SaltNaCl	0.00	99.50	0.10	0.10	0.11	0.18
Trace Premix	0.00	95.97	0.02	0.02	0.02	0.04
Vitamin Premix	0.00	95.75	0.02	0.02	0.02	0.04
CalciumSulfateDihyd	0.00	99.50	0.00	0.00	0.00	0.00
Total			95.62	54.42		

Macro Nutrients

Nutrient	DM	AF
Dry Matter (%)	100.00	56.91
Forage (%)	59.08	33.65
Crude Prot (%)	16.88	9.61
RUP (%CP)	44.81	44.81
RDP (%CP)	55.19	55.19
RDP (%)	9.32	5.30
Sol Prot (%CP)	27.39	27.39
ME (mCal/lb)	1.18	0.67
NEl (mCal/lb)	0.76	0.43
Nem (mCal/lb)	0.76	0.43
NEg (mCal/lb)	0.49	0.28
ADF (%)	20.44	11.64
NDF (%)	32.98	18.77
For NDF (%NDF)	71.46	40.67
Forage NDF (%)	23.57	13.41
peNDF (%)	22.01	12.52
Lignin (%)	3.74	2.13
NFC (%)	39.30	22.37
Sil Acids (%)	3.40	1.93
Sugar (%)	2.87	1.63
Starch (%)	24.08	13.71
Sol Fiber (%)	8.95	5.10
EE Total (%)	5.25	2.99
EE 1 (%)	4.78	2.72
EE 2 (%)	0.01	0.01
EE 3 (%)	0.47	0.27
LCFA Total (%)	4.27	2.43
Ash (%)	8.58	4.89
Cost ($/d)	0.00	0.00
Cost ($T)	0.00	0.00

Minerals and Vitamins

Nutrient	DM	AF
Dry Matter (%)	100.00	56.91
Calcium (%)	0.73	0.42
Phosphorus (%)	0.46	0.26
Magnesium (%)	0.34	0.19
Potassium (%)	1.53	0.87
Sulfur (%)	0.33	0.19
Sodium (%)	0.32	0.18
Chlorine (%)	0.30	0.17
Iron (ppm)	243.05	138.33
Zinc (ppm)	45.44	25.86
Copper (ppm)	12.23	6.96
Manganese (ppm)	38.61	21.97
Selenium (ppm)	0.12	0.07
Cobalt (ppm)	0.20	0.12
Iodine (ppm)	0.33	0.19
Vitamin A (KIU/lb)	1.06	0.60
Vitamin D (KIU/lb)	0.27	0.15
Vitamin E (IU/lb)	8.51	4.84
DCAD1 (meq/100g)	23.73	13.51
DCAD2 (meq/100g)	28.21	16.05
Cost ($/d)	0.00	0.00
Cost ($T)	0.00	0.00

CPM Diet Analysis of Calcium Sulfate Treatment (Jersey)

CPM-Dairy | CNCPS Evaluation | 10/2/2015 1:43:38 PM

File: F:\Studies\1504DA\Diet formulations\EXP. 1504DA 20RFDDGSplusCalciumSulfate-4Jersey

Farm: UNL Dairy	BW: 1536 lb	DIM: 140
Ration: 1504-DA Calcium Sulfate Grain Mix 1505	BCS: 3.50	Milk: 85.00 lb
Ration By: Paul Kononoff & Jared Judy	Growth: 0.62 lb/d	Fat: 3.50 %
Organization: University of Nebraska	Lact#: 3	TP: 3.10 %

Cost ($)	0.00	IOF ($)	0.00			Ingredient	DM (lb/d)
DMI (lb/d)	54.6	Model	52.1	% Model	104.7	Corn Silage	21.250
ME Bal (mCal)	1.4	CP (%)	17.0	NDF (%)	33.0	Alfalfa Hay	9.900
MP Bal (g)	10.0	RUP (% CP)	44.8	ForageNDF (% NDF)	71.2	BrmdHy15Cp55Ndf7LNdf	1.000
NP / MP (%)	64.6	LCFA (%)	3.1	ForageNDF (% DM)	23.5	CornGrainGrndMed	6.900
BactMP (% MP)	49.0	EE (%)	3.9	peNDF (%)	22.0	SoybeanHullsGrnd	0.300
Rumen N Balance				Lignin (%)	3.7	Reduced Fat DDGS	10.940
Pept (g)	25	Pept & NH3 (g)	70	NFC (%)	40.1	SoybeanML47.5Solv	0.000
% rqd	112	% rqd	119	Sil Acids (%)	3.4	Soy Pass	2.500
Amino Acid Balance				Sugar (%)	2.9	BloodMeal	0.250
Met (g)	3.0	Lys (g)	1.8	Starch (%)	24.9	CalciumCarbonate	0.100
Met (% rqd)	106	Lys (% rqd)	101	Sol Fiber (%)	9.0	FatCornOil	0.000
Met (% mp)	1.92	Lys (% mp)	5.96	Lys:Met	3.11:1	SodiumBicarbonate	0.340
Possible production due to ME and MP						Megalac	0.300

	Milk(lb)	Fat (%)	TP (%)	Milk(lb)	Fat (%)	TP (%)	
Trg:	85.0	3.50	3.10	85.0	3.50	3.10	MagOx 0.130
		Yield Constant			Composition Constant		SaltNaCl 0.100
ME:	85.0	n/a	n/a	87.8	3.50	n/a	Trace Premix 0.020
MP:	85.0	n/a	3.12	85.5	3.50	3.10	Vitamin Premix 0.022
Adjustments based on Rulquin AA Ratios:							CalciumSulfateDihyd 0.510
	85.0	n/a	-0.16	-4.4	3.50	3.10	Total 54.562

n/a - Equations not available

Ration DM (%) 56.92 Forage (% DM) 58.92

CPM-Dairy | Diet Summary - Both | 10/2/2015 1:43:38 PM

File: F:\Studies\1504DA\Diet formulations\EXP. 1504DA 20RFDDGSplusCalciumSulfate-4Jersey

Farm: UNL Dairy | BW: 1536 lb | DIM: 140

Ration: 1504-DA Calcium Sulfate Grain Mix 1505 | BCS: 3.50 | Milk: 85.00 lb

Ration By: Paul Kononoff & Jared Judy | Growth: 0.62 lb/d | Fat: 3.50 %

Organization: University of Nebraska | Lact#: 3 | TP: 3.10 %

	Cost		AF	DM		
Ingredient	$ / T	DM %	lb/d	lb/d	% AF	% DM
Corn Silage	0.00	36.40	58.38	21.25	60.91	38.95
Alfalfa Hay	0.00	86.50	11.45	9.90	11.94	18.14
BrmdHy15Cp55Ndf7LNdf	0.00	88.10	1.14	1.00	1.18	1.83
CornGrainGrndMed	0.00	88.00	7.84	6.90	8.18	12.65
SoybeanHullsGrnd	0.00	91.00	0.33	0.30	0.34	0.55
Reduced Fat DDGS	0.00	90.19	12.13	10.94	12.66	20.05
SoybeanML47.5Solv	0.00	90.00	0.00	0.00	0.00	0.00
Soy Pass	0.00	90.14	2.77	2.50	2.89	4.58
BloodMeal	0.00	90.00	0.28	0.25	0.29	0.46
CalciumCarbonate	0.00	99.50	0.10	0.10	0.10	0.18
FatCornOil	0.00	99.00	0.00	0.00	0.00	0.00
SodiumBicarbonate	0.00	99.50	0.34	0.34	0.36	0.62
Megalac	0.00	97.00	0.31	0.30	0.32	0.55
MagOx	0.00	99.50	0.13	0.13	0.14	0.24
SaltNaCl	0.00	99.50	0.10	0.10	0.10	0.18
Trace Premix	0.00	95.97	0.02	0.02	0.02	0.04
Vitamin Premix	0.00	95.75	0.02	0.02	0.02	0.04
CalciumSulfateDihyd	0.00	99.50	0.51	0.51	0.53	0.93
Total			95.85	54.56		

Macro Nutrients		
Nutrient	DM	AF
Dry Matter (%)	100.00	56.92
Forage (%)	58.92	33.57
Crude Prot (%)	16.97	9.66
RUP (%CP)	44.85	44.85
RDP (%CP)	55.15	55.15
RDP (%)	9.36	5.33
Sol Prot (%CP)	27.33	27.33
ME (mCal/lb)	1.15	0.65
NEl (mCal/lb)	0.74	0.42
Nem (mCal/lb)	0.74	0.42
NEg (mCal/lb)	0.47	0.26
ADF (%)	20.45	11.64
NDF (%)	33.03	18.80
For NDF (%NDF)	71.18	40.52
Forage NDF (%)	23.51	13.38
peNDF (%)	22.00	12.52
Lignin (%)	3.74	2.13
NFC (%)	40.14	22.85
Sil Acids (%)	3.39	1.93
Sugar (%)	2.88	1.64
Starch (%)	24.92	14.18
Sol Fiber (%)	8.95	5.10
EE Total (%)	3.92	2.23
EE 1 (%)	3.45	1.96
EE 2 (%)	0.01	0.01
EE 3 (%)	0.46	0.26
LCFA Total (%)	3.10	1.77
Ash (%)	8.95	5.10
Cost ($/d)	0.00	0.00
Cost ($T)	0.00	0.00

Minerals and Vitamins		
Nutrient	DM	AF
Dry Matter (%)	100.00	56.92
Calcium (%)	0.71	0.40
Phosphorus (%)	0.47	0.27
Magnesium (%)	0.34	0.19
Potassium (%)	1.53	0.87
Sulfur (%)	0.49	0.28
Sodium (%)	0.32	0.18
Chlorine (%)	0.30	0.17
Iron (ppm)	241.43	137.43
Zinc (ppm)	45.64	25.98
Copper (ppm)	12.25	6.98
Manganese (ppm)	36.88	20.99
Selenium (ppm)	0.12	0.07
Cobalt (ppm)	0.20	0.12
Iodine (ppm)	0.33	0.19
Vitamin A (KIU/lb)	1.05	0.60
Vitamin D (KIU/lb)	0.27	0.15
Vitamin E (IU/lb)	8.48	4.83
DCAD1 (meq/100g)	14.08	8.01
DCAD2 (meq/100g)	22.15	12.61
Cost ($/d)	0.00	0.00
Cost ($T)	0.00	0.00

APPENDIX G

CONTROL AND EXTRUDED BYPRODUCT CONTAINING FLAXSEED DIETS FOR CHAPTER 4 TREATMENTS AS CALCULATED USING THE CPM DAIRY RATION ANALYZER (2000)

CPM Diet Analysis of Control Treatment

CPM-Dairy — CNCPS Evaluation — 10/6/2017 3:03:11 PM

File: F:\Studies\1606DA\Diets\EXP. 1606DA control ration-3

Farm: UNL Dairy | BW: 864 lb | DIM: 270
Ration: 1606-DA control ration | BCS: 3.25 | Milk: 55.00 lb
Ration By: Paul Kononoff & Jared Judy | Growth: 0.15 lb/d | Fat: 5.50 %
Organization: University of Nebraska | Lact#: 3 | TP: 3.63 %

Cost ($)	0.00	IOF ($)	0.00			Ingredient	DM (lb/d)
DMI (lb/d)	38.2	Model	36.4	% Model	105.0	Corn Silage	10.500
ME Bal (mCal)	0.3	CP (%)	18.3	NDF (%)	31.8	Alfalfa Hay	8.000
MP Bal (g)	77.1	RUP (% CP)	43.1	ForageNDF (% NDF)	68.4	BrmdHy15Cp55Ndf7LNdf	0.600
NP / MP (%)	61.6	LCFA (%)	4.3	ForageNDF (% DM)	21.8	CornGrainGrndMed	7.700
BactMP (% MP)	47.9	EE (%)	5.1	peNDF (%)	21.8	SoybeanML47.5Solv	2.110
Rumen N Balance				Lignin (%)	3.9	Linpro-R	0.000
Pept (g)	57	Pept & NH3 (g)	68	NFC (%)	40.4	SoybeanHullsGrnd	2.000
% rqd	138	% rqd	125	Sil Acids (%)	2.4	CanolaMealSolv	3.500
Amino Acid Balance				Sugar (%)	3.5	Soy Pass	2.000
Met (g)	4.1	Lys (g)	24.4	Starch (%)	25.7	BloodMeal	0.100
Met (% rqd)	111	Lys (% rqd)	120	Sol Fiber (%)	8.7	CalciumCarbonate	0.308
Met (% mp)	1.94	Lys (% mp)	6.80	Lys:Met	3.52:1	SodiumBicarbonate	0.255
Possible production due to ME and MP						Megalac	0.225
	Milk(lb)	Fat (%)	TP (%)	Milk(lb)	Fat (%) TP (%)	MagOx	0.098
Trg:	55.0	5.50	3.63	55.0	5.50 3.63	SaltNaCl	0.075
		Yield Constant			Composition Constant	Trace Premix	0.015
ME:	55.0	n/a	n/a	55.5	5.50 n/a	Vitamin Premix	0.017
MP:	55.0	n/a	3.83	58.0	5.50 3.63	FatTallowBeef	0.680
Adjustments based on Rulquin AA Ratios:						FatSoybeanOil	0.000
	55.0	n/a	-0.07	-1.1	5.50 3.63	FatTallowPorcine	0.000
n/a - Equations not available						FatCornOil	0.000
Ration DM (%)	62.81		Forage (% DM)		50.02	Total	38.183

CPM-Dairy | Diet Summary - Both | 10/6/2017 3:03:11 PM

File: F:\Studies\1606DA\Diets\EXP. 1606DA control ration-3
Farm: UNL Dairy | BW: 864 lb | DIM: 270
Ration: 1606-DA control ration | BCS: 3.25 | Milk: 55.00 lb
Ration By: Paul Kononoff & Jared Judy | Growth: 0.15 lb/d | Fat: 5.50 %
Organization: University of Nebraska | Lact#: 3 | TP: 3.63 %

Ingredient	Cost $ / T	DM %	AF lb/d	DM lb/d	% AF	% DM
Corn Silage	0.00	35.30	29.75	10.50	48.93	27.50
Alfalfa Hay	0.00	87.30	9.16	8.00	15.07	20.95
BrmdHy15Cp55Ndf7LNdf	0.00	87.50	0.69	0.60	1.13	1.57
CornGrainGrndMed	0.00	88.00	8.75	7.70	14.39	20.17
SoybeanML47.5Solv	0.00	90.00	2.34	2.11	3.86	5.53
Linpro-R	0.00	94.27	0.00	0.00	0.00	0.00
SoybeanHullsGrnd	0.00	91.00	2.20	2.00	3.62	5.24
CanolaMealSolv	0.00	90.17	3.88	3.50	6.39	9.17
Soy Pass	0.00	90.14	2.22	2.00	3.65	5.24
BloodMeal	0.00	90.00	0.11	0.10	0.18	0.26
CalciumCarbonate	0.00	99.50	0.31	0.31	0.51	0.81
SodiumBicarbonate	0.00	99.50	0.26	0.26	0.42	0.67
Megalac	0.00	97.00	0.23	0.22	0.38	0.59
MagOx	0.00	99.50	0.10	0.10	0.16	0.26
SaltNaCl	0.00	99.50	0.08	0.08	0.12	0.20
Trace Premix	0.00	95.97	0.02	0.02	0.03	0.04
Vitamin Premix	0.00	95.75	0.02	0.02	0.03	0.04
FatTallowBeef	0.00	99.00	0.69	0.68	1.13	1.78
FatSoybeanOil	0.00	99.00	0.00	0.00	0.00	0.00
FatTallowPorcine	0.00	99.00	0.00	0.00	0.00	0.00
FatCornOil	0.00	99.00	0.00	0.00	0.00	0.00
Total			60.79	38.18		

