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Effect of Fat and Fiber on Methane Production and Energy Utilization in Lactating Dairy Cows

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EFFECT OF FAT AND FIBER ON METHANE PRODUCTION AND ENERGY UTILIZATION IN LACTATING DAIRY COWS

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Due to rising concerns of greenhouse gases and that ruminants are the largest livestock methane producers, an emphasis has been put on developing methane mitigation strategies to reduce methane emissions in ruminants. Other than reducing methane, maximizing the energy utilization of cattle is also important for producer's overall productivity and profitability.

In the first experiment, fat or cellulose was added to fiber isolated from dried distiller's grains and solubles. Isolated NDF residue from an in vitro setting was fermented 1) alone (control); 2) with feed grade corn oil at 20%; or 3) with cellulose powder microcrystalline at 20% using the in vitro gas production technique. Results suggested that the addition of oil or cellulose to NDF residue resulted in a decrease or no effect on methane production and total gas production, respectively. These observations further suggest that diets may be manipulated to mitigate methane from ruminant livestock.

A second experiment was conducted using, eight multiparous, lactating Jersey cows in a twice replicated 4 × 4 Latin square using a headbox type indirect calorimetry to determine the effects of feeding different concentrations of fat and hemicellulose on

energy utilization and methane production. For fat concentration manipulation, tallow was included at either approximately 0 or 2 % of the diet DM. For hemicellulose concentration manipulation, the inclusion rates of corn silage, alfalfa hay, and soybean hulls were changed and resulted in diets containing either 11.3 % or 12.7 % hemicellulose (DM basis). The factorial arrangement of the treatments were both high and low fat and hemicellulose (LFLH, LFHH, HFLH, and HFHH). Results suggest that methane production was not affected by treatment however methane produced per unit of DMI tended to decrease with inclusion of fat. Fiber digestibility improved with increasing concentration of hemicellulose. Methane per unit of digested NDF tended to decrease with increasing concentration of hemicellulose. Energy utilization overall was improved as net energy of lactation was improved with increasing hemicellulose in low fat diets.

"Which of all these does not know that the hand of the Lord has done this? In his hand is the life of every creature and breath of all mankind"

-Job 12:9-10 NIV

"God made the wild animals according to their kinds, the livestock according to their kinds, and all the creatures that move along the ground according to their kinds. And God saw that it was good"

-Genesis 1:25 NIV

"You have brains in your head. You have feet in your shoes. You can steer yourself in any direction you chose. You're on your own. And you know what you know. You are the guy who'll decide where to go"

-Dr. Seuss

"Your're off to great places! Today is your day! Your mountain is waiting, so…get on your way!

-Dr. Seuss

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INTRODUCTION

Ruminants are unique because with the aid of rumen microbes they can directly ferment and utilize fibrous material such as cellulose which is the most abundant carbohydrate in the world (Van Soest, 1994). Unlike ruminants, monogastrics cannot utilize these fibrous materials. Because of this unique trait, ruminants can be considered as net contributors to the global supply of human food. Global consumption of meat is expected to increase from 229 to 465 million tons and milk from 580 to 1043 million tons by 2050 (Morgavi et al., 2010). Therefore, livestock and specifically dairy cattle will be important to accommodate the global increase in milk and meat consumption. However, ruminants do produce methane as a by-product of fermentation in the rumen. Along with carbon dioxide and nitrous oxide, methane is a major greenhouse gas (GHG). These greenhouse gases can have negative effects on the environment by enhancing the effects of solar and thermal radiation on the surface of the earth therefore increasing global temperatures and contributing to climate change (Knapp et al., 2014). Because of this, over the decades a focus has been placed on developing methods to mitigate methane production.

Modern dairy practices require less animals, feed, water and land to produce the same 1 billion kg of milk compared to 1944. They also produce 43 % of methane per billion kg of milk as compared to historical dairy practices. In 1944, there was a total population of 25.6 million cows producing a total of 53.0 billion kg of milk annually in the US. In 2007, there were 9.2 million cows producing 84.2 billion kg of milk annually in the US (Capper et al., 2009). When looking at individual cows, there has been a 175 %

increase in methane emissions from approximately 300 L/cow/day in 1944 to 600 L/cow/day in 2007 (Chase, 2011). In the modern era, ruminants as a whole produce 24.9 % of total methane emissions or 2.2 % of total US GHG emissions (Chase, 2014). Looking at the dairy industry as a whole regarding methane emissions, dairy cattle provide 24.8 % of enteric emissions that is 0.54 % of total US GHG emissions (Chase, 2014). Total methane emissions from the dairy cattle industry has decreased by 38 % from 1944 to 2007 (Chase, 2011). So, over the years, the dairy industry has reduced its methane emissions. However, there is still an urgent need to reduce the production of GHG even more. In 2009, the United States Department of Agriculture and the Innovation Center for US Dairy signed a memorandum of understanding that the dairy industry plans to reduce GHG emissions by 25 % by 2020 (Innovation Center for Dairy, 2013). There are many methods to reduce methane production and one of these methods may be through manipulation of feed ingredients included in the diet.

Fats are often fed to cattle because they increase the energy density of the diet. Another benefit of fats is they may reduce methane production in the rumen. Inclusion of a high concentration of fat has been shown to decrease rumen methane by as much as 40 % but, 10 to 25 % is more likely in practice depending on other factors (Beauchemin et al., 2008). There are multiple mechanisms behind this reduction of methane. Methane can be mitigated by decreasing fermentability of substrates, reducing or inhibition of methanogens or protozoa, use of hydrogen for biohydrogenation of unsaturated fatty acids or enhanced propionic acid production (Johnson and Johnson, 1995; Beauchemin et al., 2007). Fat supplementation can result in maintained livestock production yet the

challenge is finding a fat source that is also cost effective (Grainger and Beauchemin, 2011). However, the inclusion of fat may result in negative effects on DMI, rumen fermentation or fiber digestibility (Andrews et al., 1991; Beauchemin et al., 2007; Eugéne et al., 2008). As compared to fat, increasing the fiber content of diets often increases methane production (Johnson and Johnson, 1995; Shibata and Terada, 2010). Cattle fed diets with low (10 to 30 %) fiber produced less methane (2 to 3 % GEI) (Knapp et al., 2014). This is because forages or fiber favor the production of acetate and butyrate and lower propionate production. Acetate and butyrate are not hydrogen sinks as compared to propionate. So the excess hydrogen and reducing equivalents from digestion and fermentation cannot be utilized. Therefore, the hydrogen builds up and is utilized in the production of methane. Improving quality and digestibility of forages would likely decrease methane (Hristov et al., 2013).

Of the fibrous components of the cell wall components, cellulose may contribute more to methane production than hemicellulose (Holter and Young, 1992). One reason for this is that compared to cellulose, hemicellulose can be less digestible in ruminants but there are some exceptions (Keys et al., 1969). Another reason for greater methane production with cellulose is although hemicellulose can also be more digestible than cellulose, hemicellulose is often more associated with lignin which is indigestible by ruminants. Lignin is cross-linked with hemicellulose by phenolic constituents such as ester and other glyosidic linkages (Van Soest, 1994). Therefore, less hydrogen can come from the digestion of hemicellulose because of lignin reducing digestibility. This may result in less hydrogen to be used in methanogenesis as compared to cellulose which has

a smaller association with lignin. In an analysis of calorimeter data Moe and Tyrrell (1979) observed that digested hemicellulose resulted in 37% less methane than digested cellulose (Knapp et al., 2014) and suggested that methane produced per gram of digested cellulose is approximately 3 times greater than each gram of digested hemicellulose (Moe Tyrell, 1979). Furthermore, neutral detergent fiber (NDF), which contains both cellulose and hemicellulose, will also have an effect on methane. Neutral detergent fiber digestibility would be expected to have a greater impact on methane production because fermentation of cellulose and hemicellulose is greater than non-fiber carbohydrates such as starch (Knapp et al., 2014). The highly digestible NDF contained in corn milling by-products such as dried distillers grains and solubles (DDGS), which have a high hemicellulose: cellulose ratio, results in lower methane production compared to other forages (Johnson and Johnson, 1995; Knapp et al., 2014). The NDF fraction of DDGS ranges from 30 to 55 % (Dong and Rasco, 1987). More recently the NRC (2001) states the NDF fraction of DDGS is 38.8 % and the hemicellulose fraction is approximately 19%. When looking at the hemicellulose content of forages such as alfalfa hay which is approximately 9 %, grass hay which is approximately 25 %, and corn silage which is approximately 17 %, DDGS are generally higher in hemicellulose content to other forages (NRC, 2001). The hemicellulose content of other by-products includes dried brewers grains which is approximately 25 %, corn gluten meal which is approximately 3 % or citrus pulp which is approximately 2 % (NRC, 2001). Compared to other byproducts the hemicellulose content is generally higher for DDGS.

Reducing methane is important for reducing greenhouse gas production but, it also represents a source of energy loss. By reducing methane, energy that would have been lost can be utilized elsewhere in the animal's body and energy partitioning scheme to improve overall energy utilization. Any lactating animal, which includes dairy cows, have a high requirement for energy to meet both maintenance and milk production and there are many complex biological pathways needed to achieve this. If the requirements are not met, body reserves are utilized resulting in a negative energy balance. Consequently, a major goal in feeding a lactating dairy cow is to feed a balanced diet to help minimize time in a negative energy balance by improving utilization of energy sources from feed. Energy utilization is complex because it may be affected by many different factors such as type of ration, level of intake and production, stage of lactation, environmental conditions and animal size that adds variability (Saama et al., 1993).

To date, a number of studies have evaluated the influence of fat and fiber on methane production and energy utilization but, information is lacking on understanding how these diets affect methane production when dairy cattle are fed diets containing high concentrations of DDGS. Therefore, the objectives of this work were to 1) isolate and test the effects of fat and fiber separately and together, on methane production and 2) more closely study how changing the proportion of fat and hemicellulose affects methane production and energy utilization in lactating dairy cattle.

CHAPTER 1

LITERATURE REVIEW

Rumen Fermentation

Rumen fermentation is a result of the activity of microbes namely bacteria, protozoa and fungi (Williams, 2000). End products of anaerobic microbial fermentation include volatile fatty acids (VFA's) in the rumen and serve as a major energy source for the host animal. The three main VFA's are acetate which is produced in the greatest amount in most diets, propionate which also serves as a hydrogen sink that reduces methane and butyrate. The production of these individual VFA's depend on the substrate consumed by the cow. For example, forage based diets favor acetate and butyrate production however starch based diets favor greater propionate production (Knapp et al., 2014).

Rumen Digestion of Fiber. Fiber is defined as the carbohydrate fraction resistant to digestion by enzymes produced by cattle and is the predominant carbohydrate of the plant cell wall (Blezinger, 2013; Corrigan, 2011). Fiber is mostly comprised of cellulose, hemicellulose and lignin (Van Soest, 1994). Cattle do not produce the enzymes required to break down fiber therefore they rely on microbes to break down the fiber. Cattle contribute to microbial digestion by chewing and ruminating feed particles and this physically breaks the fiber particles and increases surface area available for microbial digestion. Fiber digestion can occur in the rumen and large intestine; however, only a

small amount of fiber will be digested in the large intestine (Corrigan, 2011). Fibrolytic bacteria ferment the fiber and from this fermentation acetic acid (VFA) is produced and is absorbed through the rumen wall. Acetate or acetic acid is used by the cow for energy and for the synthesis of milk fat (Blezinger, 2013). Some of the fiber fermented by the microbes is utlized as energy for the microbial cell (Corrigan, 2011).

The amount and size of fiber particles are important to maintaining rumen function. Longer fiber particles form a mat layer in the rumen. This mat layer is where fiber particles are entangled because they are too long to pass to the lower gut. Fiber will then be regurgitated and chewed producing a large amount of salvia which contributes to the buffering capacity of the rumen (Blezinger, 2013). Increasing fiber levels has been shown to increase chewing activity which results in increased salvia, rumen pH, and milk fat levels which will improve rumen function (Kononoff et al., 2003). Physical reduction of fiber through rumination will increase the density because the cellular structure is broken (Van Soest, 1994). The chewed particles create a larger surface area for the microbes to utilize, consequently improving fiber digestion in the rumen (Blezinger, 2013).

The pH in the rumen may also affect fiber digestion. Inadequate fiber concentrations of fiber or fiber that is too fine may result in reduced chewing time therefore reducing salvia production and reduced ruminal pH. Fibrolytic bacteria grows best when the pH of the rumen is 6.2 to 6.8. When rumen pH drops below 6.0 – 6.2, fiber digestion begins to decline because fibrolytic bacteria activity is reduced. If the pH drops below 5.8 – 5.9 fiber digestion may be severely impaired (Blezinger, 2013).

Compared to non-fiber carbohydrates such as starch, fiber is generally less energy dense and less digestible (Knapp et al., 2014). Furthermore, there are animal factors that affect rumen fiber digestion. For example, at high levels of intake, ruminal fiber digestion may be suppressed because passage rate increases and rumen microbes have less time to digest fiber. There are also plant factors that affect fiber digestion. One limitation is the physical and chemical nature of plants which may serve as a barrier to complete digestion, especially lignin (Varga and Kolver, 1997). Lignin is part of the cell wall in forages and it is largely indigestible by rumen microbes and thus cannot be used as an energy source for the animal. As plants mature the concentration of lignin increases and will reduce fiber digestibility (Blezinger, 2013; Van Soest, 1994). Another limitation is cellulose crystallinity. This high order of structure may impair digestibility (Van Soest, 1994). Maturity of plants will affect fiber digestion. Immature plants are more digestible than mature plants. Finally, location will influence fiber digestibility. Forages grown in warmer places have more lignin and therefore are less digestible than forages grown in temperate places (Blezinger, 2013).

Effects of Fat on Rumen Fermentation and Fiber Digestion. Dietary fat may have negative effects on rumen fermentation and fiber digestion (Alstrup et al., 2015; Andrews et al., 1991; Jenkins, 1993). High supplementation rates of fat can reduce fiber digestibility (Beauchemin et al., 2007; Hoover and Miller, 1992a). Supplementation of fat often replaces easily digestible carbohydrates thus reducing fermentation in the rumen (Alstrup et al., 2015). It is largely believed that unsaturated fatty acids are toxic for rumen

microbes therefore will decrease fiber digestion and rumen fermentation (Jenkins, 1993; Onetti et al., 2001). Fatty acids, especially polyunsaturated fatty acids inhibit growth of ruminal microbes therefore reducing fiber digestion as you increase fatty acid content of the diet (Holter et al., 1992). Several mechanisms have been proposed on the effect of fat on rumen fermentation and fiber digestion; however, there are two theories that have received the most attention. The first and most popular theory is the coating theory which theorizes that fat forms a lipid layer over feed particles that inhibits digestion. The second theory is the direct antimicrobial effects theory (Jenkins, 1993).