Macro Nutrients		
Nutrient	DM	AF
Dry Matter (%)	100.00	62.81
Forage (%)	50.02	29.54
Crude Prot (%)	18.33	11.51
RUP (%CP)	43.07	43.07
RDP (%CP)	56.93	56.93
RDP (%)	10.44	6.56
Sol Prot (%CP)	26.51	26.51
ME (mCal/lb)	1.22	0.77
NEl (mCal/lb)	0.79	0.49
Nem (mCal/lb)	0.79	0.49
NEg (mCal/lb)	0.52	0.33
ADF (%)	22.01	13.82
NDF (%)	31.81	19.98
For NDF (%NDF)	68.41	42.97
Forage NDF (%)	21.76	13.67
peNDF (%)	21.83	13.71
Lignin (%)	3.88	2.43
NFC (%)	40.41	25.38
Sl Acids (%)	2.42	1.52
Sugar (%)	3.51	2.20
Starch (%)	25.74	16.17
Sol Fiber (%)	8.73	5.48
EE Total (%)	5.13	3.22
EE 1 (%)	2.85	1.79
EE 2 (%)	1.79	1.12
EE 3 (%)	0.50	0.31
LCFA Total (%)	4.29	2.69
Ash (%)	7.72	4.85
Cost ($/d)	0.00	0.00
Cost ($T)	0.00	0.00

Minerals and Vitamins		
Nutrient	DM	AF
Dry Matter (%)	100.00	62.81
Calcium (%)	0.82	0.51
Phosphorus (%)	0.40	0.25
Magnesium (%)	0.34	0.21
Potassium (%)	1.50	0.94
Sulfur (%)	0.24	0.15
Sodium (%)	0.29	0.18
Chlorine (%)	0.21	0.13
Iron (ppm)	194.66	122.27
Zinc (ppm)	50.52	31.73
Copper (ppm)	13.56	8.52
Manganese (ppm)	44.55	27.98
Selenium (ppm)	0.13	0.08
Cobalt (ppm)	0.23	0.14
Iodine (ppm)	0.36	0.22
Vitamin A (KIU/lb)	1.16	0.73
Vitamin D (KIU/lb)	0.29	0.19
Vitamin E (IU/lb)	9.37	5.88
DCAD1 (meq/100g)	30.17	18.95
DCAD2 (meq/100g)	34.80	21.86
Cost ($/d)	0.00	0.00
Cost ($T)	0.00	0.00

CPM Diet Analysis of Extruded Byproduct Containing Flaxseed Treatment

CPM-Dairy — CNCPS Evaluation — 10/6/2017 3:03:44 PM

File: F:\Studies\1606DA\Diets\EXP. 1606DA linpro-R ration-3
Farm: UNL Dairy — BW: 864 lb — DIM: 270
Ration: 1606-DA LinPro-R(linseed) ration — BCS: 3.25 — Milk: 55.00 lb
Ration By: Paul Kononoff & Jared Judy — Growth: 0.15 lb/d — Fat: 5.50 %
Organization: University of Nebraska — Lact#: 3 — TP: 3.63 %

Cost ($)	0.00	IOF ($)	0.00			Ingredient	DM (lb/d)
DMI (lb/d)	38.2	Model	36.4	% Model	105.0	Corn Silage	10.500
ME Bal (mCal)	0.0	CP (%)	18.3	NDF (%)	31.9	Alfalfa Hay	8.000
MP Bal (g)	15.4	RUP (% CP)	43.4	ForageNDF (% NDF)	68.1	BrmdHy15Cp55Ndf7LNdf	0.600
NP / MP (%)	64.3	LCFA (%)	4.3	ForageNDF (% DM)	21.8	CornGrainGrndMed	6.600
BactMP (% MP)	45.8	EE (%)	5.1	peNDF (%)	21.0	SoybeanML47.5Solv	2.400
Rumen N Balance				Lignin (%)	3.7	Linpro-R	4.000
Pept (g)	75	Pept & NH3 (g)	84	NFC (%)	40.4	CanolaMealSolv	1.000
% rqd	154	% rqd	133	Sil Acids (%)	2.4	Soy Pass	2.000
Amino Acid Balance				Sugar (%)	3.4	SoybeanHullsGrnd	2.000
Met (g)	2.4	Lys (g)	17.0	Starch (%)	25.2	CalciumCarbonate	0.308
Met (% rqd)	106	Lys (% rqd)	114	Sol Fiber (%)	9.4	SodiumBicarbonate	0.255
Met (% mp)	1.91	Lys (% mp)	6.65	Lys:Met	3.48:1	Megalac	0.225
Possible production due to ME and MP						BloodMeal	0.100

	Milk(lb)	Fat (%)	TP (%)	Milk(lb)	Fat (%)	TP (%)	
Trg:	55.0	5.50	3.63	55.0	5.50	3.63	MagOx 0.098
	Yield Constant			Composition Constant			SaltNaCl 0.075
							Vitamin Premix 0.017
ME:	55.0	n/a	n/a	55.0	5.50	n/a	Trace Premix 0.015
MP:	55.0	n/a	3.67	55.6	5.50	3.63	FatCornOil 0.000
Adjustments based on Rulquin AA Ratios:							FatSoybeanOil 0.000
	55.0	n/a	-0.10	-1.5	5.50	3.63	FatTallowBeef 0.000
n/a - Equations not available							FatTallowPorcine 0.000
Ration DM (%)	62.98		Forage (% DM)		50.01		Total 38.193

CPM-Dairy | Diet Summary - Both | 10/6/2017 3:03:44 PM

File: F:\Studies\1606DA\Diets\EXP. 1606DA linpro-R ration-3
Farm: UNL Dairy | BW: 864 lb | DIM: 270
Ration: 1606-DA LinPro-R(linseed) ration | BCS: 3.25 | Milk: 55.00 lb
Ration By: Paul Kononoff & Jared Judy | Growth: 0.15 lb/d | Fat: 5.50 %
Organization: University of Nebraska | Lact#: 3 | TP: 3.63 %

Ingredient	Cost $ / T	DM %	AF lb/d	DM lb/d	% AF	% DM
Corn Silage	0.00	35.30	29.75	10.50	49.05	27.49
Alfalfa Hay	0.00	87.30	9.16	8.00	15.11	20.95
BrmdHy15Cp55Ndf7LNdf	0.00	87.50	0.69	0.60	1.13	1.57
CornGrainGrndMed	0.00	88.00	7.50	6.60	12.37	17.28
SoybeanML47.5Solv	0.00	90.00	2.67	2.40	4.40	6.28
Linpro-R	0.00	94.27	4.24	4.00	7.00	10.47
CanolaMealSolv	0.00	90.17	1.11	1.00	1.83	2.62
Soy Pass	0.00	90.14	2.22	2.00	3.66	5.24
SoybeanHullsGrnd	0.00	91.00	2.20	2.00	3.62	5.24
CalciumCarbonate	0.00	99.50	0.31	0.31	0.51	0.81
SodiumBicarbonate	0.00	99.50	0.26	0.26	0.42	0.67
Megalac	0.00	97.00	0.23	0.22	0.38	0.59
BloodMeal	0.00	90.00	0.11	0.10	0.18	0.26
MagOx	0.00	99.50	0.10	0.10	0.16	0.26
SaltNaCl	0.00	99.50	0.08	0.08	0.12	0.20
Vitamin Premix	0.00	95.75	0.02	0.02	0.03	0.04
Trace Premix	0.00	95.97	0.02	0.02	0.03	0.04
FatCornOil	0.00	99.00	0.00	0.00	0.00	0.00
FatSoybeanOil	0.00	99.00	0.00	0.00	0.00	0.00
FatTallowBeef	0.00	99.00	0.00	0.00	0.00	0.00
FatTallowPorcine	0.00	99.00	0.00	0.00	0.00	0.00
Total			60.65	38.19		

Macro Nutrients		
Nutrient	DM	AF
Dry Matter (%)	100.00	62.98
Forage (%)	50.01	29.61
Crude Prot (%)	18.27	11.51
RUP (%CP)	43.38	43.38
RDP (%CP)	56.62	56.62
RDP (%)	10.35	6.52
Sol Prot (%CP)	27.83	27.83
ME (mCal/lb)	1.22	0.77
NEl (mCal/lb)	0.78	0.49
Nem (mCal/lb)	0.78	0.49
NEg (mCal/lb)	0.52	0.33
ADF (%)	21.52	13.55
NDF (%)	31.94	20.11
For NDF (%NDF)	68.13	42.91
Forage NDF (%)	21.76	13.70
peNDF (%)	21.03	13.24
Lignin (%)	3.66	2.30
NFC (%)	40.40	25.44
Sil Acids (%)	2.42	1.53
Sugar (%)	3.39	2.14
Starch (%)	25.17	15.85
Sol Fiber (%)	9.41	5.93
EE Total (%)	5.11	3.22
EE 1 (%)	4.61	2.90
EE 2 (%)	0.01	0.00
EE 3 (%)	0.50	0.31
LCFA Total (%)	4.29	2.70
Ash (%)	7.60	4.79
Cost ($/d)	0.00	0.00
Cost ($T)	0.00	0.00

Minerals and Vitamins		
Nutrient	DM	AF
Dry Matter (%)	100.00	62.98
Calcium (%)	0.79	0.50
Phosphorus (%)	0.36	0.23
Magnesium (%)	0.34	0.21
Potassium (%)	1.52	0.96
Sulfur (%)	0.22	0.14
Sodium (%)	0.28	0.18
Chlorine (%)	0.21	0.13
Iron (ppm)	197.10	124.13
Zinc (ppm)	50.25	31.65
Copper (ppm)	14.28	8.99
Manganese (ppm)	43.03	27.10
Selenium (ppm)	0.13	0.08
Cobalt (ppm)	0.23	0.14
Iodine (ppm)	0.35	0.22
Vitamin A (KIU/lb)	1.16	0.73
Vitamin D (KIU/lb)	0.29	0.19
Vitamin E (IU/lb)	9.37	5.90
DCAD1 (meq/100g)	31.63	19.92
DCAD2 (meq/100g)	36.56	23.02
Cost ($/d)	0.00	0.00
Cost ($T)	0.00	0.00

APPENDIX H

EXPERIMENTAL DIET FOR CHAPTER 5 TREATMENTS AS CALCULATED USING THE CPM DAIRY RATION ANALYZER (2000)

CPM Diet Analysis of the Experiment Diet

CPM-Dairy | CNCPS Evaluation | 10/6/2017 3:06:24 PM

File: F:\Studies\1701DA\1701DA Control Ration
Farm: UNL Dairy Research Unit | BW: 900 lb | DIM: 220
Ration: 1701DA Control with 1608 Grain | BCS: 3.25 | Milk: 70.00 lb
Ration By: Kononoff & Judy | Growth: 0.10 lb/d | Fat: 5.30 %
Organization: University of Nebraska-Lincoln | Lact#: 3 | TP: 3.70 %

Cost ($)	4.89	IOF ($)	-4.89			Ingredient	DM (lb/d)
DMI (lb/d)	43.0	Model	42.0	% Model	102.5	Alfalfa hay	6.000
ME Bal (mCal)	0.0	CP (%)	18.9	NDF (%)	29.0	BrmdHy10Cp70Ndf9LNdf	1.100
MP Bal (g)	82.6	RUP (% CP)	45.5	ForageNDF (% NDF)	77.3	Corn silage	16.200
NP / MP (%)	62.2	LCFA (%)	4.6	ForageNDF (% DM)	22.4	Ground Corn	7.365
BactMP (% MP)	44.7	EE (%)	5.5	peNDF (%)	22.0	SoybeanML47.5Solv	6.012
Rumen N Balance				Lignin (%)	3.0	RFDDGS	0.000
Pept (g)	54	Pept & NH3 (g)	76	NFC (%)	41.1	CanolaMealSolv	0.000
% rqd	131	% rqd	125	Sil Acids (%)	3.0	SoybeanHullsGrnd	1.102
Amino Acid Balance				Sugar (%)	3.5	Soy Pass	2.004
Met (g)	11.3	Lys (g)	35.5	Starch (%)	27.5	FatTallowBeef	0.852
Met (% rqd)	125	Lys (% rqd)	125	Sol Fiber (%)	7.0	CalciumCarbonate	0.601
Met (% mp)	2.19	Lys (% mp)	7.08	Lys:Met	3.24:1	BloodMeal	0.671
Possible production due to ME and MP						Megalac	0.351
	Milk(lb)	Fat (%)	TP (%)	Milk(lb)	Fat (%) TP (%)	SodiumBicarbonate	0.250
Trg:	70.0	5.30	3.70	70.0	5.30 3.70	CalciumPhosDi	0.150
		Yield Constant			Composition Constant	MagOx	0.140
ME:	70.0	n/a	n/a	70.1	5.30 n/a	SaltNaCl	0.110
MP:	70.0	n/a	3.87	73.2	5.30 3.70	Vitamin Premix	0.022
Adjustments based on Rulquin AA Ratios:						Trace Premix	0.017
	70.0	n/a	0.02	0.4	5.30 3.70	Agipro-L	0.022
n/a - Equations not available						SmartamineM	0.030
Ration DM (%)	57.06		Forage (% DM)	54.19		Total	43.000

CPM-Dairy | Diet Summary - Both | 10/6/2017 3:06:24 PM

File: F:\Studies\1701DA\1701DA Control Ration
Farm: UNL Dairy Research Unit | BW: 900 lb | DIM: 220
Ration: 1701DA Control with 1608 Grain | BCS: 3.25 | Milk: 70.00 lb
Ration By: Kononoff & Judy | Growth: 0.10 lb/d | Fat: 5.30 %
Organization: University of Nebraska-Lincoln | Lact#: 3 | TP: 3.70 %

	Cost		AF	DM		
Ingredient	$ / T	DM %	lb/d	lb/d	% AF	% DM
Alfalfa hay	110.00	89.90	6.67	6.00	8.86	13.95
BrmdHy10Cp70Ndf9LNdf	60.00	88.00	1.25	1.10	1.66	2.56
Corn silage	33.00	35.50	45.63	16.20	60.55	37.67
Ground Corn	130.00	88.00	8.37	7.36	11.10	17.13
SoybeanML47.5Solv	435.80	90.00	6.68	6.01	8.86	13.98
RFDDGS	120.00	89.00	0.00	0.00	0.00	0.00
CanolaMealSolv	310.00	90.17	0.00	0.00	0.00	0.00
SoybeanHullsGrnd	123.60	91.00	1.21	1.10	1.61	2.56
Soy Pass	404.80	90.14	2.22	2.00	2.95	4.66
FatTallowBeef	752.60	99.00	0.86	0.85	1.14	1.98
CalciumCarbonate	26.80	99.50	0.60	0.60	0.80	1.40
BloodMeal	1247.00	90.00	0.75	0.67	0.99	1.56
Megalac	1500.60	97.00	0.36	0.35	0.48	0.82
SodiumBicarbonate	543.80	99.50	0.25	0.25	0.33	0.58
CalciumPhosDi	26.80	99.50	0.15	0.15	0.20	0.35
MagOx	799.60	99.50	0.14	0.14	0.19	0.33
SaltNaCl	243.80	99.50	0.11	0.11	0.15	0.26
Vitamin Premix	26.80	95.75	0.02	0.02	0.03	0.05
Trace Premix	26.80	95.97	0.02	0.02	0.02	0.04
Agipro-L	0.00	97.00	0.02	0.02	0.03	0.05
SmartamineM	0.00	98.00	0.03	0.03	0.04	0.07
Total			75.36	43.00		

Macro Nutrients		
Nutrient	DM	AF
Dry Matter (%)	100.00	57.06
Forage (%)	54.19	32.23
Crude Prot (%)	18.88	10.77
RUP (%CP)	45.53	45.53
RDP (%CP)	54.47	54.47
RDP (%)	10.28	5.87
Sol Prot (%CP)	22.83	22.83
ME (mCal/lb)	1.26	0.72
NEl (mCal/lb)	0.81	0.46
Nem (mCal/lb)	0.81	0.46
NEg (mCal/lb)	0.55	0.31
ADF (%)	19.47	11.11
NDF (%)	28.96	16.53
For NDF (%NDF)	77.33	44.12
Forage NDF (%)	22.40	12.78
peNDF (%)	22.00	12.55
Lignin (%)	2.96	1.69
NFC (%)	41.05	23.42
Sil Acids (%)	2.99	1.71
Sugar (%)	3.50	1.99
Starch (%)	27.53	15.71
Sol Fiber (%)	7.04	4.01
EE Total (%)	5.48	3.13
EE 1 (%)	2.76	1.57
EE 2 (%)	2.04	1.16
EE 3 (%)	0.69	0.39
LCFA Total (%)	4.64	2.65
Ash (%)	8.59	4.90
Cost ($/d)	4.89	4.89
Cost ($T)	227.49	129.80

Minerals and Vitamins		
Nutrient	DM	AF
Dry Matter (%)	100.00	57.06
Calcium (%)	1.02	0.58
Phosphorus (%)	0.40	0.23
Magnesium (%)	0.35	0.20
Potassium (%)	1.37	0.78
Sulfur (%)	0.24	0.14
Sodium (%)	0.28	0.16
Chlorine (%)	0.23	0.13
Iron (ppm)	211.89	120.90
Zinc (ppm)	49.24	28.10
Copper (ppm)	13.89	7.93
Manganese (ppm)	44.68	25.50
Selenium (ppm)	0.14	0.08
Cobalt (ppm)	0.26	0.15
Iodine (ppm)	0.34	0.20
Vitamin A (KIU/lb)	1.34	0.76
Vitamin D (KIU/lb)	0.34	0.19
Vitamin E (IU/lb)	21.02	11.99
DCAD1 (meq/100g)	25.88	14.77
DCAD2 (meq/100g)	32.17	18.35
Cost ($/d)	4.89	4.89
Cost ($T)	227.49	129.80

APPENDIX I: GAS SYSTEM PROTOCOL

CALIBRATION

1. Plug in the analyzer and flip the switch in the back to turn it on
   1.1. For best results, turn analyzer on at least 24 hours before analyzing gases (1 hr min)
2. Change the Drierite so its fresh each morning (to regenerate drierite, place in oven at 425 °F or 210 °C for 1 to 2 hours)
3. Calibration of the analyzer (**DO NOT TURN ON PUMP PLATE UNTIL AFTER CALIBRATION**)
   3.1. Turn on gas tanks and set valve to read from the nitrogen tank (Figure A.1)
      3.1.1. Allow gas to purge for two minutes
         3.1.1.1. Make sure the PSI is at 15 on the pump plate (Figure A.2)
      3.1.2. On the analyzer, push the home tab (Figure A.3)
         3.1.2.1. Then press the enter arrow (Figure A.4)
         3.1.2.2. Go to control and push enter arrow again (Figure A.5)
         3.1.2.3. For nitrogen tank, do Zero calibration for channel 1 (CO_2)
            3.1.2.3.1. Push enter on the zero calibration tab (Figure A.6)
            3.1.2.3.2. Move channel to channel 1 for CO_2 and press enter (Figure A.7)
            3.1.2.3.3. Move arrow down to start, press enter arrow (Figure A.8) and allow the system to purge and zero
      3.1.3. Push the home tab (Figure A.3)
         3.1.3.1. Then press the enter arrow (Figure A.4)
         3.1.3.2. Go to control and push enter arrow again (Figure A.5)
         3.1.3.3. For nitrogen tank, do Zero calibration for channel 2 (CH_4)
            3.1.3.3.1. Push enter on the zero calibration tab (Figure A.6)
            3.1.3.3.2. Move channel to channel 2 for CH_4 and press enter (Figure A.9)
            3.1.3.3.3. Move arrow down to start, press enter arrow (Figure A.10) and allow the system to purge and zero
      3.1.4. Push home tab (Figure A.3.)
   3.2. Turn nobs at the end of the cart to O_2 for the high Oxygen tank (Figure A.11)
      3.2.1. Allow gas to purge for two minutes
         3.2.1.1. Make sure the PSI is at 15 on the pump plate (Figure A.2)
      3.2.2. On the analyzer, push the home tab (Figure A.3.)
         3.2.2.1. Then press the enter arrow (Figure A.4)
         3.2.2.2. Go to control and push enter arrow again (Figure A.5)
         3.2.2.3. For high Oxygen tank, do Span calibration for channel 3 (O_2)
            3.2.2.3.1. Push enter on the span calibration tab (Figure A.12)
            3.2.2.3.2. Move channel to channel 3 for O_2 and press enter (Figure A.13)
            3.2.2.3.3. Move arrow down to start, press enter arrow (Figure A.14) and allow the system to purge and span
         3.2.2.4. Push Home button (Figure A.3)
   3.3. Turn nobs at the end of the cart to Mixed Gas for the high CO_2 and high CH_4 and low O_2 tank (Figure A.15)
      3.3.1. Allow gas to purge for two minutes

3.3.1.1. Make sure the PSI is at 15 on the pump plate (Figure A.2)

3.3.2. On the analyzer, push the home tab (Figure A.3)

3.3.2.1. Then press the enter arrow (Figure A.4)

3.3.2.2. Go to control and push enter arrow again (Figure A.5)

3.3.2.3. For Mixed Gas tank, do Span calibration for channel 1 (CO_2)

3.3.2.3.1. Push enter on the span calibration tab (Figure A.12)

3.3.2.3.2. Move channel to channel 1 for CO_2 and press enter (Figure A.7)

3.3.2.3.3. Move arrow down to start, press enter arrow (Figure A.16) and allow the system to purge and span

3.3.2.4. Press Home button (Figure A.3)

3.3.3. On the analyzer, push the home tab (Figure A.3)

3.3.3.1. Then press the enter arrow (Figure A.4)

3.3.3.2. Go to control and push enter arrow again (Figure A.5)

3.3.3.3. For Mixed Gas tank, do Span calibration for channel 2 (CH_4)

3.3.3.3.1. Push enter on the span calibration tab (Figure A.12)

3.3.3.3.2. Move channel to channel 2 for CH_4 and press enter (Figure A.9)

3.3.3.3.3. Move arrow down to start, press enter arrow (Figure A.17) and allow the system to purge and span

3.3.3.4. Press Home button (Figure A.3)

3.3.4. On the analyzer, push the home tab (Figure A.3)

3.3.4.1. Then press the enter arrow (Figure A.4)

3.3.4.2. Go to control and push enter arrow again (Figure A.5)

3.3.4.3. For Mixed Gas tank, do Zero calibration for channel 3 (O_2)

3.3.4.3.1. Push enter on the zero calibration tab (Figure A.6)

3.3.4.3.2. Move channel to channel 3 for O_2 and press enter (Figure A.13)

3.3.4.3.3. Move arrow down to start, press enter arrow (Figure A.18) and allow the system to purge and zero

3.3.4.4. Push the home Tab (Figure A.3)

4. Turn the knobs back to nitrogen tank (Figure A.1)

4.1. Allow tank to purge for 2 minutes, then record the numbers (Figure A.19) on the screen into UNL Gas data sheet in the Gas sheet tab under the VO^2 spots for CO_2 and CH_4 Column B (Figure A.20)

4.1.1.

CO_2, Bag $V0^2$
CH_4, Bag $V0^2$

5. Turn the knobs to high O_2 tank (Figure A.11)

5.1. Allow tank to purge for 2 minutes, then record the numbers on the screen (Figure A.19) into UNL Gas data sheet in the Gas sheet tab under the VO^2 spot for O_2 Column B (Figure A.20)

5.1.1.

O_2, Bag $V0^2$

6. Turn the knobs to Mixed Gas tank (Figure A.15)

6.1. Allow tank to purge for 2 minutes, then record the numbers on the screen (Figure A.19) into UNL Gas data sheet in the Gas sheet tab under the VO^1 spot for CO_2, CH_4, and O_2 Column B (Figure A.20)

6.1.1.

7. Turn the knobs to Bag (Figure A.21)
8. **THEN EITHER DO GAS COLLECTION BAGS OR CONTINOUS SYTEM PROTOCOLS**

FOR GAS COLLECTION BAGS

9. Turn on the pump plate and make sure the PSI is 15 (Figure A.2)
10. Hook up each bag (ONE BY ONE) and open the stopcock valve and allow for a two minute purge and then sample the numbers located on the front of the analyzer and put them into UNL Gas data sheet under the bag number for each bag (Figure A.20)
11. Go through each bag one time in order, and then go back the reverse way so each bag has two numbers in the spreadsheet
12. Turn off pump plate when your last bag is done
13. Then go back through the gas tanks in reverse order
14. Turn the knobs to Mixed Gas tank (Figure A.15)
    14.1. Allow tank to purge for 2 minutes, then record the numbers on the screen (Figure A.19) into UNL Gas data sheet in the Gas sheet tab under the VO^1 spot for CO_2, CH_4, and O_2 in column C (Figure A.20)
    14.1.1.

O_2, Bag $V0^1$
CO_2, Bag $V0^1$
CH_4, Bag $V0^1$

15. Turn the knobs to O2 tank (Figure A.11)
    15.1. Allow tank to purge for 2 minutes, then record the numbers on the screen (Figure A.19) into UNL Gas data sheet in the Gas sheet tab under the VO^2 spot for O_2 Column C (Figure A.20)
    15.1.1.

O_2, Bag $V0^2$

16. Turn the knobs back to nitrogen tank (Figure A.1)
    16.1. Allow tank to purge for 2 minutes, then record the numbers on the screen (Figure A.19) into UNL Gas data sheet in the Gas sheet tab under the VO^2 spots for CO_2 and CH_4 Column C (Figure A.20)
    16.1.1.

CO_2, Bag $V0^2$
CH_4, Bag $V0^2$

17. TURN OFF ALL GAS TANKS

FOR CONTINOUS GAS SYSTEM

18. Turn on the pump plate and make sure the PSI is 15 at 9:55a.m. or 5 minutes before the collections start (Figure A.2)
19. Open Daisy lab2016 on computer (Figure A.22)
    - 19.1. Go to file, then open the last or CONTINOUS GAS COLLECTION (Figure A.23)
    - 19.2. Push the green play button at 10a.m. (Figure A.24)
    - 19.3. Make sure the numbers are being read in the worksheet tab (Figure A.25)
    - 19.4. Check drierite tubes (If desiccant is pink for 75% of tube, change it)
        - 19.4.1. To Change the Drierite, move the daisylab screen to layout (Figure A.26)
            - 19.4.1.1. Check which valve is currently on and DO NOT CHANGE THE DRIERITE IN THAT TUBE
    - 19.5. STOP system at 9a.m.(Figure A.27)
    - 19.6. Go to documents (Figure A.28; Figure A.29; Figure A.30; Figure A.31; Figure A.32: Figure A.33; Figure A.34; Figure A.35
        - 19.6.1. Daisy lab → 14.0.0 → eng → Data → Move Valve spreadsheets to continuous data → Create new folder for the day that the analyzer started → put the valve spreadsheets into the new folder for the date → Rename valve spreadsheets to correlate to the correct headbox
            - 19.6.1.1. Valve 1 = Headbox 1; Valve 2 = Headbox 2; Valve 3 = Headbox 3; Valve 4 = Headbox 4; Valve 5 = ambient air

20. TURN ON GAS TANKS

21. Then go back through the gas tanks in reverse order
22. Turn the knobs to Mixed Gas tank (Figure A.15)
    - 22.1. Allow tank to purge for 2 minutes, then record the numbers on the screen (Figure A.19) into UNL Gas data sheet in the Gas sheet tab under the VO^1 spot for CO_2, CH_4, and O_2 in column C (Figure A.20)
        - 22.1.1.

O_2, Bag $V0^1$
CO_2, Bag $V0^1$
CH_4, Bag $V0^1$

23. Turn the knobs to O2 tank (Figure A.11)
    - 23.1. Allow tank to purge for 2 minutes, then record the numbers on the screen (Figure A.19) into UNL Gas data sheet in the Gas sheet tab under the VO^2 spot for O_2 Column C (Figure A.20)
        - 23.1.1.

O_2, Bag $V0^2$

24. Turn the knobs back to nitrogen tank (Figure A.1)
    - 24.1. Allow tank to purge for 2 minutes, then record the numbers on the screen (Figure A.19) into UNL Gas data sheet in the Gas sheet tab under the VO^2 spots for CO_2 and CH_4 Column C (Figure A.20)
        - 24.1.1.