The type of fat determines if it reduces rumen fermentation and fiber digestion. For example, calcium salts of long chain fatty acids (Ca-LCFA) don't negatively affect rumen fermentation and fiber digestion in lactating dairy cows (Andrews et al., 1991). Ca-LCFA are rumen bypass fat and therefore can't affect rumen fermentation or fiber digestion occurring in the rumen. Long chain fatty acids inhibit cellulolytic microbes, with degree of unsaturation and rate of release in the rumen positively related to decreased ruminal fermentation. Therefore, feeding sources of saturated fatty acids such as tallow may not have negative effects on fiber digestion. Also, unsaturated long chain fatty acids may not reduce fiber digestion (Beauchemin et al., 2007). Blended fat sources may improve fermentation compared with single fat sources such as commercial blends of animal fat and vegetable oil because they resemble ruminally inert fats. With inclusion of less than 10% fat supplementation can reduce ruminal digestion of structural carbohydrates by 50% or more (Jenkins, 1993). The current recommendations for dairy

ration crude fat is not to exceed 6 to 7 % DM (Knapp et al., 2014). Low levels of dietary fat (≤ 5 %) do not negatively affect microbial growth (Hoover and Miller, 1992b).

Methane Production in the Rumen

Methane along with carbon dioxide and nitrous oxide are considered greenhouse gases (GHG) that contribute to global warming. There has been a rising concern and emphasis put on ways to mitigate these GHG, especially methane in the livestock industry since ruminants produce more methane than any other livestock. The Innovation Center for US Dairy is striving to reduce GHG from fluid milk by 25% by 2020 (Innovation Center, 2013). Greenhouse gases are either directly (e.g. enteric fermentation and manure management) or indirectly (e.g. feed production activities) produced from livestock (Hristov et al., 2013). In 1995, it was estimated that over the subsequent 50 years methane would be responsible for 15 – 17 % of global warming while 2 % was expected to be from cattle (Johnson and Johnson, 1995). More recently Knapp et al. (2014) suggested that methane causes 3.3 % of the total GHG emissions and cattle contributed 6.3 % of these GHG emissions (Hristov et al., 2015). Overall agriculture is responsible for 29 % of global methane sources, with 17 % of methane coming from enteric fermentation, 2 % of methane coming from manure production and 7 % of global GHG sources (Knapp et al., 2014). Dairy cattle specifically produce 24.8 % of enteric methane emissions which is 0.54 % of the total US GHG emissions (Chase, 2014). Importantly the world population is growing and because of that livestock numbers are projected to increase also. According to Grainger and Beauchemin (2011) if methane

emissions increase parallel to the projected increase in livestock numbers, then global methane emissions from livestock are expected to increase 60 % by 2030. In summary, it is clear that ruminants contribute to increasing methane emissions and in turn global GHG emissions. Consequently, there is a need to discover methods to mitigate methane emissions without impacting animal and whole-farm productivity (Grainger and Beauchemin, 2011).

Methane production, is a natural component of the digestive processes in ruminants. Microbes occupy the animal's digestive system that ferments feed consumed by the animal. This digestive microbial fermentation process is often referred to as enteric fermentation and produces methane as a byproduct. This methane is then ultimately exhaled which can be called eructation or loss via flatulence by animals. The volume of methane an animal will emit and is dependent on characteristics of the individual animal such as size of animal's digestive system and the amount or type of feed they consume. Ruminant animals emit large volumes of methane and this is because of the extent of rumen fermentation. The rumen which is an anaerobic environment allows microbial fermentation to break down the feed ruminants have consumed into specific products that can be absorbed and metabolized. This microbial fermentation in the rumen allows ruminants to digest plant material that non-ruminants cannot and consequently ruminant animals have the highest methane emitted per unit of body mass among all animal types (EPA, 2015).

Cattle begin to eructate methane at about 4 weeks of age and this coincides with the consumption of solids, a developing reticulorumen and establishment of rumen

microbes. Fermentation and methane production rates are rapidly increasing during reticulorumen development (Johnson and Johnson, 1995). Cattle produce 60 to 160 L of methane, per year, though size of animal and DMI will have an effect (Hristov et al., 2013). Lactating dairy cattle specifically will produce 109 to 126 L of methane per year (Johnson and Johnson, 1995). Beauchemin et al. (2008) reported that dairy cattle consuming grain and forage diets produce approximately 500 to 600 L/d of methane. Though methane and carbon dioxide are natural by-products of ruminants, they do require a fair amount of energy from cattle. Generally, 6 to 8%, but up to 12% of the gross energy in feed is converted to methane in the rumen (Beauchemin et al., 2007). Therefore, reducing methane production in the rumen is generally also believed to improve energetic and production efficiency of the cattle.

Microbes have a large effect on daily function of cattle and methane production in the rumen is no different. Methane and carbon dioxide are natural by-products of microbial fermentation of carbohydrates and to a smaller degree amino acids in the rumen plus the hindgut of farm animals (Hristov et al., 2013). Enteric methane is produced by ruminants during the process of microbial digestion of feed (Beauchemin et al., 2007). Ruminant animals and microbes have a unique relationship that allows for conversion of complex plant carbohydrates to energy that is beneficial to both ruminant and microbes. In the reticulo-rumen, carbohydrates are converted to 5- and 6- carbon sugars by microbial enzymes. Some fermentation occurs in the hind gut but the extent of this activity is much lower than the rumen. Carbohydrates in the rumen are then fermented to volatile fatty acids (VFA's) (primarily acetate, propionate and butyrate) by

microbes including bacteria, protozoa and fungi that obtain energy and produce reducing equivalents (e.g metabolic hydrogen, NADH or $FADH_2$) in the process. A small amount of these reducing equivalents will be used in lipid synthesis and fatty acid biohydrogenation Synthesis of amino acids can use or produce reducing equivalents also (Knapp et al., 2014). Or the reducing equivalents can go to methane production, often referred to as methanogenesis (Equation 1):

$$CO_2 + H_2 \rightarrow CH_4 \quad [1]$$

A visual representation of the complete microbial fermentation in the rumen is illustrated in Figure 1.1. Formation of VFAs also influence methane production. Formation of propionate is negatively related to methane production in the rumen and consumes hydrogen while the formation of acetate and butyrate is positively related to methane production in the rumen and generates hydrogen. Propionate is a known major hydrogen sink in rumen, whereas acetate releases hydrogen that is used by methanogens as a substrate for methane production (Hassanat et al., 2014). Increasing the propionate concentration or shifting VFA production towards propionate is generally believed to be the most efficient way to reduce methane emissions. Unfortunately, 55 to 90 % of total VFA produced in the rumen is acetate which increases methane production in the rumen.

The rumen is an anaerobic, methanogenic environment. Microbes thrive in this environment especially methanogens. Methanogens belong to the domain archaea. They use 3 major substrates to produce methane which are carbon dioxide, compounds containing a methyl group or acetate (Morgavi et al. 2010). In the rumen, the most common pathway is using carbon dioxide as the carbon source and hydrogen as the main

electron donor. Formate can also be used an electron donor and may account for up to 18% of methane produced in the rumen. Compounds containing a methyl group is the 2nd substrate. Methylamines and methanol produced in the rumen can also be used by methylotrophic methanogens. There is a small proportion of methanogens that can do this. The last substrate is acetate which methane can be produced via the aceticlastic pathway; however, only a small number of methanogens are able to use this pathway (Morgavi et al. 2010). Ruminants and the microbes inside the rumen have a symbiotic relationship with each other especially methanogens and protozoa related to methane creation. The methanogen and protozoa symbiosis is that the hydrogen produced by protozoa during fermentation of feed in the rumen is used by methanogens for methane production (Benchaar et al., 2013). The hydrogen produced by protozoa is a fermentation by-product that is produced in the hydrogenosome. The hydrogen is then utilized by the methanogens that are found inside or in close association with protozoal cells. Protozoa may also serve as hosts for methanogens (Morgavi et al. 2010). Johnson and Johnson (1995) suggest that ruminal methanogens have been observed to attach to protozoal species suggesting possible interspecies hydrogen transfer. Ten to twenty percent of methanogens are attached to protozoa (Stumm et al., 1982) and methanogens account for 9 to 25% of methanogensis occurring in the rumen (Newbold et al., 1995).

Effect of Fat. Fats or lipids are often fed to cattle because of their ability to increase the energy density of the diet (NRC, 2001). Lipid sources can have other benefits as well such as altering the fatty acid composition of meat and milk, reducing the

dustiness of feed, and increasing the absorption of fat-soluble nutrients (Beauchemin et al., 2007). One major advantage to the addition of lipids in the diet is they can also reduce methane production. Some consider fat supplementation to be one of the most promising dietary methods to reduce enteric methane production (Alstrup, 2015). The are some factors that will affect how much methane is emitted which include fat source, fatty acid profile, inclusion rate and diet composition (Beauchemin et al., 2008; Eugéne et al., 2008; Knapp et al., 2014, Onetti et al., 2001). Beauchemin et al. (2008), states a reduction of methane ≥ 40% is possible with high levels of lipid supplementation, but reductions of 10-25% are more likely.

There are multiple ways that lipid supplementation can decrease or inhibit methane production. Johnson and Johnson (1995) found that the reduction in methane production from fat is due to a decrease in fermentability of substrates rather than directly affecting methanogenesis. According to Beauchemin et al. (2007) lipid supplementation of diets reduces methane emissions by decreasing the amount of organic matter fermented in the rumen, reducing the activity of methanogens, reducing the number of ruminal protozoa and through the use of hydrogen during the biohydrogenation process. Similarly, Johnson and Johnson (1995) suggest that fat additions to ruminant diets impact methane reduction through several mechanisms such as biohydrogenation of unsaturated fatty acids, improved propionic acid production and protozoal inhibition. In a study by van Zijderveld et al. (2011), total number of protozoa was decreased by 63 % with 31 g/kg of DM of supplemental fat. McGinn et al (2009) reported that added fats lowered methane emissions by exerting toxic effects on cellulolytic bacteria. Biohydrogenation of

unsaturated fatty acids uses 1 % of the total metabolic hydrogen while reduction of carbon dioxide to methane uses approximately 48 %, followed by VFA synthesis (33 %) and bacterial cell synthesis (12 %) (Johnson and Johnson, 1995). In general, most of the metabolic hydrogen is used for methane production; therefore, discovering methods to reduce metabolic hydrogen will reduce overall methane production. Reducing microbe numbers and biohydrogenation of fatty acids appear to be the main two mechanisms used by fats.

Among possible lipid sources that can be utilized by producers in practice, there has been a fair amount of variation in methane reduction between sources. Beauchemin et al (2008) found that refined oils high in medium chain fatty acids (MCFA) were effective in reducing methane. Similarly, Eugéne et al (2008) reported that some in vitro experiments showed that medium chain fatty acids (8 to 16 C) caused a greater decrease in methane production compared with short (< 8 C) or long (≥ 18 C) chain fatty acids. The methods used by MCFA to reduce methane is through toxicity on rumen methanogens. However, refined oils containing MFCA are less likely to be used by producers due to the cost. Another possibility is long-chain fatty acids (LCFA), oilseeds and animal fats that are usually less expensive than refined oils. Although pure oils are more effective in reducing methane production, the same amount of lipid supplied via oilseeds is preferred because there are fewer side effects in relation to intake and fiber digestibility (Beauchemin et al., 2008). Also, a reduction of methane was observed when a calcium salt of long-chain fatty acids was added to the diet of lactating cows at 2.95 % DM basis (Andrews et al., 1991). With a broad range of conditions over 17 studies,

Beauchemin et al (2008) found methane was reduced by 5.6 % with each 1 % addition of supplemental fat. In regards to lactating dairy cows, daily methane production was decreased by approximately 9 % with lipid supplemented diets containing an average of 6.4 % crude fat compared with control diets with an average dietary crude fat of 2.5 % (Eugéne et al., 2008). Different sources and amounts of lipid supplementation can have different effects on methane production. Thus, producers must consider both cost, amount and type of fat that can be supplemented to livestock without reducing productivity.

Effect of Cellulose and Hemicellulose. The amount of methane that will be produced largely depends on the substrate being utilized. Forage based diets which are high in fiber components (i.e cellulose, hemicellulose, lignin) favor the production of acetate and butyrate whereas starch based diets favor production of propionate (Knapp et al. 2014). Therefore, forage based diets are expected to produce more methane than starch based diets. Of the major structural carbohydrates, it is generally believed that cellulose will produce more methane then hemicellulose. Moe and Tyrrell (1979) created an equation to predict methane production using calorimetry data and observed that methane produced per gram of digested cellulose is 3 times greater than the methane produced per gram of digested hemicellulose. They also observed that methane produced per gram of digested cellulose is 5 times greater than methane produced per gram of digested non-fiber carbohydrates (i.e. starch) (Moe and Tyrrel1, 1979). Observations of Holter and Young (1992) support these estimates and further suggest the cellulose could

be the fiber fraction that contributes most to methane production in lactating dairy cows consuming mixed forage-concentrate diets.

In-Vitro Procedures to Study Rumen Fermentation and Rumen Digestion

Gas Production. From the early 1940's to the modern era, the in-vitro gas production technique (IVGPT) has dramatically changed. The early technique involved incubating samples of feedstuffs in gas-tight flasks and measuring gas production using a manometer. El-Shazly and Hungate (1965) employed a technique where gas production was measured by using a syringe attach to a flask in which substrate was fermented. The Hohenheim group was the first to use large glass syringes to conduct the fermentation process in as illustrated in Figure 1.2. With this technique, the total volume produced was recorded after 24 hours (Williams, 2000).

Automated systems have also been developed. Pell and Schofield (1993), from Cornell University were the first to describe the use of computerized pressure sensors to monitor gas production. Equipment used for this automated gas production system is illustrated in Figure 1.3. This technique provided real time measurements of gas accumulation which allowed for a better understanding of fermentation kinetics (Yáñez-Ruiz et al., 2016). An example of a more advanced automated system is called the IGER automated pressure evaluation system as shown in Figure 1.4. This system was developed at Institute of Grassland and Environmental Research (IGER) in Aberystwyth, Wales and was first reported by Davies et al. (1995). The system uses gas-tight bottles each fitted with a pressure sensor and solenoid valve linked to a computer. During fermentation of

the substrate when the pressure sensor reaches a pre-set gas pressure the solenoid valves open to release accumulated gas (Williams, 2000; Yáñez-Ruiz et al., 2016). The number of vents and time between each vent are used to plot a cumulative gas profile (Williams, 2000). With these automated systems, gas composition analysis still requires manual injection of gas sample into a gas analyzer such as a gas chromatography (GC) (Yáñez-Ruiz et al., 2016). Today wireless in-vitro gas production techniques commonly are used. This technique is manufactured and marketed by Ankom (Ankom Technology, Macedon, NY) and combines traits of previously described techniques. The system uses individual bottles with individual gas production modules that continuously measure pressure from fermentation occurring in the bottle and release gas when it reaches a fixed pressure. Bottles are incubated in a water bath. Data is then wirelessly transferred from the modules to a computer that plots cumulative pressure over time as illustrated in Figure 1.5 (Storm et al., 2012). Even with this system the composition of gas produced must be determined by manual collection and then injection of gas into a gas analyzer. More recently an automated system has been developed that uses the vented and released gases directly to measure gas composition via a computer controlled GC instead of releasing the gas into the air once the pressure threshold has been hit (Yáñez-Ruiz et al., 2016).