CO_2, Bag $V0^2$
CH_4, Bag $V0^2$

25. REPEAT GAS CALIBRATION IF THE SYSTEM WILL BE USED THAT DAY

26. TURN OFF ALL GAS TANKS OR ANALYZE THE GAS BAGS IF NEEDED AND THEN TURN OFF THE GAS TANKS

SHUT OFF THE SYSTEM WHEN DONE

FIGURES

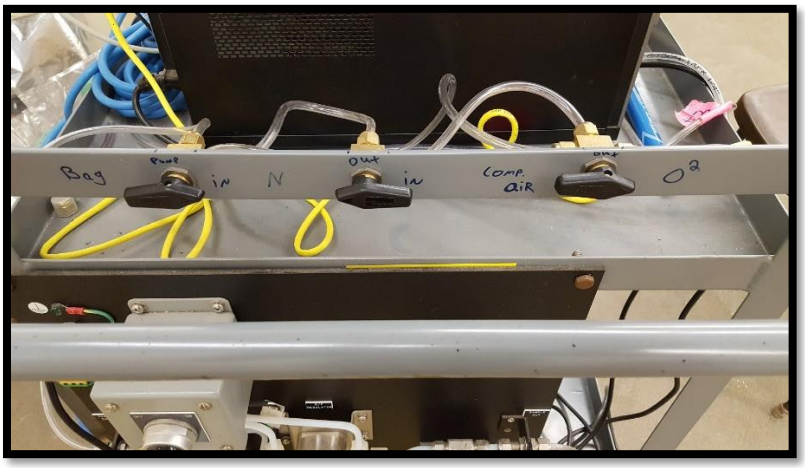


Figure A.1. Valve set up for nitrogen tank gas analysis and calibration

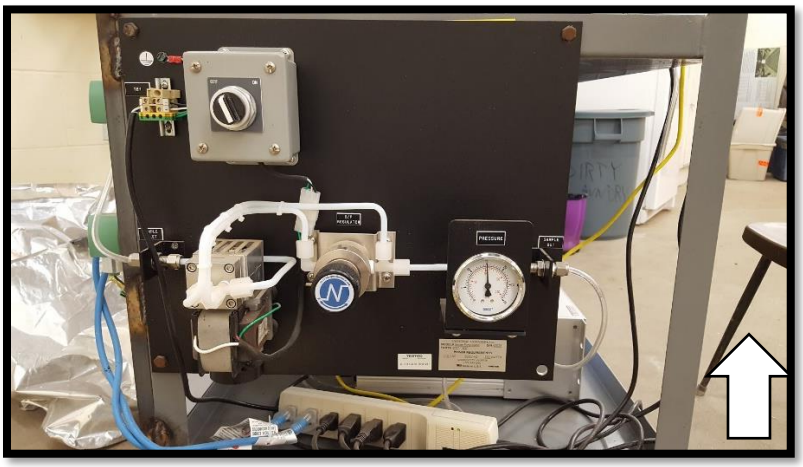


Figure A.2. Pump plate set to 15 PSI for gas analysis

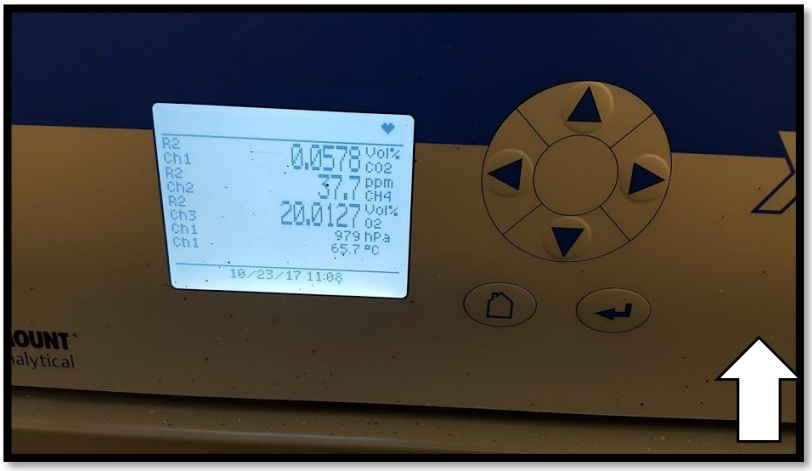


Figure A.3. Home screen and home tab.

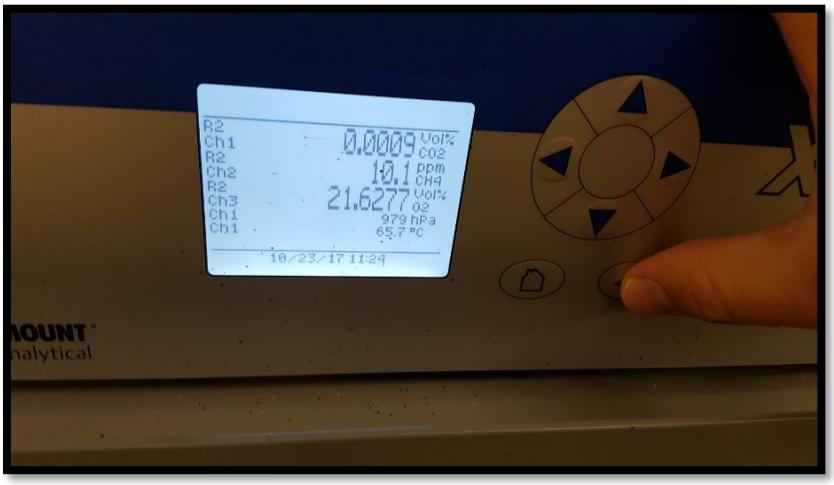


Figure A.4. Man pressing the enter arrow from the home screen

Figure A.5. Press enter on the control option

Figure A.6. Press enter on zero calibration

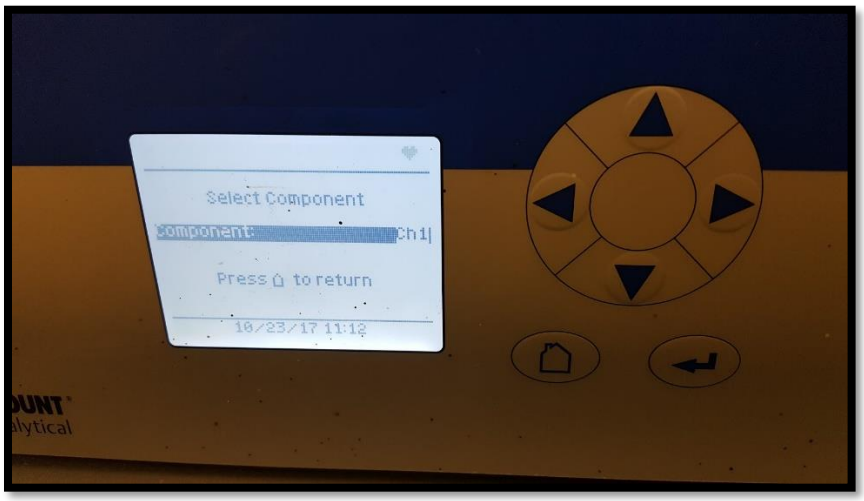


Figure A.7. Move component to channel 1 for CO_2 and press enter

Figure A.8. Move highlighted section to Start and press enter for Ch1

Figure A.9. Move component to channel 2 for CH_4 and press enter

Figure A.10. Move highlighted section to Start and press enter for Ch2

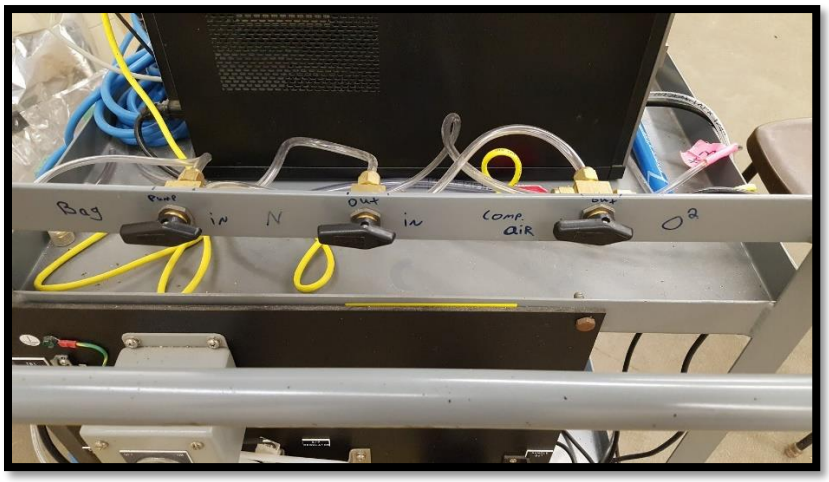


Figure A.11. High Oxygen (O_2) valve for tank analysis

Figure A.12. Span calibration, push enter on span calibration

Figure A.13. Move channel to 3 for O_2 and press enter

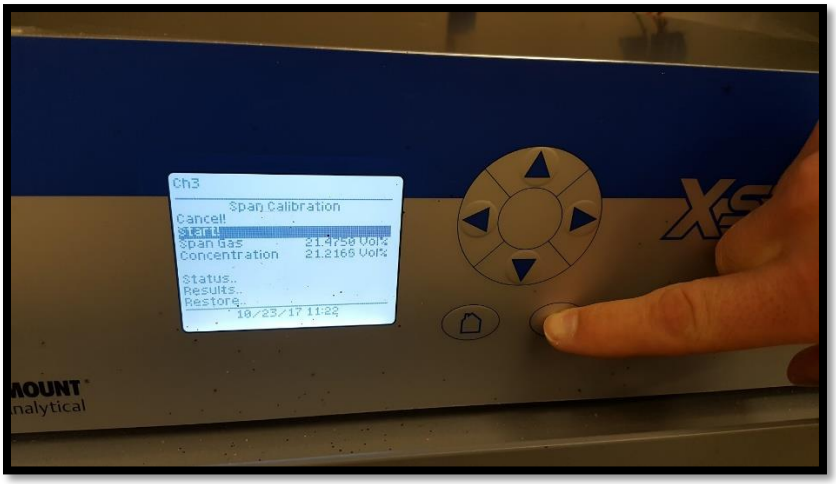


Figure A.14. Span start for high O_2, Move highlighted area to start and press enter

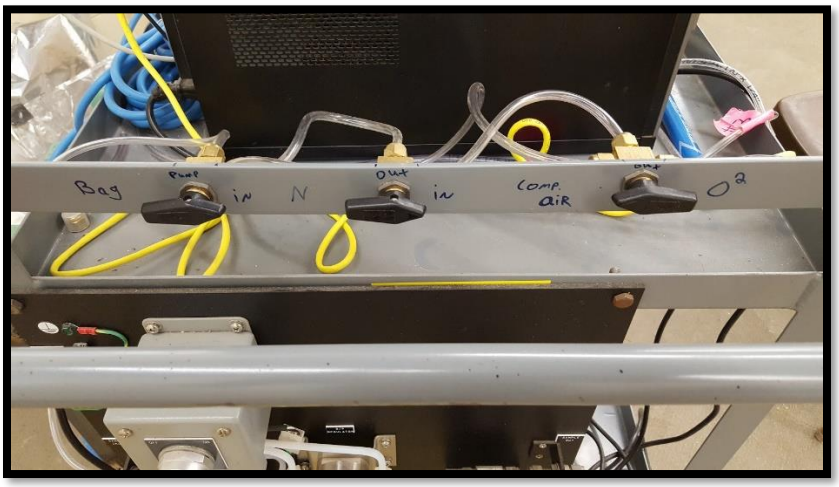


Figure A.15. High CO_2, High CH_4, and low O_2 valve for tank analysis

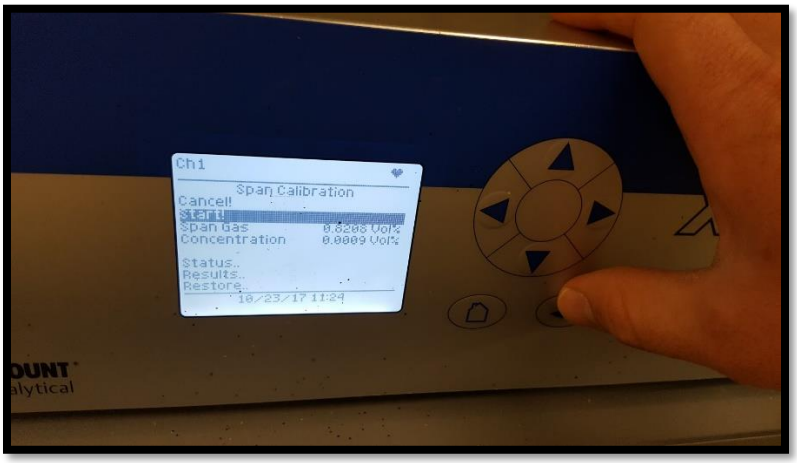


Figure A.16. Span calibration start for high CO_2, move to start and press enter

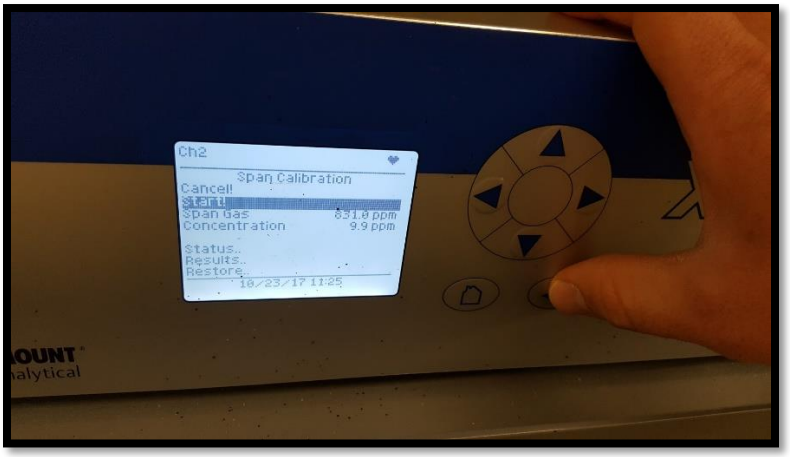


Figure A.17. Span start for high CH_4, move to start and press enter

Figure A.18. Zero start for low O_2, move to start and press enter

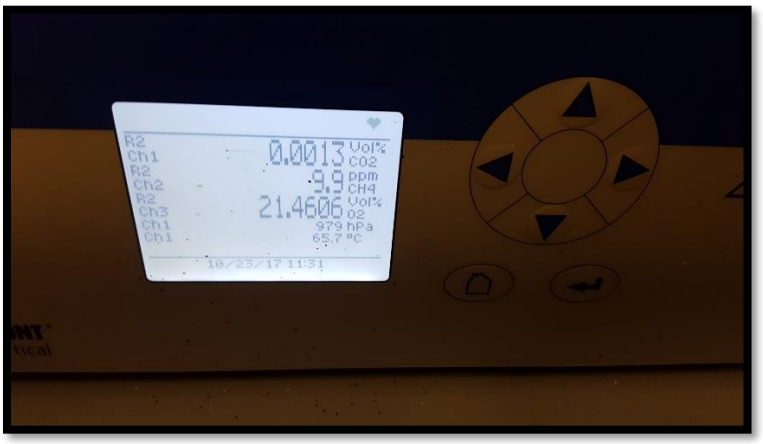


Figure A.19. Home screen to retrieve the gas concentration numbers

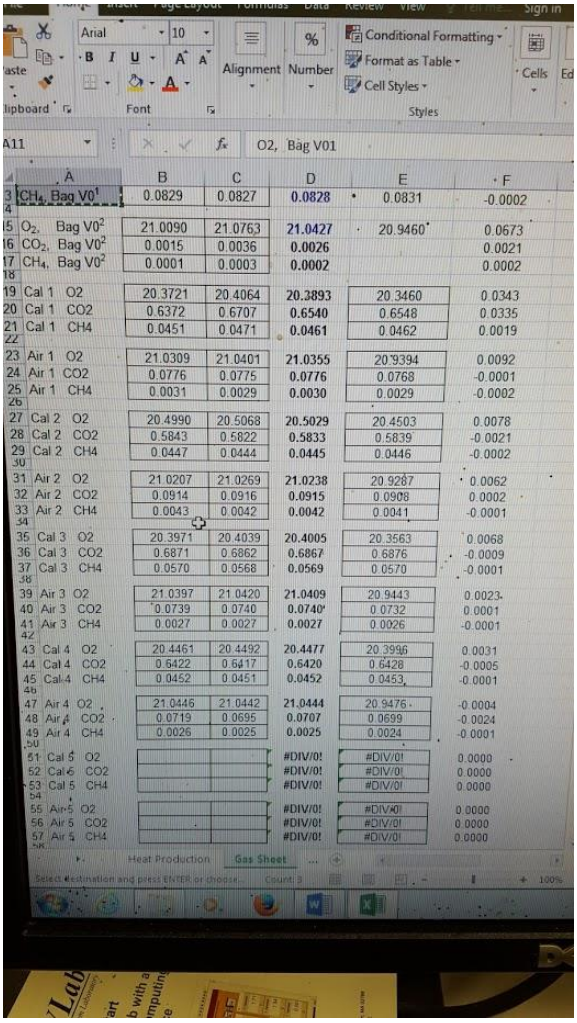


Figure A.20. Gas spreadsheet to enter the numbers from the analyzer

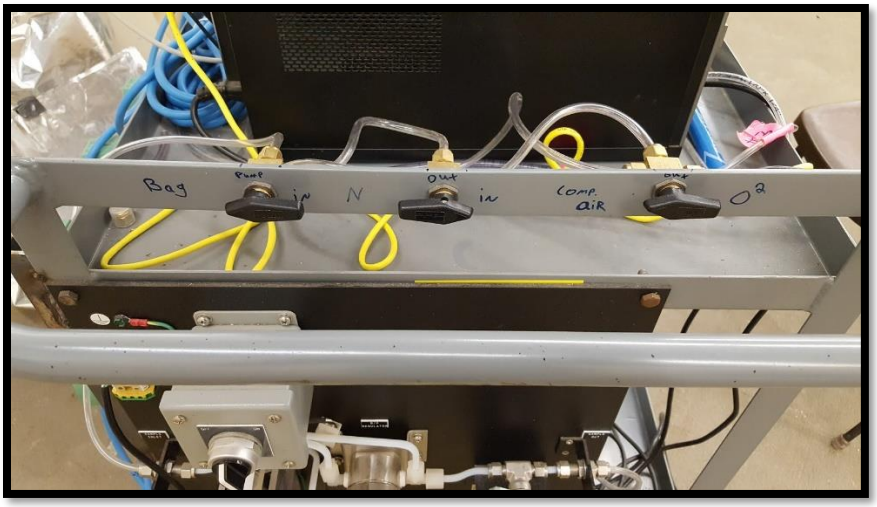


Figure A.21. Set valves to bag and turn on pump plate for Gas Bag Analysis

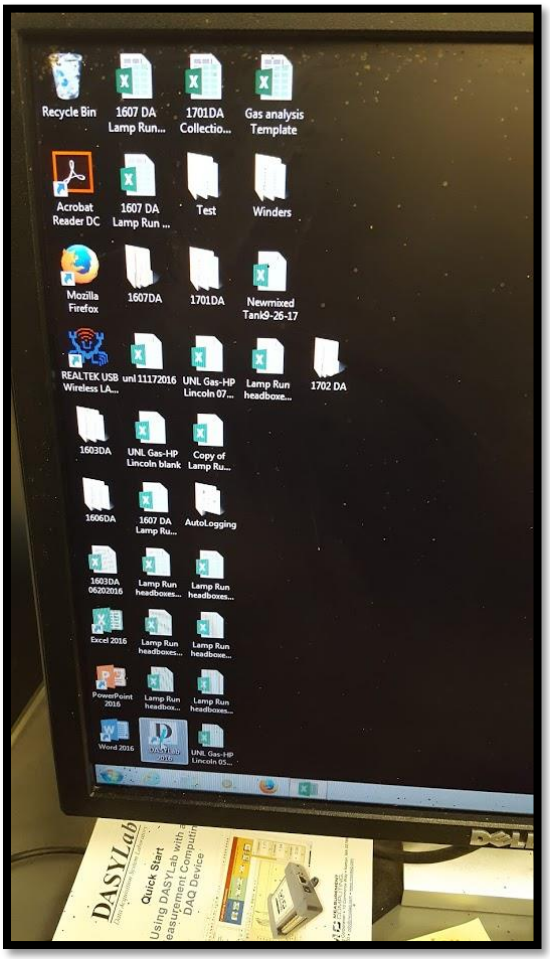


Figure A.22. Daisylab program on desktop

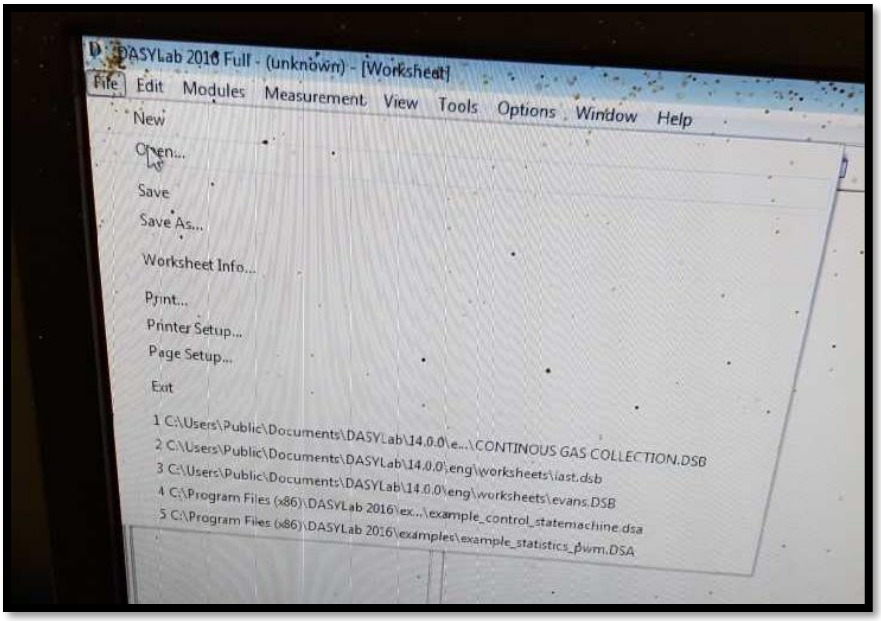


Figure A.23. Click on file then open

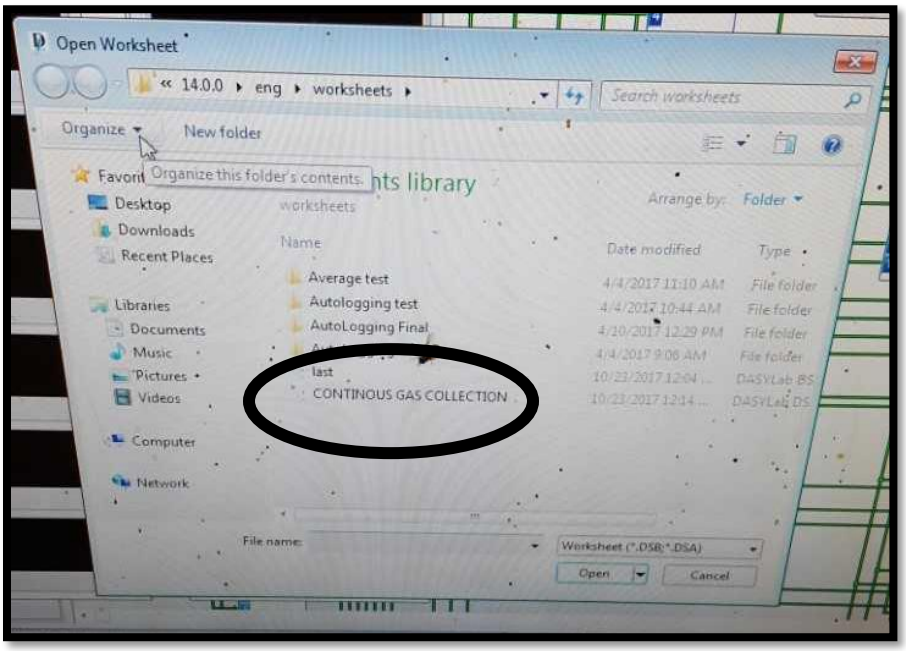


Figure A.24. Open file and go to either last or continuous gas collection

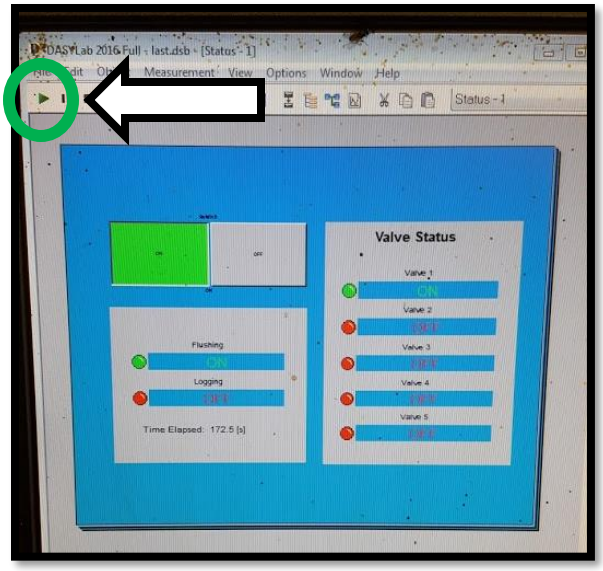


Figure A.25. Push the green button to start the continuous measurements

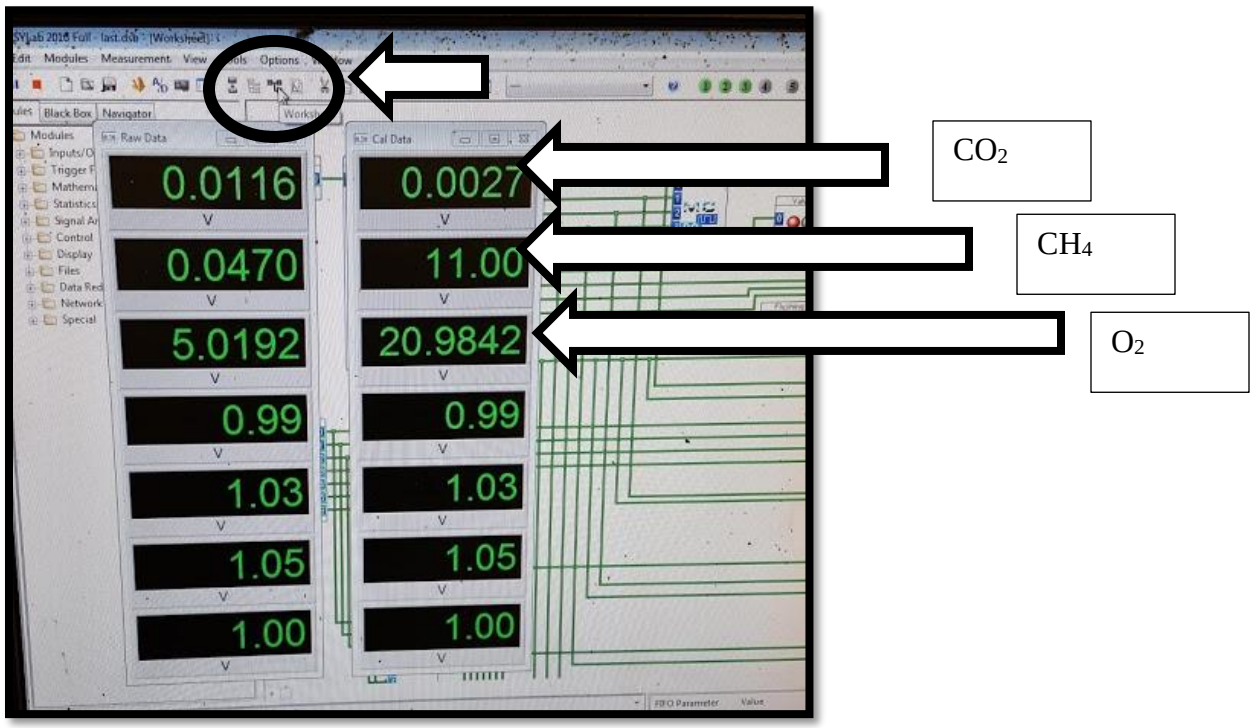


Figure A.26. Move the cursor to the worksheet tab and click on it to measure to make sure the gases are correct for the valve/headbox its on (Ambient air will be high in oxygen like in this figure, headboxes will be low in headboxes around 20.2 to 20.5)

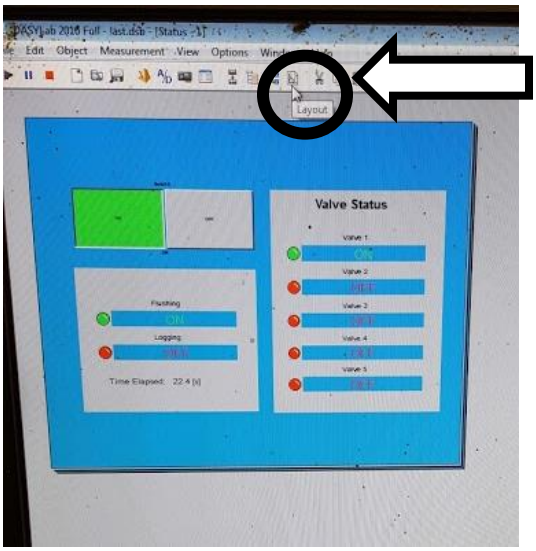


Figure A.27. Move to the layout tab to change the drierite and check that it is NOT analyzing gas from that headbox