The IVGPT has been used to study ruminal fermentation of feedstuffs using natural rumen microbes (Storm et al., 2012). It has also has been used to evaluate nutritive value of ruminant feeds (Yáñez-Ruiz et al., 2016). Feedstuffs are incubated at 39°C in a mix of rumen fluid and a buffer for fixed period of time. The volume of total gas produced is measured and composition analyzed (Storm et al., 2012). The gases

composed in the rumen are mainly carbon dioxide (65 %) and methane (26 %) but also include nitrogen (7 %) and small amounts of hydrogen (0.2 %) and oxygen (0.5 %) (Sniffen and Herdt, 1991). These values are just general averages but may be changed depending upon the feed consumed. This IVGPT is a robust approach to characterize feedstuffs and observations may be at least in part transposable to live animals (Storm et al., 2012).

One advantage of IVGPT is that results may be generated more rapidly and cheaper than in-vivo experiments (Storm et al., 2012; Williams, 2000). A typical in-vitro experiment may take 1-4 weeks to conduct and contains no animal variation that is inherent of an in-vivo experiment (Storm et al., 2012). Another advantage is the sample size required is generally smaller (Williams, 2000; Yáñez-Ruiz et al., 2016). A third advantage is that although a live animal is needed to donate inoculum fewer animals are needed. A final and important advantage is the system allows one to measure kinetics of fermentation. The largest disadvantage of the system is it only stimulates gas production in the rumen rather than generating estimates of total tract digestibility of the whole animal (Storm et al., 2012; Williams, 2000). Another disadvantage is IVGPT is this system is a batch system which feed is added just at the beginning instead of continuous flow of feed systems that add more feed over time like it would be in the rumen. This makes it simpler to conduct but IVGPT will be less accurate because of the buildup of the acid from VFA since VFAs can't be absorbed. Fermentation will eventually fall off and dissipate. A finally disadvantage is the lack of uniformity in methodology used by different people which makes it difficult to compare results with others (Williams, 2000).

Cattani et al (2014) further suggests that it is difficult to compare observations between laboratories because of the influence of several confounding sources of variation including different operative conditions, type of buffer used, ratios among feed samples size, headspace volume, and type of gas production equipment used.

Methane Production. More recently the IVGPT has been utilized to measure methane production and to assess the potential of the feed or diet sample to reduce methane emissions (Storm et al., 2012; Yáñez-Ruiz et al., 2016). This is also becoming more popular because for some research groups, in-vitro methods may be the only option available to screen for compounds that may mitigate methane production (Yáñez-Ruiz et al., 2016). In this technique, total gas produced is measured and a sample of that is used to test the composition of gas. It is possible also to look at degradation of feedstuffs with this method. Outputs are generally reported as methane per gram of dry matter intake, methane per gram of degraded DM or methane per gram degraded NDF (Storm et al., 2012).

Fiber Digestion. Using a computerized monitoring system Pell and Schofield (1993) developed a technique to measure forage digestion in vitro by measuring gas production. This system replaces fiber (NDF) disappearance as a measure of carbon metabolism. Traditional in vitro methods follow disappearance of one component of the substrate while gas measurements focus on appearance of fermentation products. With intact forages, gas produced during in vitro digestion comes from both soluble and fiber

fractions (Pell and Schofield, 1993). Unfortunately, gas data is more difficult to interpret than NDF disappearance because gas in produced from many different substrates, including the soluble and fiber components. Fiber digestion is difficult to describe mathematically because fibers are generally a mix of components. For example, NDF includes hemicellulose, cellulose and lignin with each possessing very different rates of digestion. Mathematical models, although complex, may still be too simplistic to describe the complex nature of microbial digestion of natural feeds (Schofield et al., 1994).

Energy Utilization

Energy Balance. The gross energy content of feed, milk, feces and urine are determined using the bomb calorimeter. Gross energy intake (GEI) is the amount of energy an animal consumes (Equation 2). This is the first energy branch on the energy partitioning diagram as illustrated in Figure 1.6. Most of the gross energy will be digested and absorbed, yet some energy can be indigestible and will be lost without being utilized (Foth, 2014). Intake of digestible energy (DEI) accounts for fecal loss, therefore energy lost from fecal output will be subtracted from GEI as illustrated in Figure 1.6 (Equation 3). Digestible energy is considered to be less accurate and less precise than measuring GE since it requires measurement of fecal output (Weiss, 2007). Metabolizable energy (ME) accounts for energy loss from feces, urine excretion and eructation of methane, therefore DE, urine and methane will be subtracted out as illustrated in Figure 1.6 (Equation 4). Metabolizable energy is less accurate and less precise than DE because of the inherent error from DE calculation and measurement of urine and methane energy can be difficult

(Weiss, 2007). Finally, net energy, specifically net energy of lactation, will be calculated. Net energy (NE) is the energy required for maintenance plus production (lactation (NE_L), gestation or growth (NEg) in the animal as illustrated in Figure 1.6. Maintenance is when an animal is neither gaining nor losing body tissue (Flatt and Moe, 1969). Body heat is produced as a byproduct of digestion and NE accounts for heat loss plus all other energy losses mentioned above as illustrated in Figure 1.6 (Equation 5). Heat production (HP) is determined through calorimetry and nitrogen excretion in urine is also accounted for. Heat increment (HI) is defined as the increase in heat production following the consumption of food when an animal is in thermoneutral environment and is determined by estimating an assumed certain amount of heat is equal to maintenance or through regression of ME intake on HP (Flatt and Moe, 1969; Weiss, 2007). Both HP and HI are needed for measurement of NE. Net energy systems are based on the first law of thermodynamics which states energy cannot be created or destroyed. If NE of the diet is accurately estimated and we know the NE requirement, energy balance therefore is also known. Measurement of NE includes all the inherent error of GE, DE, and ME plus errors associated with measuring heat; therefore, it is the least accurate and precise measurement of diet energy. However, it does have a theoretical advantage over the other expressions of energy because it allows different efficiency values to be used at different physiological states (Weiss, 2007).

$$\text{GEI} = \text{intake of feed} \times \text{GE of feed} \quad [2]$$

$$\text{DE} = \text{GEI} - \text{fecal energy} \quad [3]$$

$$\text{ME} = \text{DE} - \text{urinary energy} - \text{gaseous energy} \quad [4]$$

$$NE_L = ME - HP \quad [5]$$

Milk synthesis is observed to be a more energetically efficient process than the deposition of body fat or growth. This is most likely because amino acids are incorporated into the proteins of milk and consequently little energy is expended in the synthesis of urea. Further reason for the greater efficiency is that the fatty acids of milk are of a shorter length than those of deposited fat. The cost of increasing the length of fatty acid chains is energetically expensive. A final reason is the synthesis of lactose from glucose is not energetically expensive (Blaxter, 1967).

Energy Input. The main pathway for energy input into cattle is from feed intake. Energy from feed intake is usually estimated from the intake of total digestible nutrients (TDN) which is calculated from individual feed composition (NRC, 2001). Experimentally, energy from feed intake can be calculated from direct measure and calculation. First the gross energy content of the feed ingredients or total mixed ration along with the refusals is determined using the bomb calorimeter. Next the gross energy intake is calculated by taking the amount of DM offered to the cow × energy of the diet the cows given – orts energy to get GEI. Energetic losses from feces, urine, gasses and heat must be accounted for to determine digestible, metabolizable and net energy intake.

Another pathway for energy input is through mobilizing body tissue. Lactating dairy cows will often mobilize body tissue during early lactation to support the energy requirements for milk production. This is especially true during early and mid lactation and these reserves will generally be replenished in late lactation (NRC, 2001). Because of

this increased demand for energy in early lactation, cows often will go into negative energy balance (NEB) the first few weeks of lactation. During this time body tissue reserves (mainly fat) are mobilized to compensate for the NEB. If cows are unable to recover from NEB, issues such as metabolic disorders will result. Mobilization and replenishing the tissue is a normal physiological process that occurs in all lactating mammals, therefore it is expected for the cow to recover from NEB and lactation energy needs (NRC, 2001).

Energy Loses and Utilization.

Heat. Heat produced is measured using a calorimeter by either direct measure or indirect measure through gaseous exchange. Heat is considered the second most variable energetic loss with fecal losses being the first (Weiss, 2007). This is supported by the observations of Tine et al. (2001) who reported that in early lactation dairy cattle fed control, isogenic diets the heat produced was 34.6 Mcal/d and expressed as percent of GE was 34.1 % whereas with dry cows it was 17.2 Mcal/d and 58.2 %. Birkelo et al. (2004) observed in early to mid lactation dairy cows fed a control diet the amount of heat produced was 27.3 Mcal/d. Similar results were observed by Foth et al. (2015) where in mid to late lactation dairy cows fed a control diet the heat produced was 30.0 Mcal/d. Based on the previous numbers the approximate average range for heat production for lactating dairy cows is 25 to 35 Mcal/d.

Gaseous. In nonruminants, gaseous energy losses are generally small and therefore ignored; however, this is not the case in ruminants. To measure gaseous energy, the ruminant animal is placed in a chamber or a breathing mask can also be used to collect expelled air that can be sampled and measured for methane. Measuring methane with these systems can also be difficult to conduct making fine estimates prone to error. One factor known to be a major source of variation in gaseous energy losses is the diet fed to the animal. Diets with higher concentrations of fiber can alter the rumen microbial population so more methane will be produced. Diets with high starch often reduce methane production (Weiss, 2007). In a study by Tine et al. (2001) in early lactation dairy cattle fed control, isogenic diets the methane energy was estimated to be 5.8 Mcal/d or 5.7 % of the GE. In comparison, in dry cows methane energy losses were estimated to be 2.5 Mcal/d or 8.6 % of the GE. Birkelo et al. (2004) observed in early to mid lactation dairy cows fed a control diet the methane energy was 4.3 Mcal/d and expressed as percent of GE was 4.43 %. Similar results were observed by Foth et al. (2015) where in mid to late lactation dairy cows fed a control diet the methane energy was 4.77 Mcal/d and expressed as percent of GE was 5.72 %. In a study by Moraes et al. (2015), which included a larger dataset, for the entire lactation period of dairy cows methane energy accounted for similar energy losses as the previous studies and is listed in Table 1.1. Based on the previous numbers the approximate average range for methane energy for lactating dairy cows is 4 to 6 Mcal/d and expressed as percent of GE is also 4 to 6 %.

Milk. To measure milk energy, daily milk output is measured and then, sampled, dried and combusted in a bomb calorimeter. In a study by Tine et al. (2001) they found that in early lactation dairy cattle fed control, isogenic diets the milk energy was 22.7 Mcal/d. Birkelo et al. (2004) observed in early to mid lactation dairy cows fed a control diet milk energy was 21.3 Mcal/d. Similar results were observed by Foth et al. (2015) where in mid to late lactation dairy cows fed a control diet had a milk energy of 22.1 Mcal/d. In a study by Moraes et al. (2015), which included a larger dataset, for the entire lactation period of dairy cows milk energy accounted for lower energy losses than the previous studies and is listed in Table 1.1. Based on the previous numbers the approximate average range for milk energy for lactating dairy cows is 16 to 23 Mcal/d.

Feces. To measure fecal energy, daily fecal output is measured, sampled, dried and then combusted in a bomb calorimeter. Fecal loss generally is the largest and most variable loss (Weiss, 2007). This is partially due to the fact that the digestibility of the carbohydrate fraction of diets is extremely variable. For example carbohydrates in fiber are generally less digestible than non-fiber carbohydrates (Weiss, 2007). In a study by Tine et al. (2001) they found that in early lactation dairy cattle fed control, isogenic diets the fecal energy was 31.1 Mcal/d and expressed as percent of GE was 30.6 % whereas with dry cows it was 8.5 Mcal/d and 28.7 %. Birkelo et al. (2004) observed in early to mid lactation dairy cows fed a control diet the fecal energy was 32.3 Mcal/d and expressed as percent of GE was 33.4 %. Similar results were observed by Foth et al. (2015) where in mid to late lactation dairy cows fed a control diet the fecal energy was

27.8 Mcal/d and expressed as percent of GE was 33.1 %. In a study by Moraes et al. (2015), which included a larger dataset, for the entire lactation period of dairy cows fecal energy accounted for lower energy losses than the previous studies and is listed in Table 1.1. Based on the previous numbers the approximate average range for fecal energy for lactating dairy cows is 26 to 33 Mcal/d and expressed as percent of GE is 31 to 33 %.

Urine. To measure urine energy, daily urine output is measure, sampled, dried and then combusted in a bomb calorimeter. Collection of urine and analysis of urine through the bomb calorimeter is laborious and is also prone to some error. Diets high in protein result in increased synthesis of urea which is excreted by the urine therefore increasing loss of urinary energy (Weiss, 2007). In a study by Tine et al. (2001) they found that in early lactation dairy cattle fed control, isogenic diets the urine energy was 4.0 Mcal/d and expressed as percent of GE was 3.9 % whereas with dry cows it was 1.3 Mcal/d and 4.2 %. Birkelo et al. (2004) observed in early to mid lactation dairy cows fed a control diet the urine energy was 3.5 Mcal/d and expressed as percent of GE was 3.69 %. Similar results were observed by Foth et al. (2015) where in mid to late lactation dairy cows fed a control diet the urine energy was 3.05 Mcal/d and expressed as percent of GE was 3.62 %. In a study by Moraes et al. (2015), which included a larger dataset, for the entire lactation period of dairy cows urine energy accounted for similar energy losses as the previous studies and is listed in Table 1.1. Based on the previous numbers the approximate average range for urine energy for lactating dairy cows are approximately 3 to 4 Mcal/d and expressed as percent of GE is also 3 to 4 %.

Calorimetry Methods

For many years, calorimetry has been used as a method for determination of nutritional energetics for animals and humans. Calorimetry is a measurement of heat and specifically animal calorimetry is the science of measurement of heat transfer between an animal and its environment (Nienaber at al., 2009). Dating back to 1700's, the utilization of dietary energy has been researched starting with researchers Antoine-Laurent Lavoisier and Joseph Priestly. Later in the early 1900's, Kellner and Köhler created the starch equivalent system that is a net energy based system where energy values of feeds were determined relative to how much starch was needed to meet the animal's energy needs for growth. Since then, new systems and other methods have been created to determine energy values of feed ingredients. Throughout the years, the field of nutritional energetics has sought 3 general objectives (Johnson et al., 2003). Firstly, to describe the relationships between gas exchange and HP. Secondly, to assess feed ingredients and define energy requirements and partitioning of energy for the animal. Thirdly, to determine the nature of energy partitioning specifically how much and where energy is used in the body. Calorimetry can be used to study each of these objectives. Early calorimeters used to measure animal heat production were based on the same principle as the bomb calorimeter (Blaxter, 1967). Calorimetry is used to determine the amount of energy an animal needs for metabolism of nutrients through HP or heat loss. The most common ways to measure HP is through either direct or indirect calorimetry (Nienaber et al., 2009). Either option can be used and are generally accepted as valid and accurate

methods to study nutritional energetics for animals and humans. However they are not directly comparable because of the use of different principles to measure HP. For all methods of calorimetry, the major challenge is ensuring that the measured animals are not stressed and that the systems are accurately representing emissions from animals in their "normal" states. To do this, proper cattle adaption to the system is vital (Place et al., 2011).

Direct Calorimetry. Direct calorimetry is a direct measurement of heat produced by an animal via sensible and evaporative heat losses of the animal (Johnson et al., 2003; Nienaber et al., 2009). The first direct calorimeter was designed by Lavoisier and Laplace in which they placed a guinea pig in a chamber containing a given weight of ice and then estimated heat production. Carbon dioxide exhaled was also measured and they observed that melting a given amount of ice corresponds to exhalation of a definite amount of carbon dioxide. Lavoisier and Laplace also measured heat production of a rabbit by temperature rise in a given volume of water surrounding an animal chamber (Brody, 1945). The two methods considered as direct calorimeters are respiratory calorimeters, also called heat sink calorimeters, and gradient layer calorimeters (Blaxter, 1989; Nienaber et al., 2009). Respiratory calorimetry can be referred to as adiabatic calorimetry because no heat is lost to or from the calorimeter like in an abiabatic bomb calorimeter. They are also called heat sink calorimeters because sensible heat is collected in a type of heat sink, such as water, for measuring the heat that has been generated (Neinaber et al., 2009). Often respiratory calorimetry is carried out using whole animal chambers. The

chamber is heavily insulated to prevent heat gained or lost from the outside environment to measure sensible heat loss (Blaxter, 1989; Nienaber et al., 2009). A common design to facilitate this is having the air space between the chamber and the outside environment maintained at the same temperature as inside the chamber. This prevents sensible heat from transferring though the walls of the calorimeter (Nienaber et al., 2009). The temperature is constantly monitored and the amount of heat produced in the chamber is considered sensible heat loss from the animal (Foth, 2014).

Gradient layer calorimetry is yet another form of direct calorimeter. The general principle behind this method is that the heat produced by the animal is allowed to flow through the containing walls of the chamber and measures the total sensible heat lost through the walls (Blaxter, 1967; Nienaber at al., 2009). Sensible heat loss can be partitioned into radiation and convection by using heat flow meters placed on the walls with this method. This method, can also be referred to as partitional calorimeter. The biggest advantage of this method is its quickness to respond to changes in sensible heat loss as the animal moves around or change positions (Nienaber et al., 2009).

Indirect Calorimetry. Unlike direct calorimetry that measures heat loss, indirect calorimetry measures heat production using gas exchange measurements. Heat production is calculated using the Brouwer equation that accounts for gas composition which include oxygen, carbon dioxide and methane and urinary N loss (Brouwer, 1965) (Johnson et al., 2003, Equation 6).

$$HP = 3.866 \times O_2 + 1.200 \times CO_2 - 0.518 \times CH_4 - 1.431 \times N \quad [6]$$

This equation has been widely used to determine energy balances in ruminants. This equation was developed with fasting animals. However in many energy studies animals are not being fasted. The assumption behind the equation is that heat from oxidation of carbohydrates, fat and protein are equal to total heat given off by the animal (Foth, 2014). Therefore this equation and determination of energy balances from it may not be completely accurate because this assumption is most likely not being meet.

Another definition of indirect calorimetry is the measurement of energy exchange occurring within the animal's living tissues which includes metabolism of food or catabolism of body tissue (Nienaber et al., 2009). Lavoisier and Laplace designed the first indirect calorimeter using the direct calorimetry guinea pig experiment that demonstrated exhalation by a guinea pig of a given quantity of CO_2 corresponds to melting of a given weight of ice surrounding the animal. Therefore, indirect calorimetry is based on the fact that gas exchange is closely correlated with heat production. This can be illustrated using the oxidation of carbohydrate equation (Brody, 1945, Equation 7).

$$C_6H_{12}O_6 + 6O_2 = 6CO_2 + 6H_2O + \text{heat} \qquad [7]$$

There are two different types of systems: closed-circuit and open-circuit (Blaxter, 1989). Closed-circuit systems are often utilized with humans and smaller animals as illustrated in Figure 1.7 and some for larger animals, but has never been as widely used as the open-circuit system (Johnson et al., 2003). With the open-circuit system or Pettenkofer principle, outside air of known composition is passed through the chamber (Blaxter, 1989). With the closed-circuit system or Regnault & Reiset principle, CO_2 produced by the animal and water vapor are removed with or absorbed by absorbents

while oxygen consumed by the animal is measured and then replaced in the returning air (Blaxter, 1967; Blaxter, 1989). Finally, the major advantage of indirect calorimetry compared to direct calorimetry is various environment conditions and changes can be investigated (Nienaber et al., 2009).

Indirect Calorimetry Methods

Indirect calorimetry is versatile in that there are many different techniques that can be used making it more flexible than direct calorimetry (Nienaber et al., 2009). A common method to measure methane emissions is using whole animal respiration chambers. The main idea behind these are to collect all exhaled air from the animals and measure the gas composition (Storm et al., 2012). The most common method for lactating dairy cattle would be the open-circuit whole animal chambers as illustrated in Figure 1.8. Within the United States, chambers have been constructed at United States Department of Agriculture (USDA) research centers in Beltsville, MD and Clay Center, NE (Johnson et al., 2003). The major advantage of these systems are they are an accurate method to measure emissions from cattle including methane from ruminal and hindgut fermentation. A major disadvantage to this method is that it is costly and laborious (Johnson and Johnson, 1995).

Another method to measure methane emissions is using sulfur hexafluoride (SF_6) tracer method. This method is commonly used for grazing animals because chambers can't be utilized in this environment. In general, methane emissions can be measured as long as the volume of a tracer gas from the rumen is known. This is done by placing the SF_6 tracer gas into the rumen through a permeation tube. Sampling of gas is then

regulated through tubing that is placed at the nose and gas is collected in a canister that generally is placed around the animal's neck. The emission of methane can then be calculated (Storm et al., 2012). The sampling apparatus is as illustrated in Figure 1.9. The major advantage of this system is that it doesn't require the animal to be enclosed or restrained while a major limitation is that is doesn't measure hindgut methane emissions (Johnson and Johnson, 1995).

Headboxes. Other systems that are less expensive but labor intensive have also been constructed and used to determine gas exchange measurements such as indirect calorimeter headboxes (Johnson and Johnson, 1995). As compared to whole animal chambers, headboxes serve a similar function while only surrounding the animals head with a canvas neck cape as illustrated in Figure 1.10. The box is large enough for the animal to move their head unrestrictedly while allowing access to continuous feed and water (Johnson and Johnson, 1995). Because only the head is enclosed, the animals are given the opportunity to move freely to stand or lay down. Plexiglass encloses the box which allows the animals to see their environment around them. On one side of the plexiglass sides, is a door which allows for access and services needed for the animals. The boxes are placed on wheels therefore allowing them to be moved to cows in stanchions or stalls (Nienaber at al., 2009). Additionally, the headboxes allow lactating animals to be milked without disrupting the collection of gases in the system. Compared to the other systems, they are a lower cost alternative and viable option for energetics research (Foth, 2014). The major disadvantage is they do not account for hindgut

fermentation loss of gases which accounts for 2 to 3 % (Johnson and Johnson, 1995; Hristov et al., 2013). However, this system allows you to measure the gases coming directly from the rumen (Place et al., 2011).

SUMMARY

Rumen fermentation in dairy cattle is a result of the activity of the microbes that inhabit it. Healthy rumen fermentation is vital for good healthy dairy cattle. If the rumen is not fermenting as well as it should more than likely issues for the cow will arise. The product of rumen fermentation is VFAs which are an important energy source for ruminants. The proportion of specific VFAs produced depends on what substrate is being utilized by the cow. Rumen digestion is also important in the dairy cow. Rumen digestion of fiber and microbes have an important interaction. Cattle don't possess the enzymes required to break down fiber but, the microbes do possess the enzymes. Therefore cattle rely heavily on microbes for fiber digestion. There are some animal factors that will affect ruminal fiber digestion such as intake and gut fill. There are also some plant factors that will affect fiber digestion such as lignin concentration, cellulose crystallinity and NDF concentration. Dietary factors can have an effect on rumen fermentation and fiber digestion also. Dietary fat often will have negative effects on ruminal fermentation and fiber digestion. The two theories for this to occur is the coating theory and the direct antimicrobial effects theory.

Methane is a natural product of digestion in ruminants. A dairy cow will produce approximately 500 to 600 L/d of methane. Methane production is largely driven by

microbial fermentation and formation of VFAs, which will either increase or decrease methane production in the rumen. More methane is produced and lost from the rumen via eructation than from hindgut fermentation. Methane contributes to global warming therefore there is an increasing interest to look at methane emissions from cattle. Importantly methane represents a large amount of energy loss in cattle therefore reducing methane in the rumen will improve energetic and production efficiency of cattle. Methane production can be affected by dietary factors. The addition of fat often will reduce methane production in cattle. The mechanisms responsible for this include biohydrogenation of unsaturated fatty acids, reducing the activity of methanogens and reducing the number of ruminal protozoa. Fibrous components will have an effect on the amount of methane being produced. Cellulose will produce more methane than hemicellulose.

In-vitro procedures can also be used to study both rumen fermentation and digestion. The in-vitro gas production technique has dramatically changed over the years with automated systems commonly being used today although manual injection of gas sample into a gas analyzer is still used to determine gas composition. In-vitro gas production stimulates ruminal fermentation of feedstuffs and is a good approach of initial testing of feedstuffs before in-vivo testing. The caveat is that in-vitro and in-vivo results won't always be the same. Because of the increasing interest in methane production, the in-vitro gas production technique has been modified to measure methane production.

Energy utilization is a complex biological function in all animals. There are many different sources of variation that can be included. Energy will be partitioned to gross

energy, digestible energy, metabolizable energy and net energy where energy will be both digested and metabolizable plus undigested which is lost as feces, urine, gas or heat. Milk production has been found to be a more energetically efficient process than growth which includes the deposition of body fat. Finally, maintenance requirements of lactating dairy cattle have increased due to higher milk production in cows and therefore increased nutrient and energy needs for daily function and milk production.

Over the years many methods have been used to determine nutritional energetics. Calorimetry is the most common way to do this and there are two different ways. Direct calorimetry is a measure of heat produced by an animal. Indirect calorimetry is a measure of heat production using gas exchange from the animal. The most common way to measure energetics in lactating dairy cattle is using an open-circuit indirect calorimetry. Whole animal chambers could be considered the standard for lactating dairy cattle because it accounts for respiration gases from the cow plus eructated gas from the rumen and gas from hind gut fermentation. However, there are other systems that could be utilized which includes the SF_6 tracer method and headboxes. These systems don't account for gases produced from hindgut fermentation however the amount of gas loss from flatulence is much less than eructated gas. Therefore, the results from these systems have been shown to be accurate.

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TABLES AND FIGURES

Table 1.1. Summary of heat losses and utilization in lactating dairy cattle[1]

Item[2]	Mean	Minimum	Maximum	SD
GEI (Mcal/d)	76.1	27.1	139.7	84.1
MEI (Mcal/d)	43.5	15.8	91.8	48.1
Methane energy (Mcal/d)	4.02	0.91	7.33	5.0
Milk energy (Mcal/d)	16.4	0.07	37.4	29.3
Feces energy (Mcal/d)	26.1	5.59	55.6	34.1
Urine energy (Mcal/d)	2.53	0.69	6.12	3.3
DIM	160.2	11.0	488.0	81.5

[1]Adapted from Moraes et al., 2015.

[2]GEI = gross energy intake, MEI = metabolizable energy intake.

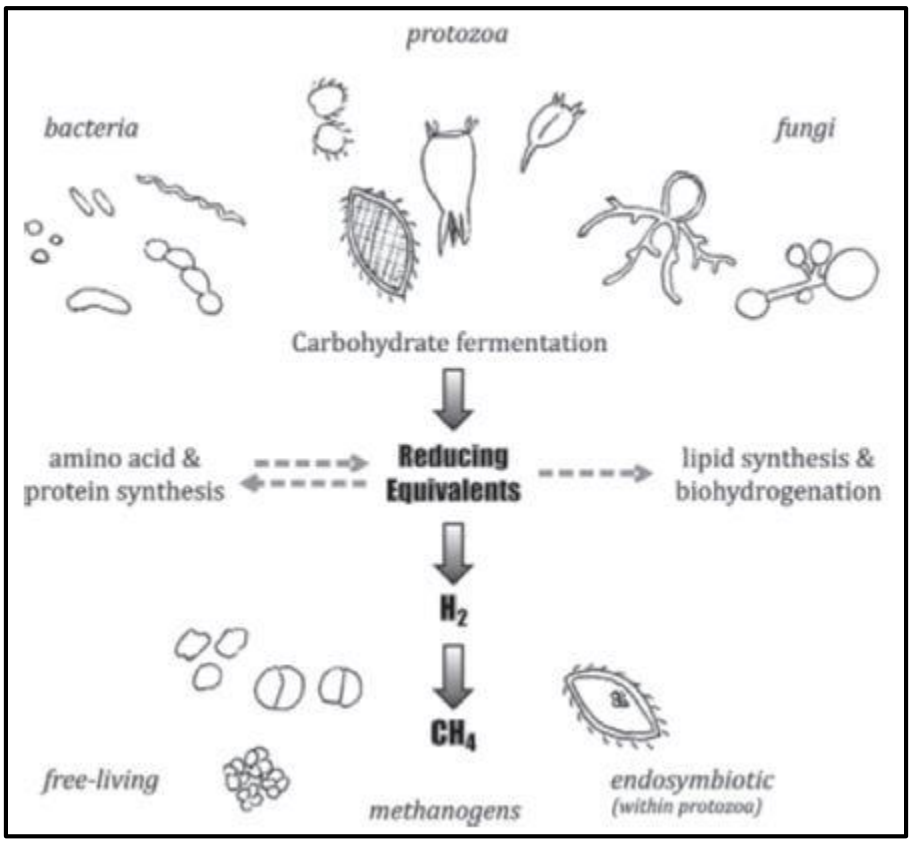

Figure 1.1. Visual representation of microbial fermentation in the rumen (Knapp et al., 2014).