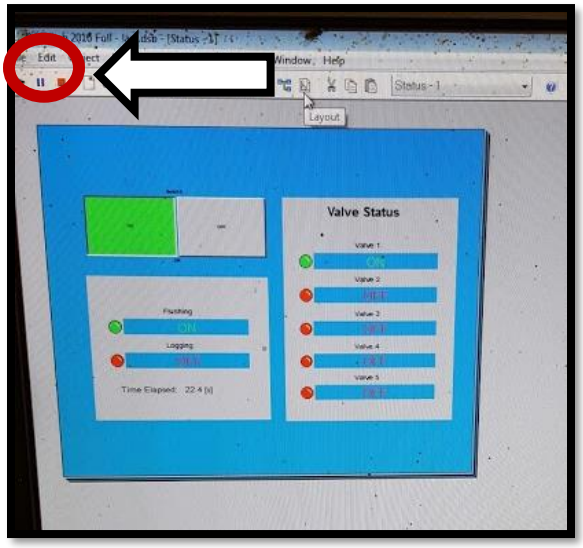


Figure A.28. Push the red button to stop the continuous measurements

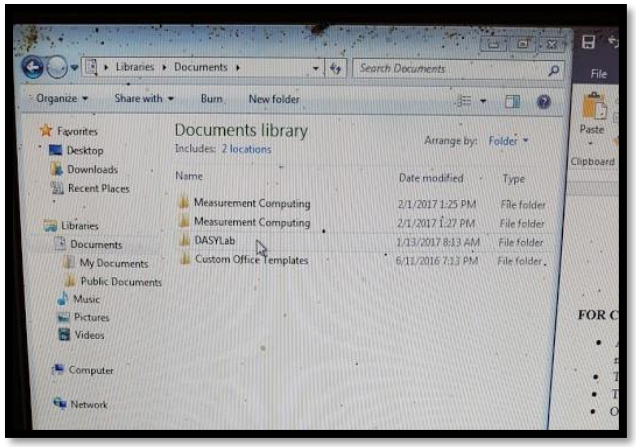


Figure A.29. Go to documents and then DAISYlab

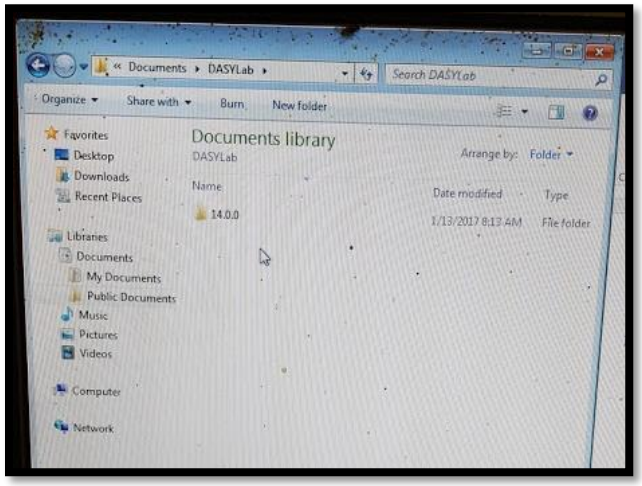


Figure A.30. Go to folder 14.0.0

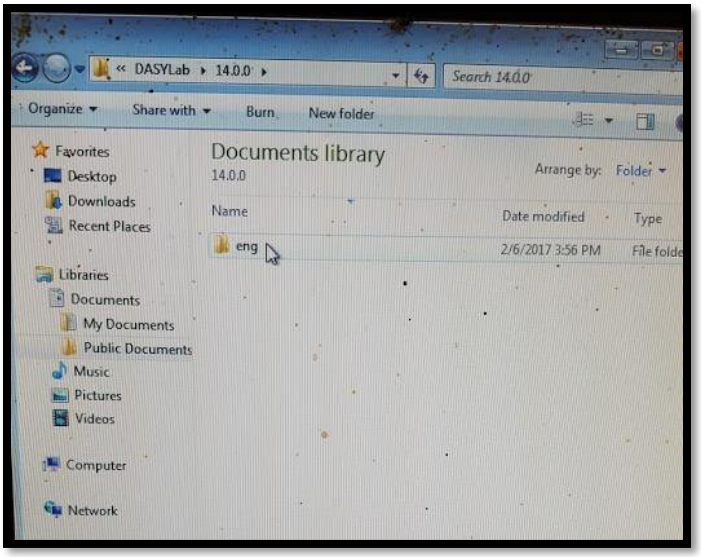


Figure A.31. Go to the folder “eng”

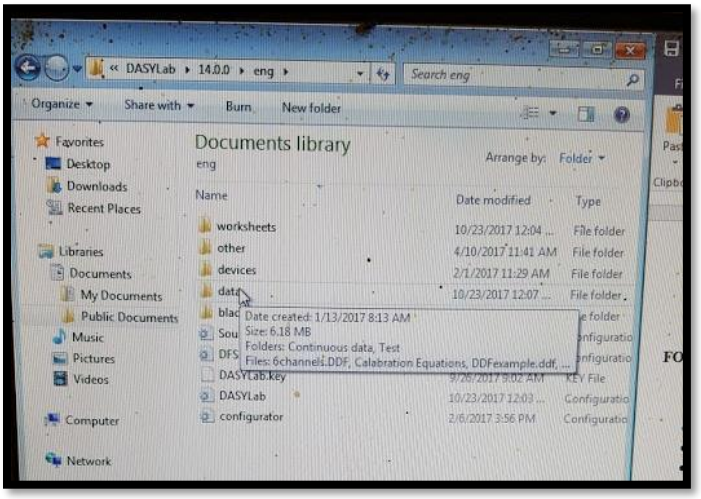


Figure A.32. Go to the folder “data”

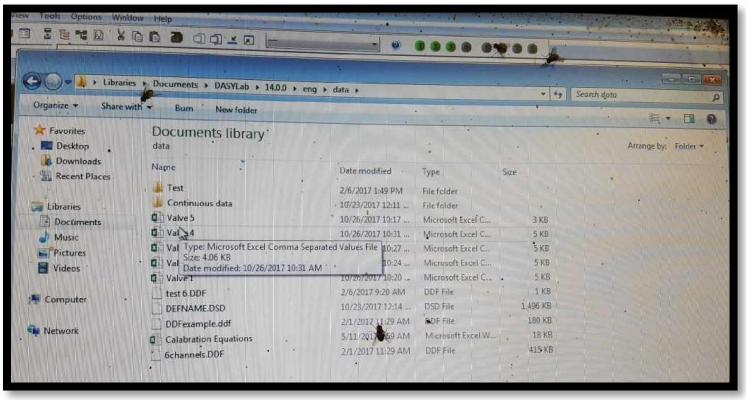


Figure A.33. Move all the valve spreadsheets to the continuous folder

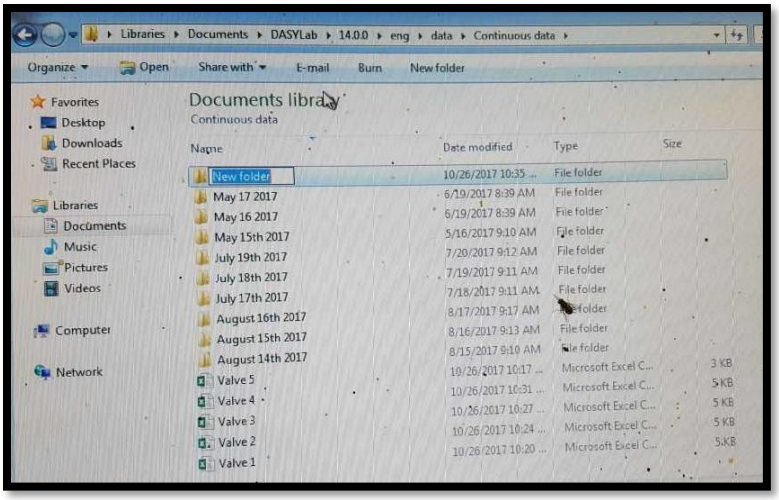


Figure A.34. Create new folder and put spreadsheets labeled valve in folder

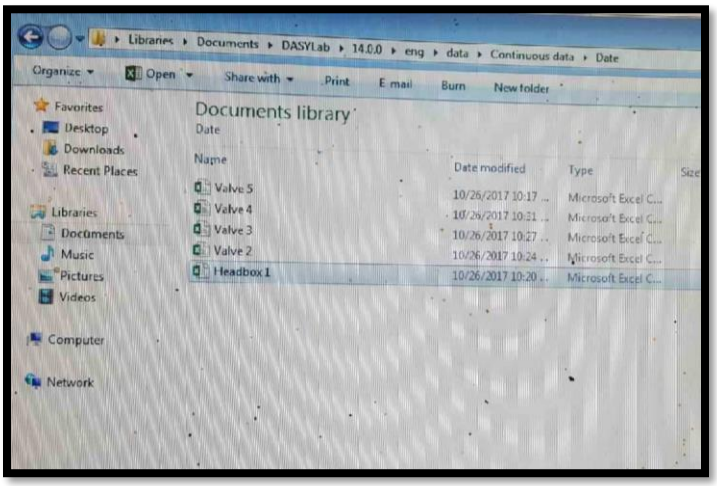


Figure A.35. Rename valve spreadsheets as Valve 1 = Headbox 1; Valve 2 = Headbox 2; Valve 3 = Headbox 3; Valve 4 = Headbox 4; Valve 5 = ambient air

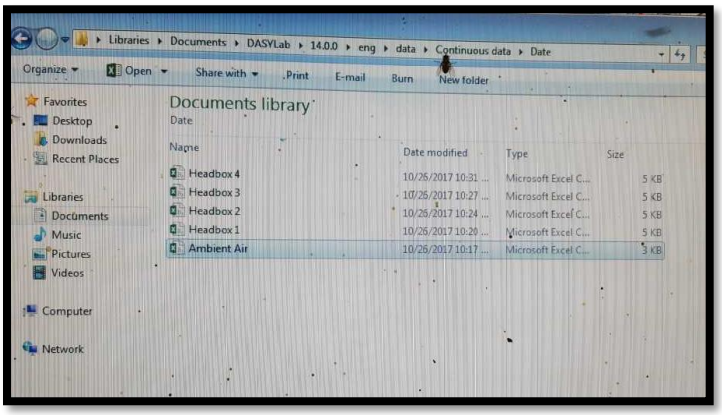


Figure A.36. This is what the folder should look like after renaming. Exit when finished

Continuous Gas System Parts List

Item	Supplier	Item Number	Cost	Contact Info	Details
Utility Cart for the Analyzer	Grainger	16D367	$517.95 each	Grainger 800-472-4643	
Michell Instruments PC33 and PC52 Humidity Probes	Instrumart	PC52-4-XX-T3-CD-F25	$336 each	1-800-884-4967	
SS-4TF-2 Filter 1/4" T Ends, 2 MIC	Swagelok	SS-4TF-2 Filter 1/4" T Ends, 2 MIC	$94.4 each	OMAHA VALVE & FITTING 12231 Cary Circle Suite 500 402-733-7636 or 800-247-7061 La Vista NE 68128	
BAROMETRIC PRESSURE SENSOR	NOVALYNX CORPORATION	110-WS-16BP	$200 each	NOVALYNZ CORPORATION PO BOX 240 GRASS VALLEY, CA 95945-0240 Phone: (530) 823-7185 Fax: (530) 823-8997 E-mail: nova@novalynx.com	
Male Elbox, 90 Deg, 1/4 in., TubexMNPT		Item no:36X117	$2.92 each		
Reducing Male Hex Nipple, Brass, MNPT		Item no: 1DGA5	$6.04 each		
Street Elbow, 90 Deg, Brass, 1/4 in., NPT		Item no: 1DGJ6	$17.66 each		
Union Tee, 1/4 in.		Item no: 11K693	$3.61 each		
Barrier Strip, 20A, 12 Pole, 300VAC		Item no: 6YH99	$4.17 each		
Hex Socket Plug, Sz 1/4 in, L 7/16 in		Item no:4WPK3	$1.32 each		
Manifold, Metal, NPT, 4in. L		Item no: 2KGZ5	$19.35 each		
Male Adapter, 1/4 in., TubeXMNPT		Item no:36X026	$2.54 each		
Male Adapter, 1/4 in., TubeXMNPT		Item no:36X027	$2.49 each		
Sample Pump Station	Universal Analyzers Inc.	6001-1637	$2,765	Universal Analyzers Inc.	18" x 15" Black Powder Coated Wall Mount Aluminum Plate, Micro Diavac Sample Pump - B161, Go Back Pressure Reg. - CPR1, 0-30PSIG Outlet Pressure Gauge, Pump
USB-based 8 Channel DAQ Module, MCC 8-Channel	Grainger	usb-1408fs, Serial Number HC1592064	$304.99	Micro-Dat P.O. Box 439 Contoocook, NH 603-746-5524 MicroDaq.com	USB-based 8 Channel DAQ Module, MCC 8-Channel 48khz Module, 4 Differential, 8 Single-ended, Analog Inputs and 2 12-bit Analog Ouput Channels,
Parker Instrumentation Ball Valves Switching Valve	MSCDIRECT.com	4z-mb4xpfa-bp 1/4 inch	$68.40	Parker Manufacturing or Mscdirect.com	
Needle Valve 1/4" NPT 5000psi SS	Grainger	5TUL9	$95.81	Grainger	
Differential Pressure Transducer	omega.com	px274-30di	$195	OMEGA.COM	
Gas Analyzer X-stream (XEGP)	Rosemount Analytical	XGEP-A-09-B40-0-C42-0-O2S-0-000-0-000-0-3-0-3-0-0-0-0-A-E-I		Rosemount Analytical-Gas Division	
Power supply and converter	MPJ	HF 60W-SL-24, Stock #16008PS	$19.95 each	MPJa.com	
SainSmart 8 Channel DC 5V Relay Module for Arduino Raspberry Pi		20-018-102-US-KS	$11.98 each		
DAISYLAB FULL Full version	Daisylab	DAISYLAB FULL MCC-399861, (HTS: 8523.49.2020 ECCN: EAR99 CoO: US)	$1,799	http://www.mccdaq.com/legal.aspx	All drivers. With all std. mods., 200
Drierite Drying Tube	WA Hammond Drierite Co. Ltd.	26930 30 g Drierite Max Flow Rate: 300 cm^3/min	$6.30 each	PO Box 460 Xenia, OH 45385-0460 email: drierite@aol.com Phone 937-376-2927 Fax 937-376-1977	¾" o.d. x 8" length hose barbs for ¼" to 3/8" i.d. flexible tubing Water capacity:3 g.

APPENDIX J: DISSERTATION DEFENSE SEMINAR POWERPOINT

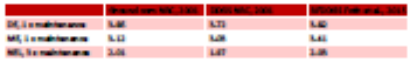
Methane From Lactating Dairy Cattle: Studies for Mitigation, Diurnal Variation, and Role in Energy Metabolism

Jared V. Judy

November 27th 2017

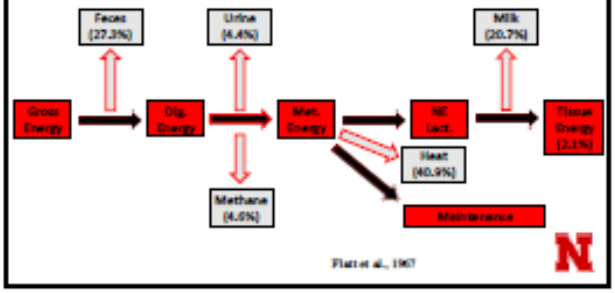
Background

- Nebraska is known for corn, and distillers grains (DDGS)
- DDGS is commonly fed
  - Energy values for reduced fat DDGS were unknown
  - Led to the work by Foth et al., 2015
    - Energy value for RFDDGS

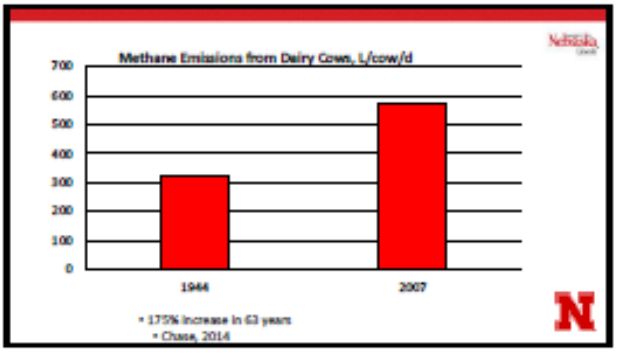


- Showed a decrease in methane when feeding RFDDGS

Energy Balance



Flatt et al., 1967



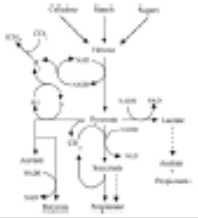
- 175% increase in 63 years
- Chase, 2014

Introduction

- The innovation center for U.S. Dairy has a goal to reduce total greenhouse gas emissions (GHG) by 25% by 2020
  - Dairy GHG emissions account for about 1.9% of the emissions in the U.S. (Thoma et al., 2013)
  - Methane reduction is an area were cattle contribute the most
    - Approximately 500 - 600 L/d and 25x the potency of CO_2 for GHG
    - Energetic loss to the animal
- Can reducing methane emissions also help total energy balance?