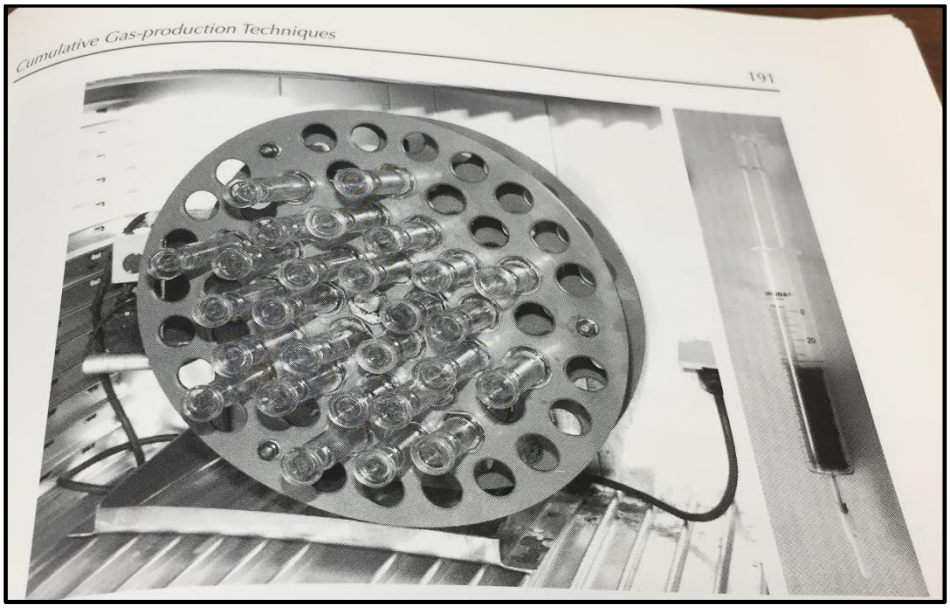

Figure 1.2. Equipment for Hohenheim gas test (Williams, 2000).

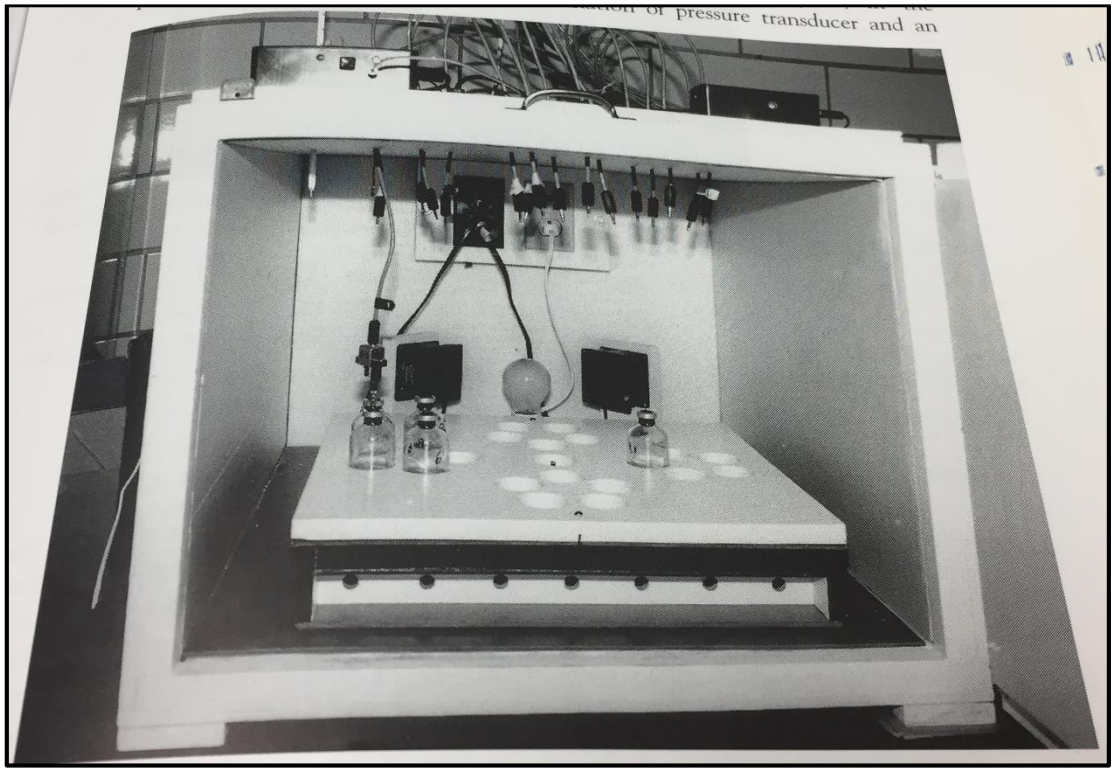

Figure 1.3. Equipment used for automated gas system from Cornell University (Pell and Schofield, 1993).

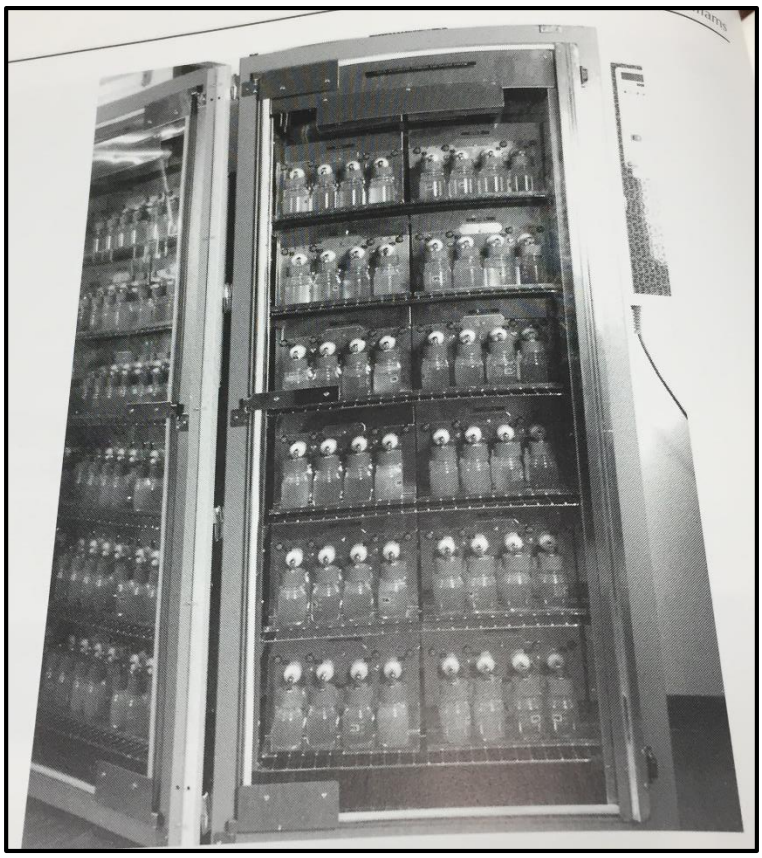

Figure 1.4. Institute of Grassland and Environmental Research automated pressure evaluation system (Williams, 2000).

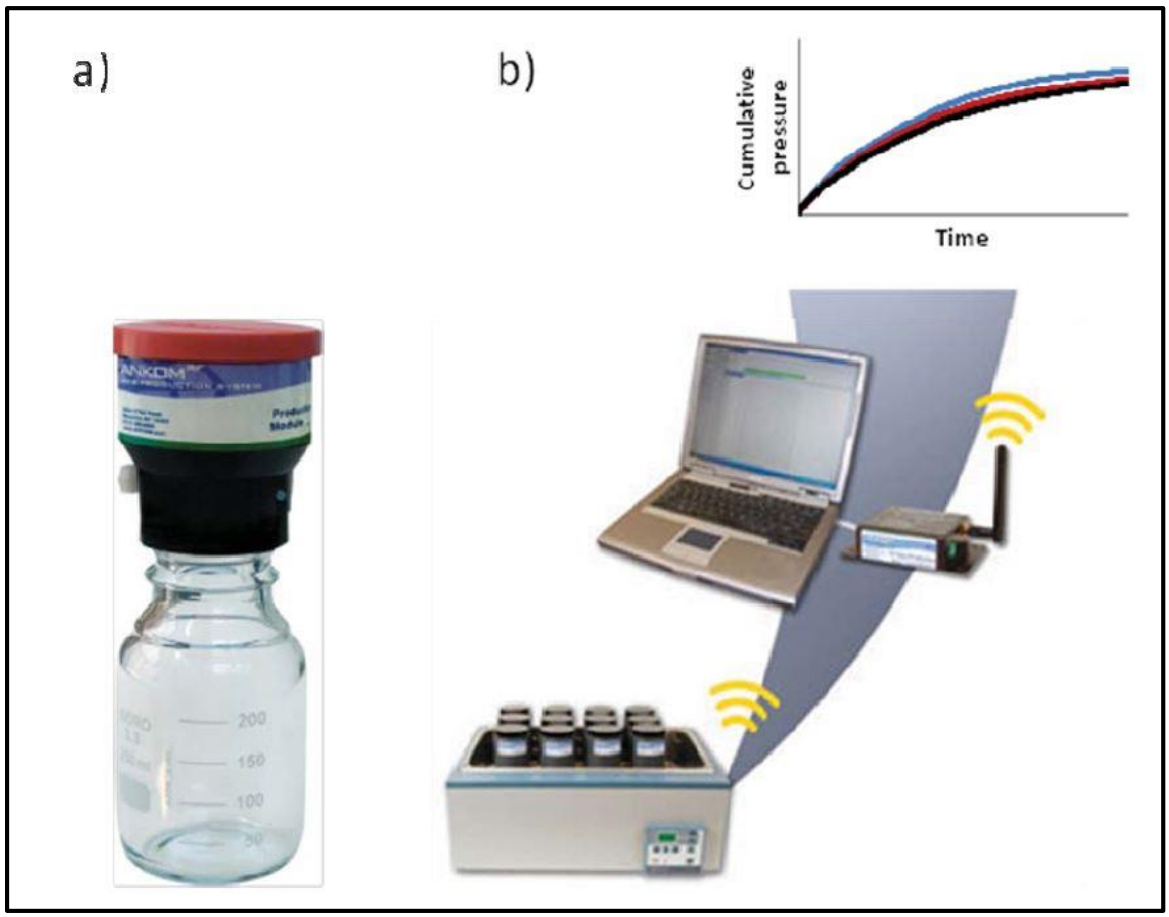

Figure 1.5. Wireless in-vitro gas production technique marketed by Ankom (Ankom Technology, Macedon, NY). Data is wirelessly transferred to a computer which will plot cumulative pressure (Storm et al., 2012).

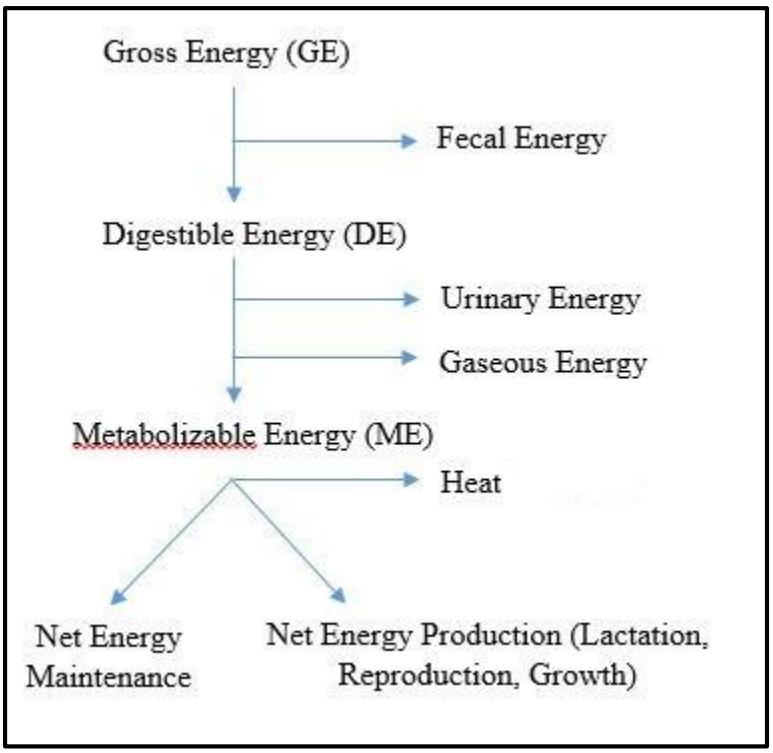

Figure 1.6. Energy partitioning diagram in animals.

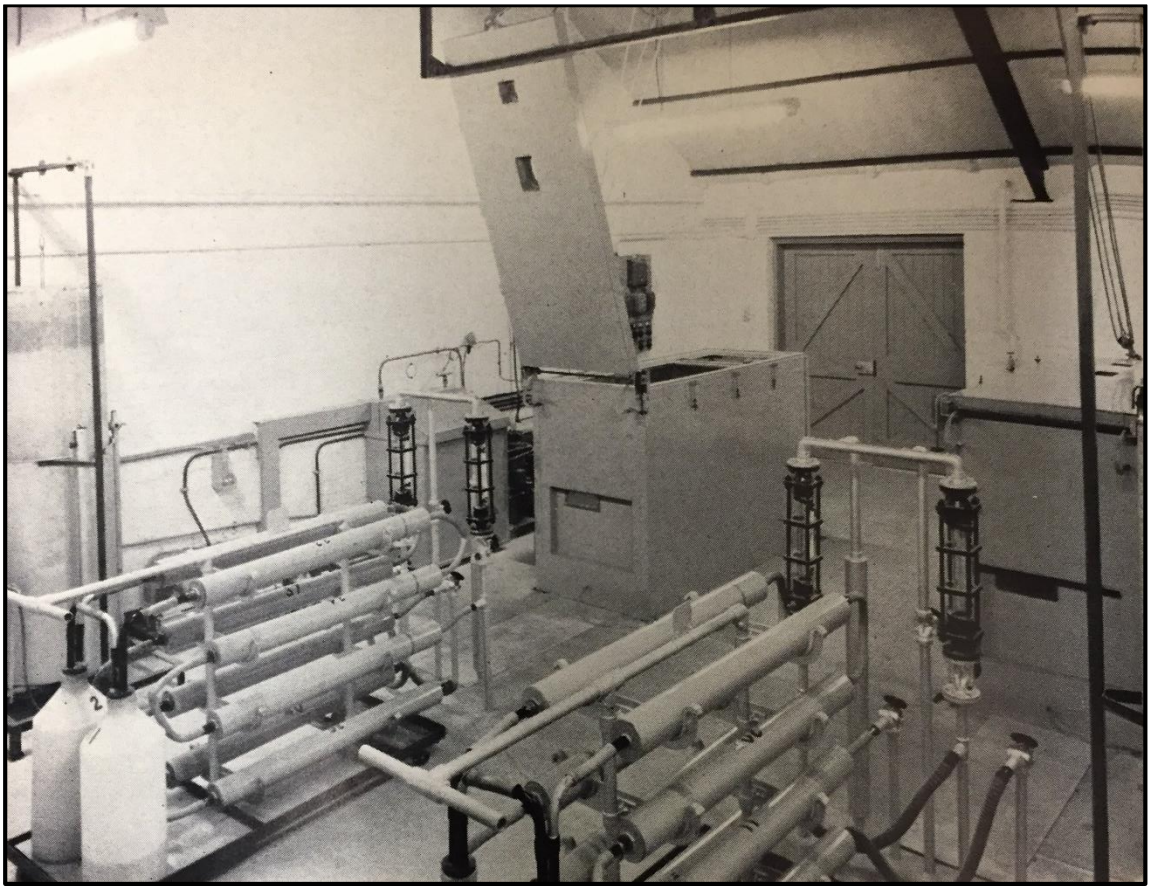

Figure 1.7. Closed circuit indirect calorimetry respiration system used for small ruminants (Blaxter, 1967).

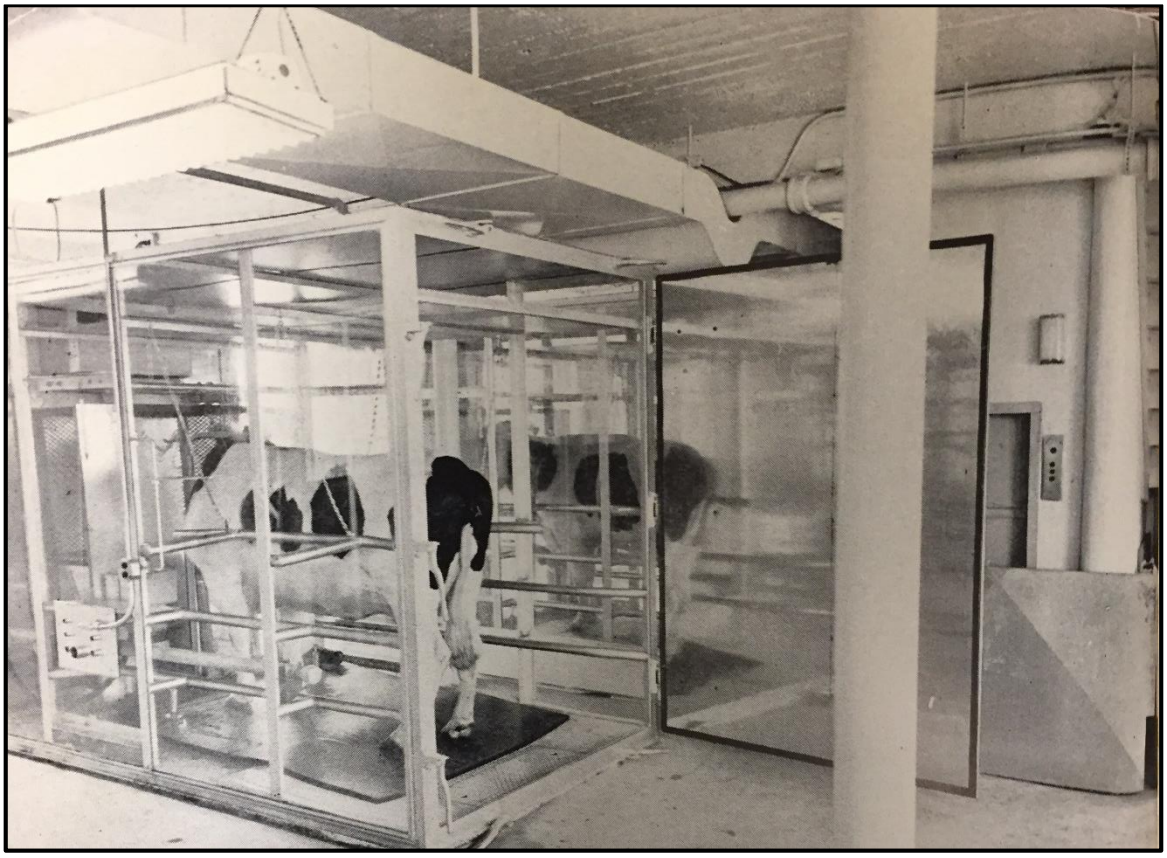

Figure 1.8. Open-circuit whole animal chambers which is a method of indirect calorimetry (Blaxter, 1967).

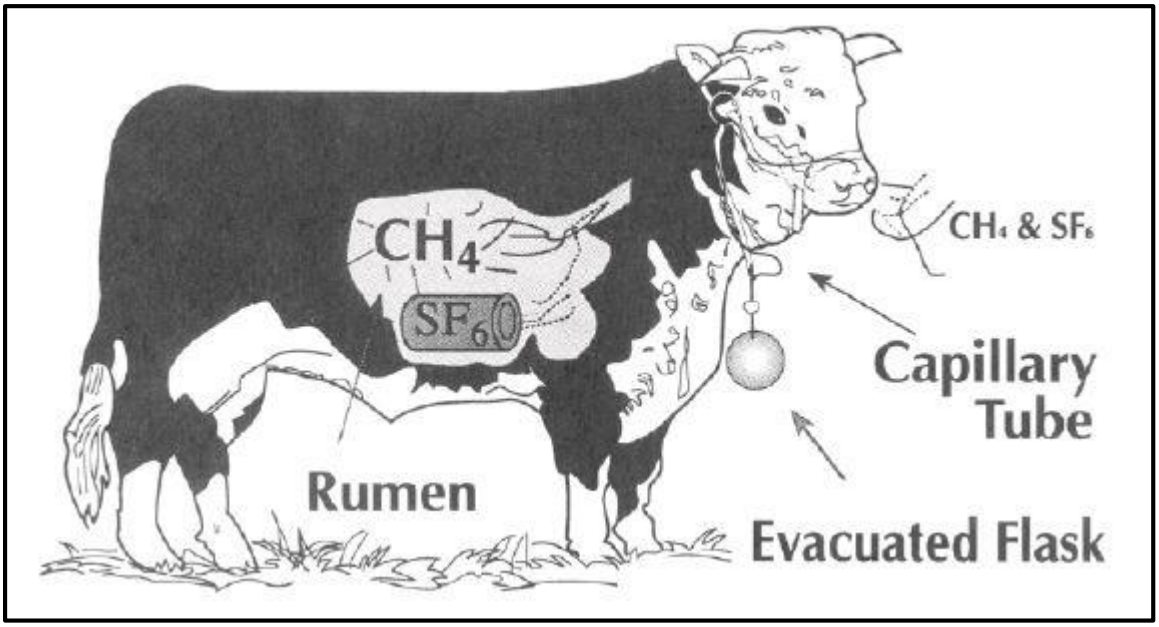

Figure 1.9. Sampling apparatus for sulfur hexafluoride (SF_6) method of indirect calorimetry (Storm et al., 2012).

Figure 1.10. Collection of gases using a headbox system from a Holstein cow (Place et al., 2011).

APPENDIX A: EQUATIONS

$CO_2 + H_2 \rightarrow CH_4$ [1]

GEI (Mcal/d) = intake of feed × GE of feed [2]

DE (Mcal/d) = GEI – fecal energy [3]

ME (Mcal/d) = DE – urinary energy – gaseous energy [4]

NE_L (Mcal/d) = ME – HP [5]

HP (Mcal/d) = 3.866 × O_2 L + 1.200 × CO_2 L – 0.518 × CH_4 L – 1.431 × N g [6]

$C_6H_{12}O_6 + 6O_2 = 6CO_2 + 6H_2O$ + heat [7]

CHAPTER 2

INTERPRETIVE SUMMARY: Drehmel *et al. (2017).* "Factors affecting methane production from ruminal fermentation of fiber isolated from dried distillers grains and solubles," illustrates the effect of adding fat and fiber to the fibrous component of dry distillers grains and solubles on total gas production and methane production in an in vitro setting that stimulates ruminal fermentation. The hope is that the results can then be translated to animal trials to reduce methane emissions.

Running Head: EFFECT OF FAT AND FIBER ON IN-VITRO GAS PRODUCTION

Factors affecting methane production from ruminal fermentation of fiber isolated from dried distillers grains and solubles

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ABSTRACT

Ruminants produce more methane than any other livestock animal. Consequently, focus has been placed on developing mitigation strategies for ruminants both in the dairy and beef industries. The objective of this study was to determine the effects of adding fat or cellulose to fiber isolated from dried distillers grains and solubles (DDGS) to investigate the effect on ruminal methane production. Three representative samples of DDGS were obtained from different commercial bio-refineries and NDF residue was isolated through an in vitro process. The isolated NDF residue was fermented 1) alone (control); 2) with feed grade corn oil; or 3) with cellulose powder microcrystalline using the in vitro gas production technique. Both cellulose and corn oil were added along with NDF residue at a 4:1 ratio (DM basis). Inoculum was obtained by collecting a mixture of rumen fluid from two steers (BW = 543.3 ± 20.6 kg) consuming a diet containing 30% concentrate and 70% roughage. For each treatment within each run, gas production was measured in real time over a 48 hour period. Using a paired but separate bottle, the concentration of methane gas produced was measured using a gas chromatograph at 0, 4, 8, 18, 24 and 48 h. Volume of methane produced at each time point was calculated by multiplying total gas produced by the concentration of methane. Three separate runs (n = 3) were conducted and data were analyzed as a randomized complete block in which run and source of DDGS were considered random effects while treatment was considered a fixed effect. Compared to the control (74.0 ± 6.04 mL/g), addition of corn oil tended (P = 0.11) to reduce total gas production (58.0 ± 6.04 mL/g), whereas the addition of cellulose did not affect (P = 0.21) total gas production (85.7 ± 6.04 mL/g). Cellulose increased (P = 0.02) total gas production compared to corn oil. Similarly, compared to the control

(0.08 ± 0.01 mL/g), the addition of corn oil tended (P = 0.12) to reduce methane production (0.04 ± 0.01 mL/g), whereas the addition of cellulose did not affect (P = 0.22) methane production (0.10 ± 0.01 mL/g). Cellulose increased (P = 0.02) methane production compared to corn oil. In an in vitro setting, the addition of oil or cellulose to NDF resulted in the decrease or no effect on methane production, which suggests that dietary components can be used to mitigate methane production in ruminant livestock.

Keywords: gas production, in vitro, methane, neutral detergent fiber

INTRODUCTION

Ruminants produce more methane than any other livestock animal. In ruminants, majority of methane production occurs in the rumen with 2 to 3 % coming from rectal emissions. Other livestock animals produce most of their methane from hindgut fermentation therefore producing less methane. For example, horses produce 18 kg/head per year while a dairy cow produces 128 kg/head per year (Hristov et al., 2013). Methane emissions from dairy cattle account for 25 %, emissions from beef cattle account for 71 %, emissions from swine account for 0.02 % and emissions from horses account for 0.01 % of total livestock methane emissions (EPA, 2015). Methane emissions from wild ruminants such as bison, elk or deer are estimated to be 4.3 % lower than the emissions from domestic ruminants (Hristov et al., 2013). According to Grainger and Beauchemin (2011) if methane emissions increase parallel to the projected increase in livestock numbers to feed an increasing world population, global methane emissions from livestock are expected to increase by 60 % by the year 2030. Consequently, a world-wide focus has been placed on developing mitigation strategies for both dairy and beef industries such as the Paris Agreement which requires all countries to make a significant commitment to address climate change (NRDC, 2015). In 2009, the US dairy industry made a voluntary goal to reduce greenhouse gas emissions from fluid milk by 25 % by 2020 through the Innovation Center for US Dairy (Innovation Center, 2009).

There are multiple diet mitigation strategies that a producer could use. Examples include manipulating the type of carbohydrate, grinding and pelleting of forages, addition of lipids and the use of ionophores (Johnson and Johnson, 1995). Additionally, feeding

more starch, high quality forages at greater feed intake (Knapp et al, 2014) and feeding higher concentrate diets to enhance propionate production results in decreased methane. Another potential option to reduce methane production is to feed dry distiller's grains and solubles (DDGS). Foth et al (2015) observed a 7 % reduction in methane production with the inclusion of DDGS and suggested that DDGS may be used as a methane reduction strategy. Similarly, Benchaar et al (2013) found that cows fed increasing amounts of DDGS that replace the corn and soybean meal component of the ration emitted an average reduction in methane production of 14 g/d and an average of 9 % less CH_4/ECM.

The effect of DDGS on methane production is variable due to composition. The composition of DDGS 15 years ago had more fat than the DDGS do today. Therefore one would expected older DDGS may reduce methane production more. Neutral detergent fraction of DDGS has also changed. In 2001 the dairy NRC states the NDF fraction is 38.8 % however the 2016 beef NRC states the NDF fraction is 33.7 %. The amount of cellulose would be different since it is a portion of NDF therefore it would be expected that older DDGS may reduce methane production more. Feeding more digestible carbohydrates can result in greater DMI with greater milk yields and reduced methane emissions in dairy cows (Knapp et al., 2014). Neutral detergent fiber (NDF) contains cellulose, hemicellulose and these are highly digestible carbohydrates. Neutral detergent fiber in byproducts such as DDGS produce less methane compared with other forages and are expected to have a larger effect on methane because the fermentation of cellulose and hemicellulose is greater (Knapp et al, 2014). The NDF fraction in DDGS ranges from 30 to 55 % (Dong and Rasco, 1987). More recently the NRC (2001) states the NDF fraction

of DDGS is 38.8 %. Furthermore, it has been thought that fat sources may reduce methane production while fiber sources may increase methane production (Beauchemin et al., 2008).

Today the corn ethanol industry is continuing to grow however little is known on the nutritive entities or components found in DDGS. Much research has been done on the effect of DDGS on milk production. Dry distillers grains and solubles are a promising feed ingredient and learning more information about them is important to help improve the dairy cattle industry. The current study evaluated the effects of adding either fat or cellulose sources to NDF isolated from DDGS to better understand the influence of DDGS on methane production. It was hypothesized that addition of corn oil will reduce methane production while the addition of cellulose will increase methane production.

MATERIALS AND METHODS

Three independent samples of corn dry distillers grains with solubles (DDGS) were obtained from separate corn-ethanol production plants. Sample 1 originated from E Energy Adams LLC., Adams, NE, sample 2 originated from POET Nutrition, LLC., Sioux Falls, SD and sample 3 was a composite sample originating from E Energy Adams LLC., Adams, NE that is different than sample 1; Flint Hills Resources, Fairmont, NE and ICM Biofuels, St. Joseph, MO. The chemical composition of the 3 DDGS individually and combined are listed in Table 2.1 and 2.2. To test the addition of corn oil and cellulose on the fermentation of NDF originating from DDGS a randomized complete block experimental design was used. Each the 3 different DDGS had 3

treatments. The treatments tested were as follows: Treatment CRTL: 100% neutral detergent fiber (NDF) residue, treatment CO: 20% corn oil plus 80% NDF residue and treatment CELL: 20% cellulose plus 80% NDF residue; in all treatments the NDF residue originated from DDGS. Samples utilized for isolation of NDF residue were unground for analysis.