Methane Energy Loss

- Methane Energy
  - Energy from Methane = methane (L) X 9.45 kcal/L



Methane Production

- Many techniques have been used to reduce methane emissions (Johnson and Johnson, 1995 & Knapp et al., 2014)
  - Supplemental fat
    - Biohydrogenation (Adding hydrogens to double bonds)
    - Decrease digestibility
  - Alter digestibility
    - Modify the amount of hemicellulose available for fermentation
  - Use alternative hydrogen sinks such as sulfur or nitrate
    - Potential challenge with producers (nitrate toxicity)
  - Increase concentrate concentration of diet
    - Increases propionate production and reduces the amount of hydrogen produced

Study 1

Methane mitigation with corn oil and calcium sulfate, responses on whole animal energy and nitrogen balance in dairy cattle consuming reduced-fat dried distillers grains plus solubles

DAIRY RESEARCH

Introduction

- Other research showed methane reduction using fats and sulfur (Beauchemin et al., 2007; Van Zijderveld et al., 2010)
  - Does the reduction occur while feeding DDGS?
    - Foth et al., (2015) showed a reduction with just RFDDGS
- Methane production is a loss for cattle
  - Does reducing methane increase or repartition energy to other productive processes?
- Very little research is available on the effects of methane mitigation strategies on energy balance

Objectives

- Determine the effects of different strategies to mitigate methane production while feeding reduced fat dry distillers grains plus solubles in lactating dairy cows and determine if added corn oil and calcium sulfate affect methane production.

Hypothesis

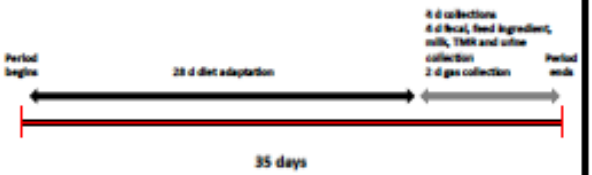
- Rations containing reduced fat dry distillers grains plus solubles with added corn oil and calcium sulfate will reduce methane production without affecting milk production, feed intake, energy and nitrogen balance

DAIRY RESEARCH

Materials & Methods

- 16 multiparous cows, 8 Holstein, 8 Jersey
  - 78 ± 15 DIM at the start of the experiment and not pregnant during study
    - 106 DIM during first gas collection
  - Holsteins BW = 428 ± 33 kg
  - Jersey BW = 593 ± 48 kg
- Repeated Latin square experimental design
- 4 squares and 2 blocks
- 4 periods
  - n = 64

Period Schedule



Materials & Methods

CONTROL (No DDGS)

DG

DG + CO

DG + CaS

Diet Formulation

Ingredient	CON	DG	CO	CaS
	% of DM			
Corn Silage	28.8	28.8	28.8	28.8
Alfalfa hay	28.8	28.8	28.8	28.8
Brome hay	3.87	3.87	3.87	3.86
Ground corn	21.8	11.8	11.8	11.8
Soybean meal	11.8	--	--	--
WDGS	--	30.0	30.0	30.0
Corn oil	--	--	1.86	--
Calcium carbonate	0.78	0.78	0.78	0.18
Calcium sulfate	--	--	--	0.65
Concentrate mix	7.87	7.87	7.87	7.87

Concentrate containing: Soybean ~3.00% of diet DM, Ground soybean hulls ~3.00% of diet DM, Dicalcium ~0.44% of diet DM, Sodium bicarbonate ~0.81% of diet DM, Calcium salts of long chain fatty acids ~ 0.69% of diet DM, Magnesium oxide ~0.24% of diet DM, Salt ~0.18% of diet DM, vitamin and mineral premix ~0.18% of diet DM

Chemical Analysis

Ingredient*	CON	DG	CO	CaS
CP, %	17.1	16.8	16.7	16.8
Crude fat, %	3.2	6.3	8.3	6.2
NDF, %	30.8	34.8	34.8	33.3
Starch, %	27.8	24.1	22.2	24.8
Sulfur, %	0.28	0.81	0.84	0.82**

*Values determined by Cumberland Valley Analytical Services, Hagerstown, MD
Mineral Research Council. 2005. Mineral Tolerance of Animals. Vol. 1nd rev. ed. Washington DC: Natl. Acad. Press.

- NRC, 2001 – Recommendation for maximum inclusion is 0.4%
- NRC, 2005 – With diet containing 40% forage, 0.5% is safe

University of Nebraska-Lincoln Dairy Metabolism Area

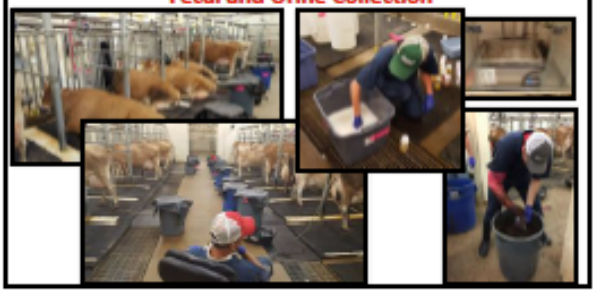
DAIRY RESEARCH

Headboxes (Indirect measure of heat production)

Milk Sampling

* 2x daily (am and pm)

Fecal and Urine Collection

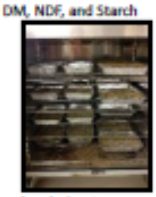


Gas Collection

- Indirect calorimeter headbox
- Two 23-h gas collection during each period
- Sampled both air entering and exiting headbox
- Analyzed air for:
  - Oxygen
  - Carbon Dioxide
  - Methane

Samples

- Feed ingredient, and TMR samples were analyzed for complete nutrient composition
  - Cumberland Valley Analytical Services
- Orts and fecal samples were analyzed for ASH, CP, DM, NDF, and Starch
  - Cumberland Valley Analytical Services
- Ingredients, orts, and feces were dried for 48 h in a 60 °C forced air oven
  - Ground to 1-mm for nutrient analysis
- Milk and Urine were lyophilized (freeze dried)
- Samples were composited by cow and period
- All samples had energy values determined using bomb calorimetry



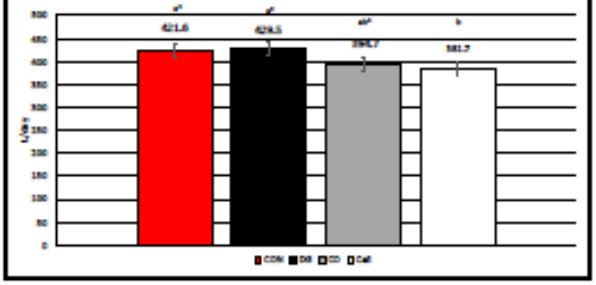
Results

DMI, milk production and composition, BW and BCS[1] of Control (CON), Reduced-fat dried distillers grains with solubles (DS), Corn Oil (CO), and Calcium Soilbie (CaS) treatments

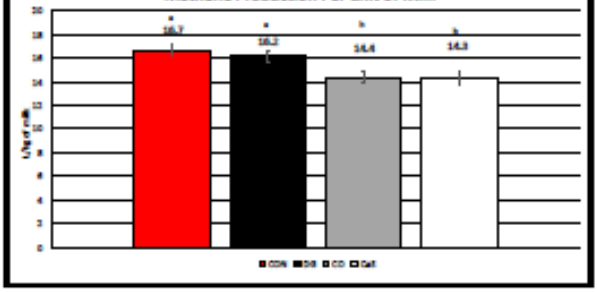
	Diet					
Item	CON	DS	CO	CaS	SEM[2]	P-value
DMI, kg/d	28.3[a]	28.1[a]	26.0[b]	28.6[a]	0.57	0.19
Milk yield, kg/d	34.3[a]	37.3[a]	28.3[b]	37.4[a]	0.87	< 0.01
ECM[3], kg/d	32.1[b]	35.4[a]	30.7[b]	35.2[a]	0.68	0.01
Fat, %	4.70	4.64	4.55	4.97	0.13	0.92
Fat Yield, kg/d	1.59	1.78	1.34	1.77	0.05	0.22
Protein, %	3.28[b]	3.26[b]	3.18[b]	3.50[a]	0.04	0.11
Protein Yield, kg/d	0.84[b]	0.97[a]	0.88[b]	0.98[a]	0.02	0.12
MUN[4], mg/dl	17.3[a]	19.0[a]	14.4[c]	18.3[b]	0.39	< 0.01
Water Intake, L/d	82.1	84.3	88.8	88.1	3.61	0.92
Body Weight, kg	708.1	703.4	719.2	710.7	11.1	0.90
BCS[1]	3.25[a]	3.15[b]	3.16[ab]	3.20[ab]	0.06	0.08

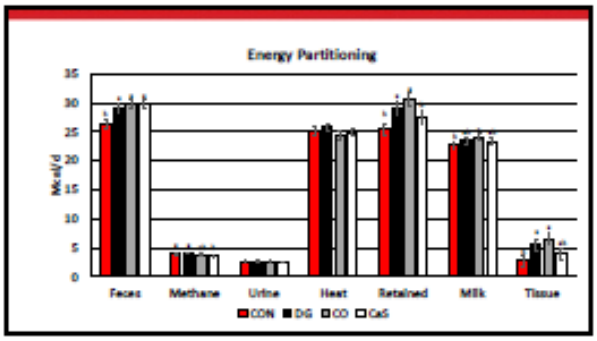
[1]BCS = Body Condition Score 1-5 scale according to Wildman et al. (1982)
[2]Lowest standard error of treatment means is shown
[3]Energy corrected milk = 0.327 × milk yield [kg] + 7.2 × protein [kg] adjusted for 3.5% fat and 3.2% total protein (DH Discovery, 2011)
[4]MUN = Milk urea nitrogen
[a,b,c]Means within rows lacking common superscript differ (P < 0.05)

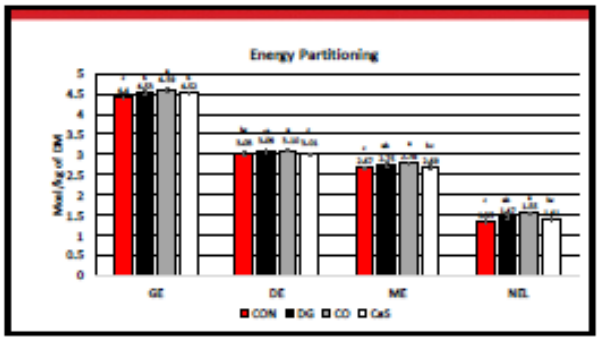
Methane Production

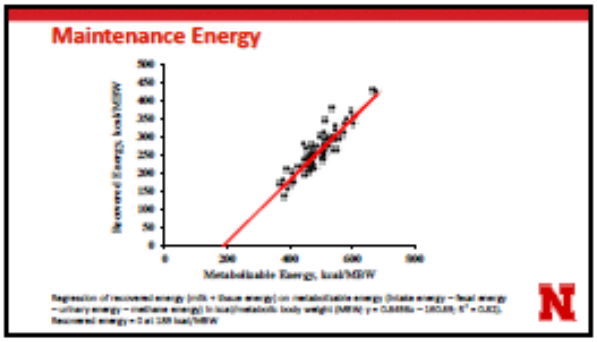


Methane Production Per unit of Milk



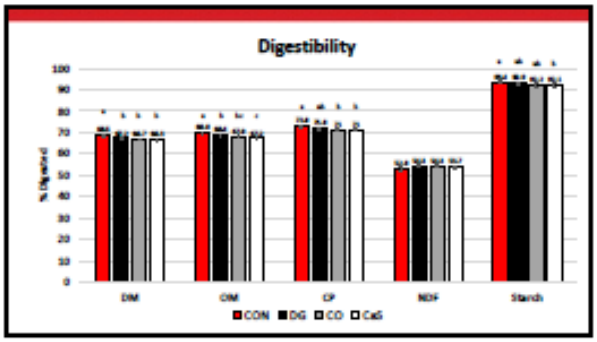






Maintenance Energy

- Greater value compared to other research (Birkelo et al. 2004; Vermorel et al. 1982)
  - Maintenance energy requirements = 189 kcal/MBW
    - Yan et al. (1997) maintenance ranged from 146 – 179 kcal/MBW
  - Efficiency of converting ME to lactation energy = 85%
    - Potentially using body stores
- Increased milk production could increase energy requirements to support increased organ function

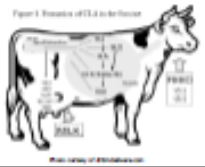


Conclusions

- Addition of CO and CaS to diets containing DDGS ↓ methane
- Feeding 20% DDGS with 1.38% added corn oil may ↑ milk yield and DMI
- Energy balance (particularly tissue energy) ↑ with distillers grains in the diet
- Diets containing 20% DDGS may ↓ overall digestibility
  - Similar to results of Foth et al. (2015)
  - May be caused by increased intake?

Background

- One strategy believed to reduce methane production by cattle is supplemental fat in the diet (Knapp et al., 2014)
  - Biohydrogenation of fat would utilize hydrogens in the rumen and potentially reduce methane production by competing with the methanogens (Johnson and Johnson, 1995)
    - Plays a role in maintaining partial pressure in the rumen
    - Uses the hydrogens so the partial pressure is reduced and biological processes may continue
  - Decreased digestibility due to the potent effect of fat on the bacterial community (Knapp et al., 2014)



Introduction

- Previous research has found that sunflower oil which is high in linoleic acid reduced methane without negatively affecting digestibility compared to tallow
  - This may suggest that biohydrogenation is occurring and that there is potential for unsaturated FA's to further reduce methane production
    - Beauchemin et al., (2007)
- Further research is needed on linolenic acid to see if it could potentially be used without affecting production, intake, or digestibility

Study 2

Increasing the concentration of linolenic acid (C18:3) in diets fed to Jersey cows in late lactation does not affect methane production

DAIRY RESEARCH

Objectives

- To determine the effects of feeding canola/tallow vs. extruded byproduct containing flaxseed as a fat source on methane production and diet digestibility in late lactation dairy cows.

Hypothesis

- Increased supplementation of linolenic acid (C18:3) would reduce methane production in lactating dairy cows without affecting milk production, milk composition and diet digestibility

DAIRY RESEARCH

Materials and Methods

- 8 multiparous Jersey cows (325 ± 17 DIM) housed in tie stall barn
  - BW averaged 485 kg
  - BCS averaged 3.75
- Two dietary treatments differing in the type of fat in the diet (detailed in Table), formulated with the CPM Model.
- 28-d periods, last 4-d of each period for data collection
  - Daily feed intake (fed once a day, allowing ~ 5% orts)
  - Daily milk production (2× milking)
  - Milk composition
  - Methane production (last 2-d of each period) collected via the indirect calorimetry method
- Data were analyzed using the MIXED procedure of SAS
  - Fixed effects: Period and Treatment
  - Random effect: Cow

Diet Formulation

Ingredient	CON, % DM	Flaxseed, % DM
Corn Silage	27.8	27.8
Alfalfa hay	21.0	21.0
Brome hay	1.87	1.87
Ground corn	20.3	17.8
Soybean meal	5.83	4.28
Canola meal	9.17	3.40
Extruded byproduct containing flaxseed	0.00	10.8
Tallow	1.78	0.00
Concentrate mixture	13.2	13.2

Concentrate contains: Soybean ~ 5.16% of diet DM, Ground soybean hulls ~ 0.24% of diet DM, Blood meal ~ 0.28% of diet DM, Calcium Carbonate ~ 0.82% of diet DM, Sodium bicarbonate ~ 0.47% of diet DM, Calcium salts of long chain fatty acids ~ 0.89% of diet DM, Magnesium oxide ~ 0.34% of diet DM, Salt ~ 0.20% of diet DM, vitamin and mineral premix ~ 0.08% of diet DM

Diet Chemical Composition

Composition and analysis of canola meal plus tallow (CON) and extruded byproduct containing flaxseed (Flaxseed) fed to lactating Jersey cows in late lactation averaging 323 ± 17 days in milk.