Isolation of NDF Residue

Fiber residues were prepared by weighing approximately 1.2500 – 1.2540 g of each DDGS sample into nylon bags (5 ×10 cm; pore size 50 μm). To isolate NDF residue, the ANKOM^{2000} Fiber Analyzer (Ankom Technology, Macedon, NY) was used. Three bags per platform were placed in a bag suspender as illustrated in Figure 2.1. Eight platforms were used for 24 bags with a ninth platform on top that contained no bags and a weight as illustrated in Figure 2.2. Because the NDF procedure was used in the Ankom, 1.25 g of sodium sulfite (Na_2SO_3) per bag or 30 g for 24 bags was placed in the Ankom device (Buckner et al., 2013). The bag suspender was placed in the Ankom device. This fully automated device was filled with approximately 1700 mL of NDF solution (Midland Scientific Inc., Omaha, NE) and operated for 1 hr and 20 minutes. In the last 20 minutes the samples were rinsed with hot water through 4 rinse cycles each lasting 5 minutes with each rinse followed by a 30 second drain cycle. The bag suspender was removed and bags were then placed in a wire rack and then placed in a 100°C oven for 24 hours. After the bags were dried, they were placed in a 4 L beaker with 3 L of hot water for 5 minutes to remove any extra NDF solution from the dried bags. After exposure to

hot water, 4 to 8 bags were placed in a 600 mL beaker with 80 mL of acetone for approximately 5 minutes to remove any excess lipid left in the sample. Once removed from acetone, the bags were rinsed with hot water to remove any excess acetone before being place in an oven to be dried. It is important to note that acetone could affect microbial fermentation however since excess acetone was removed the affect would be small. The bags were then placed in a wire rack which was then placed in a 100°C oven for 24 hours and weighed back using a desiccator. The mean amount of residue isolated from each bag was 0.43 ± 0.03 g, 0.37 ± 0.04 g, and 0.46 ± 0.03 g for DDGS1, DDGS2, and DDGS3 respectively.

In Vitro Procedure

In order to measure total gas production, 3 treatments were prepared. Treatment CRTL was prepared by weighing approximately 1.000 – 1.040 g of NDF residue into a 250 mL gas production bottle as illustrated in Figure 2.3. Treatment CO were prepared by pipetting 0.19 g of corn oil and weighing 0.8000 – 0.8040 g of NDF residue into a 250 mL gas production bottle as illustrated in Figure 2.4. Treatment CELL were prepared by weighing approximately 0.2000 – 0.2040 g of cellulose powder microcrystalline (MP Biomedicals LLC, Solon, OH) and 0.8000 – 0.8040 g of NDF residue into a 250 mL gas production bottle as illustrated in Figure 2.5. Two blank bottles were also included. There were a total of 20 bottles used: 6 bottles per sample of DDGS, 2 replications per treatment within DDGS, and 2 blank bottles to measure total gas production as illustrated in Figure 2.6.

Inoculum originated from whole rumen contents obtained from two rumen fistulated steers (BW = 543.3 ± 20.6 kg) consuming the diet listed in Table 2.3. Approximately 1.5 L of rumen contents were collected from each steer and then squeezed through 4 layers of cheese cloth into a pre-warmed Thermos (Thermos LLC, Schaumburg, IL) bottle. Filtered rumen fluid was poured into three 1 L separatory funnels. The funnels were purged with CO_2 and a stopper was then placed on the top of the funnels. The funnels were then placed in a 39°C water bath until most of the particulate matter from the rumen fluid floated to the top. The lower or liquid portion was then removed from the fluid, and mixed with reduced McDougall's Buffer, warmed to 39°C, at a 1:1 ratio with 1.5 g urea/L (Weiss, 1994). McDougall's Buffer was mixed at a fixed concentration of 176.40 g/18 L sodium bicarbonate ($NaHCO_3$), 49.94 g/18 L sodium phosphate dibasic (Na_2HPO_4), 10.26 g/18 L potassium chloride (KCl), 8.46 g/18 L sodium chloride (NaCl), 2.16 g/18 L magnesium sulfate ($MgSO_4$7H2O) and 2.90 g/18 L calcium chloride ($CaCl_2$2H_2O) to create artificial salvia. Buffer is mixed at a volume of 18 L, however for this study 1.5 L of buffer were used. It was maintained in a 39°C water bath and under constant CO_2 to reduce it until use.

One hundred mL of rumen fluid and McDougall's buffer inoculum were dispensed into 20 250 mL in vitro gas bottles as illustrated in Figure 2.7. Each bottle was flushed with carbon dioxide and the gas production modules of the ANKOM^{RF} Gas Production System (Ankom Technology, Macedon, NY) were placed tightly onto the bottles as illustrated in Figure 2.7. Prior to being placed on bottles, the rings of the modules were greased with petroleum jelly (Vaseline Englewood Cliffs, NJ) to ensure a

better seal on the bottle. The bottles were then manually gently agitated. The bottles were then placed in a water bath at 39°C for 48 hours as illustrated in Figure 2.7. The bottles were swirled at least twice daily. Once the bottles were placed in the water bath, the batteries in the modules were plugged in to start communicating with the Ankom system via a module number zero that measures only ambient pressure as illustrated in Figure 2.7. The pressure release from the bottles was set at 2 kPa. Gas production was measured continuously for 48 hours and pressure was recorded every 5 minutes in psi which was later converted to kPa. This whole system set up is illustrated in Figure 2.7.

To measure methane production, a SRI 8610C Gas Chromatograph (GC) (SRI Instruments, Torrance, CA) was utilized. Once the Ankom system commenced recording, 10 out of the 20 bottles were used to measure the concentration of methane. To do so 5 mL of gas was extracted out via the septum port on in vitro bottles with a syringe (Hamilton Co, Reno, Nevada) and then injected into the GC as illustrated in Figure 2.8. The GC was programmed to measure the concentration of methane and carbon dioxide in two minutes intervals. After 2 minutes the next bottle was sampled and the 2 minute cycle was repeated in the GC for a total of 20 minutes to measure all 10 bottles. Methane concentration was manually recorded at 0, 4, 8, 18, 24 and 48 h of fermentation. The described apparatus to measure total gas production and GC to measure methane concentration is illustrated in Figure 2.9.

Gas Calculations

To calculate total gas production, the ideal gas law ($n = p(V/RT)$) and Avogadro's law were used. For the ideal gas law, n = gas produced in moles (mol), p = pressure in kilopascal (kPa), V = headspace volume in glass bottle in litters (L), T = temperature in Kelvin (K) and R = gas constant (8.314472 $L \cdot kPa \cdot K^{-1} \cdot mol^{-1}$). Using Avogardo's law, at atmospheric pressure measured in psi (1 psi = 6.894757293 kilopascal) 1 mole will occupy 22.4 L at 273.15°K and 101.325 kPa. Gas measured in moles can be converted to gas measured in mL as follows: gas produced (mL) = $n \times 22.4 \times 1000$

Gas produced was calculated at 0, 4, 8, 18, 24 and 48 h. The cumulative pressure was measured by the ANKOM^{RF} Gas Production System (Ankom Technology, Macedon, NY) and was recorded in psi which was later converted to kPa at 39°C. This was then multiplied by 6.894757293 kPa to obtain a p (pressure) value for the ideal gas law. The V value for the ideal gas law was obtained by taking the actual volume capacity of 250 mL bottle, which was 310 mL subtracted from the amount of solution used, which was 100 mL, equaling 210 mL or 0.21 L. Liters were used for calculations. An R value was the given gas constant of 8.314472 $L \cdot kPa \cdot K^{-1} \cdot mol^{-1}$. A T value was obtained by adding 273°K plus 39°C to equal 312°K. For all calculations the same V (0.21 L), R (8.314472 $L \cdot kPa \cdot K^{-1} \cdot mol^{-1}$) and T (312°K) values were used for the (V/RT) part of the ideal gas law. The p value was then multiplied by the (V/RT) value which came out to be 0.000080952 L/ $L \cdot kPa \cdot K^{-1} \cdot mol^{-1} \times K$ to obtain the n value of the ideal gas law or gas produced in moles. The n value was then multiplied by 22.4 L/mol and 1000 mL/L to obtain the final amount of gas produced in mL. For each time point, gas production and

methane concentration were corrected by subtracting the blank from the respective values. Corrected values are used in the calculations. The following equations were used to find methane and total gas production:

Gas produced (volume) in mL = pressure in psi × 6.894757293 kPa × 0.000080952 L/ L·kPa·K^{-1}·mol^{-1} × K × 22.4 L/mol × 1000 mL/L [1]

Methane volume (mL) = CH_4 concentration (mg/kg)/1000000 × gas volume (mL) [2]

Total gas production (mL/g) = gas volume (mL)/sample amount (g) [3]

Methane production (mL/g) = (CH_4 concentration (mg/kg)/1000000)/sample amount (g) [4]

Statistical Analysis

Data were analyzed using the MIXED procedure of SAS (SAS Institute Inc., 2013, Cary, NC). Data were analyzed as a randomized complete block in which run (n = 3) and source of DDGS were considered random effects while treatment was considered a fixed effect. Using the LSMEANS option, the least square means of the treatments were found. Significance was declared at $P \leq 0.05$ and tendencies at $0.05 < P \leq 0.15$.

RESULTS AND DISCUSSION

Gas production is a result of fermentation of feed in the rumen that produces gases (Ishler, 2017). In vitro gas production i.e. methane generally is related to

digestibility, where fibrous highly digestible feeds generally produce more methane (Olivares-Palma et al., 2013). Measurements of in vitro gas production are believed to be indicative of rumen fermentation which is mainly a result of activity of microbes, rather than whole digestive tract digestibility, which is also influenced by digestive enzymes (Williams, 2000). The composition of rumen gas produced during fermentation are carbon dioxide, methane, nitrogen, oxygen and hydrogen which generally are believed to at 65.5, 26.8, 7.0, 0.05 and 0.2 % of the total gas in the rumen. A large portion of carbon dioxide will be converted to methane; therefore, the amount of carbon dioxide and methane will be more similar after feeding when the substrate has been utilized (Hoover and Miller, 1992). This technique typically tests feedstuffs and fermentation processes using freshly collected rumen fluid and an added buffer (Storm et al., 2012). Cumulative gas production during fermentation is then measured. This method is frequently used to test feed quality because it is rapid, cost effective and less laborious than using live animals. Additionally, the gas production method is informative because it can be used for digestion kinetics of rumen degradation of a feedstuff (Williams, 2000).

To date no other studies have sought to evaluate the effect of NDF isolated from DDGS (control) with the inclusion of fat and fiber sources on methane production using the in vitro gas production technique. To review, our hypothesis was that fat would reduce methane production while cellulose would increase methane production. Also noteworthy we could not anticipate the effect size of either. This is believed to be the case because previously it has been observed that fat has toxic effects on rumen microbes and also utilizes hydrogen for biohydrogenation therefore reducing methane

production (Beauchemin et al., 2008; Johnson and Johnson, 1995). It has also been observed that fermentation of fibrous carbohydrates results in greater methane production compared to non-fiber carbohydrates, because forage diets which are often high in fibrous carbohydrates favor production of acetate and butyrate (Knapp et al., 2014; Ribeiro et al., 2014). These 2 VFAs do not serve as hydrogen sinks and cannot utilize hydrogen therefore increasing hydrogen available for methane production. Also, fermentation of the cell wall fiber results in greater acetate: propionate resulting in higher methane losses (Johnson and Johnson, 1995).

The addition of fat (CO), for both total gas and methane production, decreased over a 48-hour period as illustrated in Figure 2.10 and Figure 2.11. When looking at specific time points for both total gas and methane production, there was no differences in the beginning hours and over time more differences were observed between CO and the control as listed in Table 2.4. There was a significant effect ($P = 0.05$) of treatment on both total gas and methane production. Compared to the control (74.0 ± 6.04 mL/g), the addition of CO treatment tended ($P = 0.11$) to reduce total gas production (58.0 ± 6.04 mL/g) by 24 %. Furthermore, methane production displayed a similar pattern to total gas production. Compared to the control (0.08 ± 0.01 mL/g), the addition of CO treatment tended ($P = 0.12$) to reduce methane production (0.04 ± 0.01 mL/g) by 67 %. The results from the inclusion of fat was expected and is supported by the literature. For example, in an in vitro setting, fats have been found to suppress methanogens and ciliate protozoa (Dohme et al., 2000). Methane production expressed as proportion of gross energy intake was lower for lipid supplemented diets compared to control diets fed to lactating dairy

cows (Eugène et al., 2008). The mechanisms which fat reduces methane production are not fully understood but are believed to be through enhanced propionate production, biohydrogenation of unsaturated fatty acids and reducing activity of methanogens and protozoa (Beauchemin et al., 2008; Johnson and Johnson, 1995). Furthermore, it is important to note that there is an influence of fat type on the effectiveness of reducing methane production. For example, refined oils high in medium chain fatty acids are generally more effective. Oilseeds and animal fats that are high in long chain fatty acids are not as effective at reducing methane production (Beauchemin et al., 2008).

In the case of the addition of cellulose (CELL), both total gas and methane production results were different from the effect of fat. When looking at specific time points for both total gas and methane production, there was no differences in the beginning hours and over time more differences were observed between CELL and the control as listed in Table 2.4. There was a significant effect ($P = 0.05$) of treatment on both total gas and methane production. However, compared to the control (74.0 ± 6.04 mL/g), the addition of CELL did not affect ($P = 0.21$) total gas production (85.7 ± 6.04 mL/g). Furthermore, methane production displayed a similar pattern to total gas production. Compared to the control (0.08 ± 0.01 mL/g), the addition of CELL did not affect ($P = 0.22$) methane production (0.10 ± 0.01 mL/g). As mentioned above it was expected that fibrous sources would result in higher methane production, however, this was not observed in the current study. Similar to fat it is generally believed that there is an influence of fiber type (ie hemicellulose vs cellulose) on methane production. For example, Moe and Tyrrell (1979) observed that cellulose produces more methane than

hemicellulose. Furthermore, Hindrichsen et al. (2004) observed that different fibrous carbohydrates had only minor effects on methane emissions; due to the lignification of fiber. Unlike fat, fiber did not affect methane production. This may be because the effect of fat is probably more potent on the microbes than the effect of fiber.