Item	% of DM	
	CON	Flaxseed
Chemical composition, % DM[1]		
CP	18.9	18.2
Crude fat	4.70	3.00
ADF	21.1	20.9
NDF	32.2	32.4
Ash	7.73	6.99
Starch	23.0	23.8
NFC	39.6	41.7
Fatty Acids g/d		
C18:3, intake[2]	21.1	103.8
C18:3, % of diet dry matter[2]	0.14	1.20
C18:3 Duodenal flow[3]	2.18	29.8

[1]Analyzed by Cumberland Valley Analytical Services, Hagerstown, MD
[2]Values determined by Penn State University, University Park, PA.
[3]Values formulated from Cornell-Penn-Miner dairy model V 3.0.10 (Boston et al., 2000)

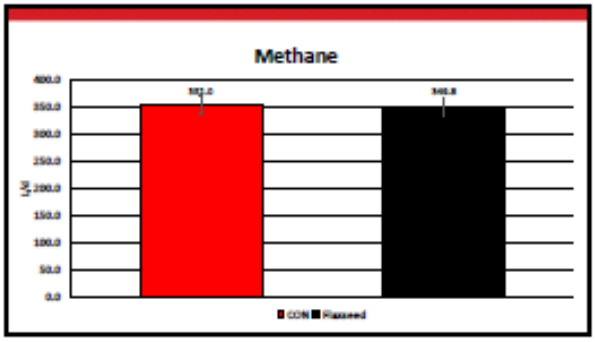
Results

DMI, milk yield and composition, BW and BCS[1] of treatments which included canola meal (CON) or extruded byproduct containing Flaxseed fed to lactating Jersey cows in late lactation averaging 323 ± 17 days in milk.

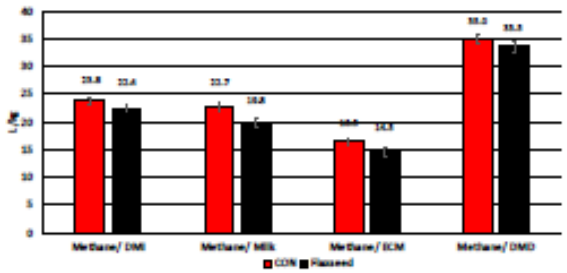
	Treatments			
Item	CON	Flaxseed	SEM[2]	P-value
DMI, kg/d	18.0	18.7	0.71	0.26
Milk yield, kg/d	16.8	17.8	1.04	0.58
ECM[3]	28.3	34.8	1.84	0.49
Feed conversion	1.92	1.97	0.18	0.93
Fat, %	5.89	5.86	0.29	0.98
Fat yield, kg/d	0.99	1.04	0.09	0.91
Protein, %	4.08	4.07	0.14	0.69
Protein yield, kg/d	0.68	0.72	0.09	0.93
Lactose, %	4.68	4.72	0.04	0.58
MUN, mg/dl[4]	20.0	19.5	1.00	0.98
Water intake, L/d	79.4	72.1	4.90	0.77
Body weight, kg	684.9	686.9	19.8	0.83
BCS[1]	3.78	3.78	0.07	1.00

[1]BCS = Body Condition Score 1-5 scale according to Wildman et al. (1982)
[2]Largest standard error of treatment means is shown
[3]Energy corrected milk = 0.327 × milk yield (kg) + 7.2 × protein (kg) adjusted for 3.5% fat and 3.2% total protein (DHI Glossary, 2014)
[4]MUN = Milk urea nitrogen

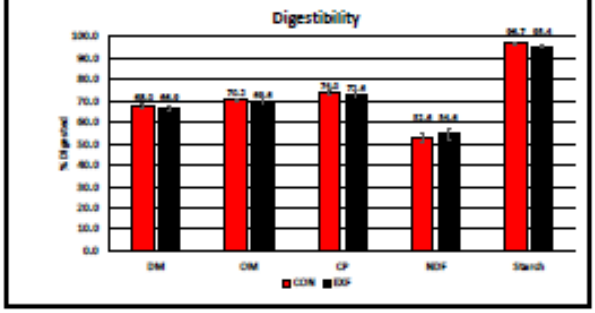
Methane



Methane Per Unit of DMI and ECM



Digestibility



Summary

- Milk production, fat yield, and protein yield were not different with the extruded byproduct containing flaxseed
- Methane production was not affected
  - Martin et al. (2008) found a 39% decrease while using extruded flaxseed
    - Difference in crude fat
  - Van Zijderveld et al. (2010) found a 10% decrease while using flaxseed oil vs. rumen inert fat
- Digestibility was unaffected by fat source

Conclusions

- Extruded by products containing flaxseed may be used without affecting production (in late lactating dairy cattle)
- Added linolenic acid did not decrease methane production compared to other fat sources
  - Small benefit on methane reduction with biohydrogenation
  - Likely the toxicity of fats on microbes plays major role
- Digestibility of the diet is not reduced when adding higher concentrations of unsaturated fatty acids

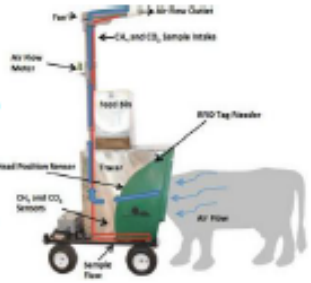
Study 3

Maintenance energy use and diurnal variation in methane production in late lactation Jersey cows

DAIRY RESEARCH

Introduction

- Methane production is very episodic (Hegarty, 2013)
- Gas production affected by...
  - Feeding frequency, fermentation rate and pattern (Braak et al., 2015)
  - Feed consumption and digestion (Crompton et al., 2011)
- Increased methane production after feeding with greater peak after a second feeding (Hollmann et al., 2013)
- Majority of maintenance energy requirements are over 50 years old and may have increased



Objectives

- Characterize diurnal methane production and estimate energy maintenance in late lactation dairy cattle being fed either once or twice daily.

Hypothesis

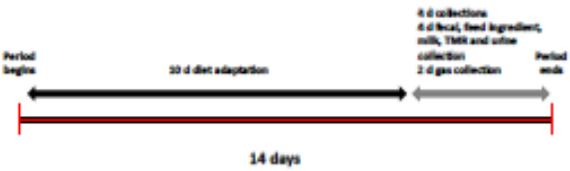
- Methane production would increase after feeding and not remain constant throughout the day

DAIRY RESEARCH

Materials & Methods

- 12 multiparous Jersey cows
  - 225 ± 16.2 DIM at the start of the experiment and not pregnant during study
  - BW = 480 ± 12.2 kg
- 3 squares
- 2 periods
  - n = 24

Period Schedule



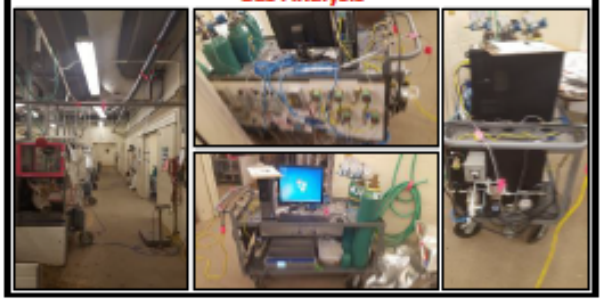
Treatments

- 2 treatments
  - Once a day feeding (1X)
    - All feed delivered at 1000 hr
  - Twice daily feeding (2X)
    - 50 % of feed delivered at 1000 hr
    - 50 % of feed delivered at 2000 hr
  - Gas measured hourly & full day

Item	Diet
Ingredient, % of DM	
Corn Silage	57.7
Alfalfa hay	14.3
Krome hay	2.94
Concentrate mix[1]	45.7
Chemical composition	
DM, %	41.8
OM, % DM	93.5
Crude Fat, % DM	4.23
ADF, % DM	16.6
NDF, % DM	28.6
Starch, % DM	28.7
Lignin, % DM	3.78
Ash, % DM	7.08

[1]Concentrate as a % of DM: soybean, ground corn – 17.1%; DDM – 14.2%; Soypass – 6.66%; Ground soybean hulls – 2.84%; Tallow – 1.99%; Monodimod – 1.04%; Ca carbonate – 1.42%; Sodium bicarbonate – 0.95%; Calcium salts of long chain fatty acids – 0.81%; CaProtaid – 0.35%; Magnesium oxide – 0.33%; Salt – 0.24%; bypass methionine – 0.07%; bypass lysine – 0.05%;Vitamin and mineral premix – 0.06%

Gas Analysis

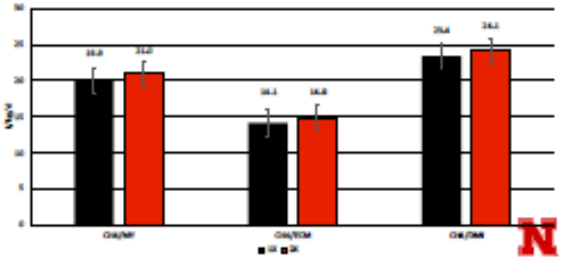


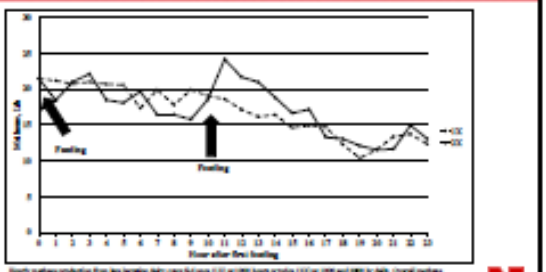
DMI, milk production and composition, body weight and SCS†, and water intake of late lactation Jersey cows (225 ± 16.3 DIM) (mean ± SE)

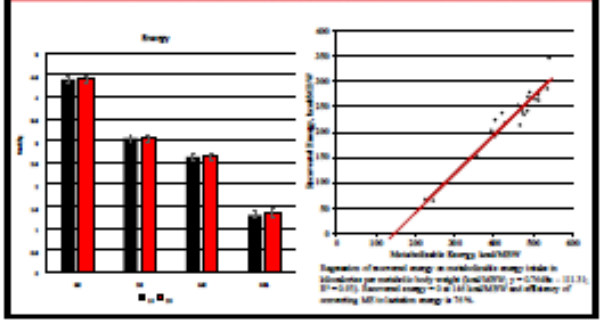
	Feeding Frequency[1]			
Item	1X	2X	SEM[2]	*P*-value
DMI, kg/d	17.4	17.1	1.00	0.393
Milk yield, kg/d	21.2	20.6	1.39	0.097
ECM†, kg/d	28.8	28.8	2.31	0.993
Fat, %	6.18	6.18	0.30	0.966
Fat yield, kg/d	1.30	1.26	0.10	0.097
FCM‡,kg/d	30.5	29.9	2.34	0.089
Protein, %	3.88	3.87	0.06	0.717
Protein yield, kg/d	0.84	0.81	0.01	0.040
Lactose, %	4.89	4.89	0.08	0.459
MUN¶, mg/dl	20.8	20.1	0.88	0.056
SCC‖, cells/mL	129.9	106.3	55.1	0.477
Free water intake, L/d	83.8	79.7	8.67	0.026
Body weight, kg	480.0	480.1	13.3	0.229
BCS#	3.37	3.43	0.11	0.148

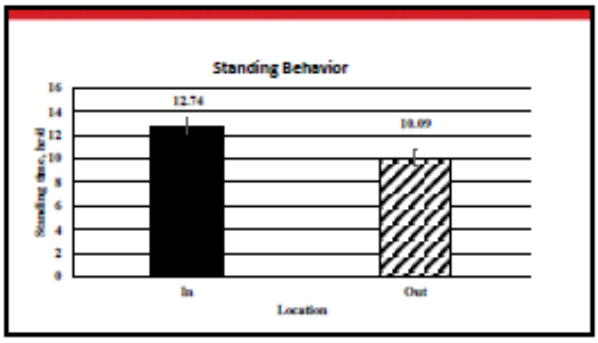
[1]Treatments: 1X = one time a day feeding; 2X = two times a day feeding.
[2]Lowest standard error of treatment means is listed.
[3]Energy corrected milk = 0.327 × milk yield [kg] + 7.2 × protein [kg] adjusted for 3.5% fat and 3.2% total protein (DH Gleason, [illegible]).
¶MUN = Milk urea nitrogen.
‖SCC = Somatic cell count.

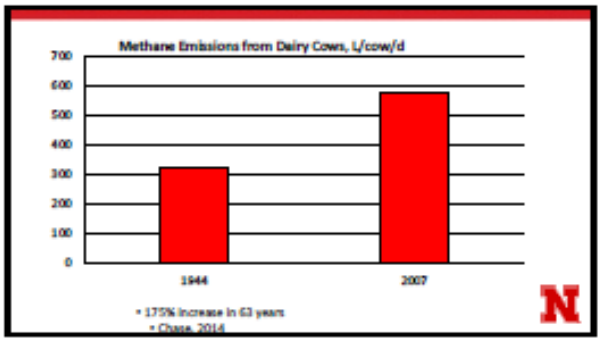
Methane Production

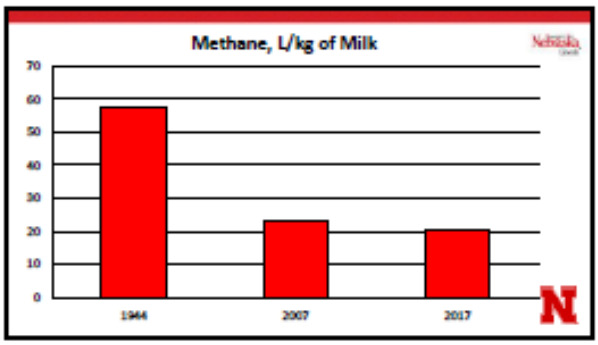












Overall Conclusions

- Added fat and sulfate may be used to reduce methane production in lactating dairy cattle
- Fat and not necessarily fat source decreases milk fat
  - Linolenic acid may be used up to 188 g/d without negative effects on milk production
- Methane production is dynamic
  - Increasing after feeding
  - Spot sampling may not accurately estimate total production

Future Research

- Utilize cannulated cattle to investigate the biohydrogenation
  - Test the limit with milk fat depression
- Heat production in dairy cattle (Particularly Jersey's)
  - Values are old and need updated
- Correspond pH values with methane reduction

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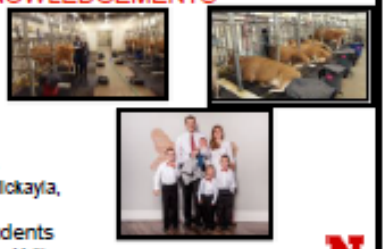


Questions?