From this study, we observed that the type of fermentation substrate added to NDF from DDGS affects methane production. Specifically, the addition of fat was found to decrease methane production while the addition of cellulose had no effect on methane production. In the current study, gas production was estimated in vitro and some caution should be exercised when trying to translate these observations to live animals. It is important to note that this system attempts to mimic only foregut fermentation while some fermentation is known to occur in the hind gut (Storm et al., 2012). Secondly it is also important to note that it may be difficult to compare the magnitude of observed effects with those from different laboratories (Williams, 2000; Cattani et al., 2014). This is because sources of variation across laboratories may include operative conditions, type of buffer used, ratios among feed sample size and fermentation fluid and type of gas production equipment used (Cattani et al., 2014). Nonetheless, the in vitro gas production technique has been widely demonstrated to be effective in studying the effects of feed chemical composition and methane production but its observations ultimately need to be supported by those using in vivo conditions.

CONCLUSIONS

In this study, it was observed that the addition of fat to NDF residue resulted in decreased total gas and methane production while the addition of cellulose resulted in no differences in total gas and methane production compared to fat. These results further suggest that manipulation of dietary ingredients can be used to mitigate methane in ruminants.

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TABLES AND FIGURES

Table 2.1. Chemical composition of individual dry distillers grains and solubles (DDGS)[1]

Item	DDGS1[2]	DDGS2[3]	DDGS3[4]
DM	89.0	94.2	90.5
CP, % DM	29.6	28.6	31.9
Soluble Protein, % DM	2.30	4.10	3.60
ADICP[5], % DM	3.36	1.80	3.02
NDICP[6], % DM	3.83	3.18	3.20
ADF, % DM	9.90	7.90	12.6
NDF[7], % DM	39.9	32.7	39.6
Lignin, % DM	4.10	1.99	2.54
NFC[8], % DM	25.8	26.8	25.4
Sugar, % DM	6.10	3.00	4.20
Starch, % DM	5.90	6.50	6.60
Crude fat, % DM	7.94	11.7	8.41
Ash, % DM	5.12	5.22	4.99
Ca, % DM	0.04	0.03	0.03
P, % DM	0.80	0.95	0.81
Mg, % DM	0.32	0.35	0.32
K, % DM	1.06	1.21	1.09
S, % DM	0.60	0.91	0.56
Na, % DM	0.12	0.13	0.18
Cl, % DM	0.15	0.13	0.15
Fe, mg/kg	90.0	78.0	93.0
Zn, mg/kg	60.0	68.0	63.0
Cu, mg/kg	3.00	2.00	4.00
Mn, mg/kg	18.0	16.0	17.0

[1]Values determined by Cumberland Valley Analytical Services, Hagerstown, MD.
[2]DDGS1 = E Energy Adams LLC (Adams, NE).
[3]DDGS2 = POET Nutrition LLC (Sioux Falls, SD) and same DDGS used in a study by Ramirez-Ramirez et al (2016).
[4]DDGS3 = composite sample from E Energy Adams LLC, (Adams, NE), Flint Hills Resources (Fairmont, NE) and ICM Biofuels (St. Joseph, MO).
[5]ADICP = Acid detergent insoluble crude protein.
[6]NDICP = Neutral detergent insoluble crude protein.
[7]NDF of isolated residue determined at the University of Nebraska – Lincoln.
[8]NFC = Nonfiber carbohydrate calculated by difference 100 – (% NDF + % CP + % Fat + % Ash).

Table 2.2. Chemical composition of dry distillers grains and solubles (DDGS) combined (n=3)

	DDGS	
Item	Mean	SD
DM	91.2	2.68
CP, % DM	30.0	1.69
Soluble Protein, % DM	3.33	0.93
ADICP[1], % DM	2.73	0.82
NDICP[2], % DM	3.40	0.37
ADF, % DM	10.1	2.36
NDF[3], % DM	37.4	2.63
Lignin, % DM	2.88	1.10
NFC[4], % DM	26.0	0.72
Sugar, % DM	4.43	1.56
Starch, % DM	6.33	0.38
Crude fat, % DM	9.35	2.05
Ash, % DM	5.11	0.12
Ca, % DM	0.03	0.01
P, % DM	0.85	0.08
Mg, % DM	0.33	0.02
K, % DM	1.12	0.08
S, % DM	0.03	0.19
Na, % DM	0.14	0.03
Cl, % DM	0.14	0.01
Fe, mg/kg	87.0	7.94
Zn, mg/kg	63.7	4.04
Cu, mg/kg	3.00	1.00
Mn, mg/kg	17.0	1.00

[1]ADICP = Acid detergent insoluble crude protein.
[2]NDICP = Neutral detergent insoluble crude protein.
[3]NDF of isolated residue determined at the University of Nebraska – Lincoln.
[4]NFC = Nonfiber carbohydrate calculated by difference 100 – (% NDF + % CP + % Fat + % Ash).

Table 2.3. Composition of the diet for fistulated donor steers

Item, % DM	Diet
Brome hay	70.5
Dry rolled corn	5.81
Dry distillers grain and solubles	23.3
Salt	0.28
Trace minerals premix[1]	0.05
Vitamin premix[2]	0.03

[1]Formulated to supply approximately 0.5 ppm Co, 7.1 ppm Cu, 0.9 ppm I, 47.0 ppm Fe, 37.6 ppm Mn, 0.3 ppm Se and 56.4 ppm Zn in diet.

[2]Formulated to supply approximately 2200 IU/kg of vitamin A, 275 IU/kg of vitamin D and 15 IU/kg of vitamin E in diet.

Table 2.4. Total gas and methane production at specific time points over 48 hours

	Treatments[1]				
	CTRL	CO	CELL	SEM[2]	*P*-value
Total Gas Production, mL/g					
0 hr	4.48×10^{-13}	-5.12×10^{-13}	6.25×10^{-13}	8.61	1.00
4 hr	5.56	6.17	3.74	8.61	0.48
8 hr	23.3	17.4	21.6	8.61	< 0.01
18 hr	97.8[a]	73.3[b]	108.3[a]	8.61	< 0.01
24 hr	145.9[a]	103.5[b]	165.0[a]	8.61	< 0.01
48 hr	172.3[b]	148.0[b]	217.6[a]	8.61	< 0.01
Methane Production, mL/g					
0 hr	3.61×10^{-16}	-6.74×10^{-15}	-5.55×10^{-17}	0.17	1.00
4 hr	0.0022	0.0012	0.0017	0.17	0.90
8 hr	0.0079	0.0026	0.0074	0.17	0.64
18 hr	0.09[ab]	0.05[b]	0.11[a]	0.17	< 0.01
24 hr	0.15[a]	0.08[b]	0.19[a]	0.17	< 0.01
48 hr	0.20[b]	0.12[c]	0.29[a]	0.17	< 0.01

[abc] Means within rows with different superscripts differ ($P < 0.05$).

[1]CRTL = Control, CO = Corn Oil, CELL = cellulose.

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

Figure 2.1. Three bags of dry distillers grains and solubles placed on one platform of the bag suspender for the ANKOM^{2000} Fiber Analyzer (Ankom Technology, Macedon, NY).

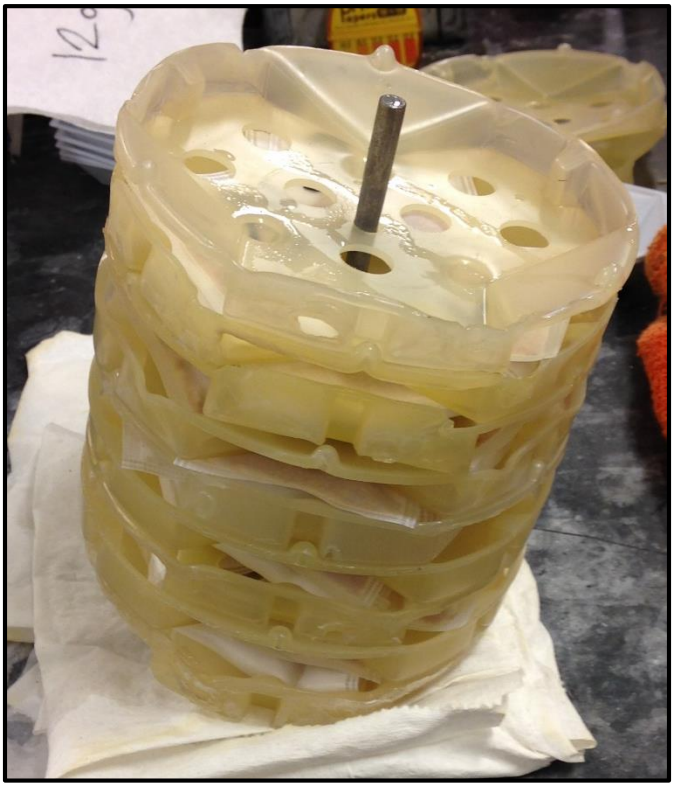

Figure 2.2. Nine platforms with 3 bags per platform of dry distillers grains and solubles except the top platform of the bag suspender for the ANKOM^{2000} Fiber Analyzer (Ankom Technology, Macedon, NY).

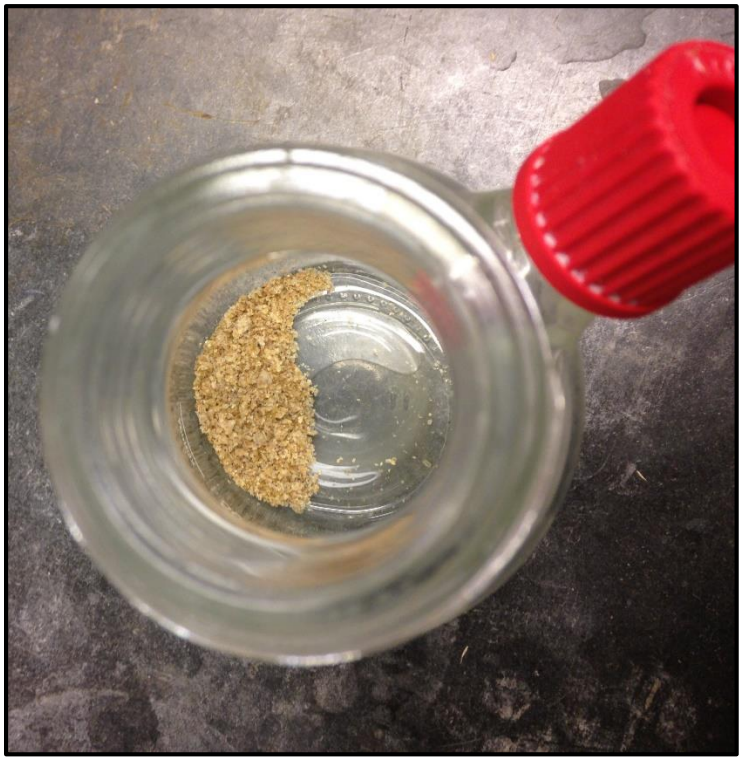

Figure 2.3. Treatment CRTL (100% or 1 g of NDF residue) prepared in the in vitro gas production bottle.

Figure 2.4. Treatment CO (80% or 0.8 g of NDF residue & 20% or 0.2 g of corn oil) prepared in the in vitro gas production bottle.

Figure 2.5. Treatment CELL (80% or 0.8 g of NDF residue & 20% or 0.2 g of cellulose powder) prepared in the in vitro gas production bottle.

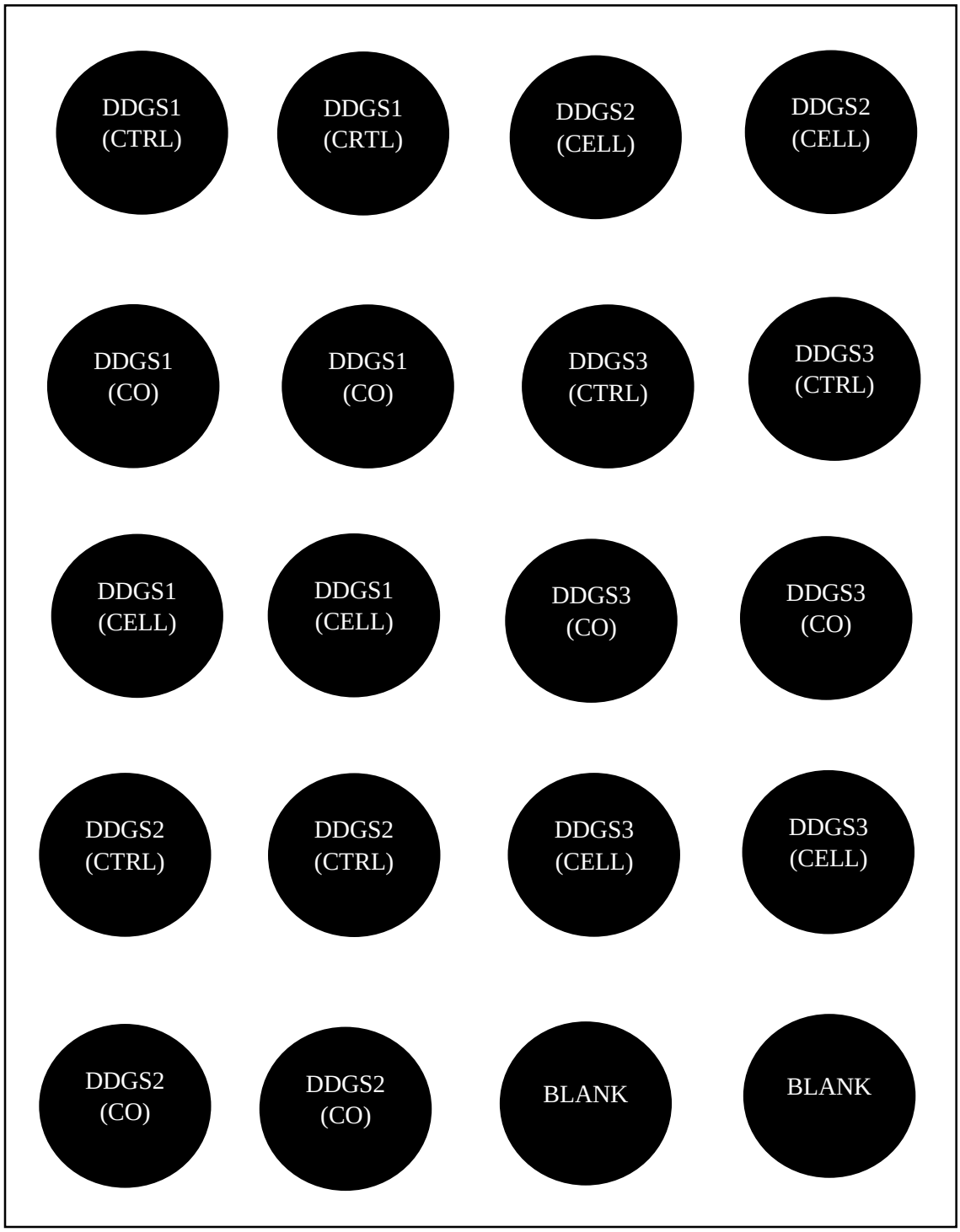

Figure 2.6. Design of dry distillers grains and solubles for measurement of total gas production.

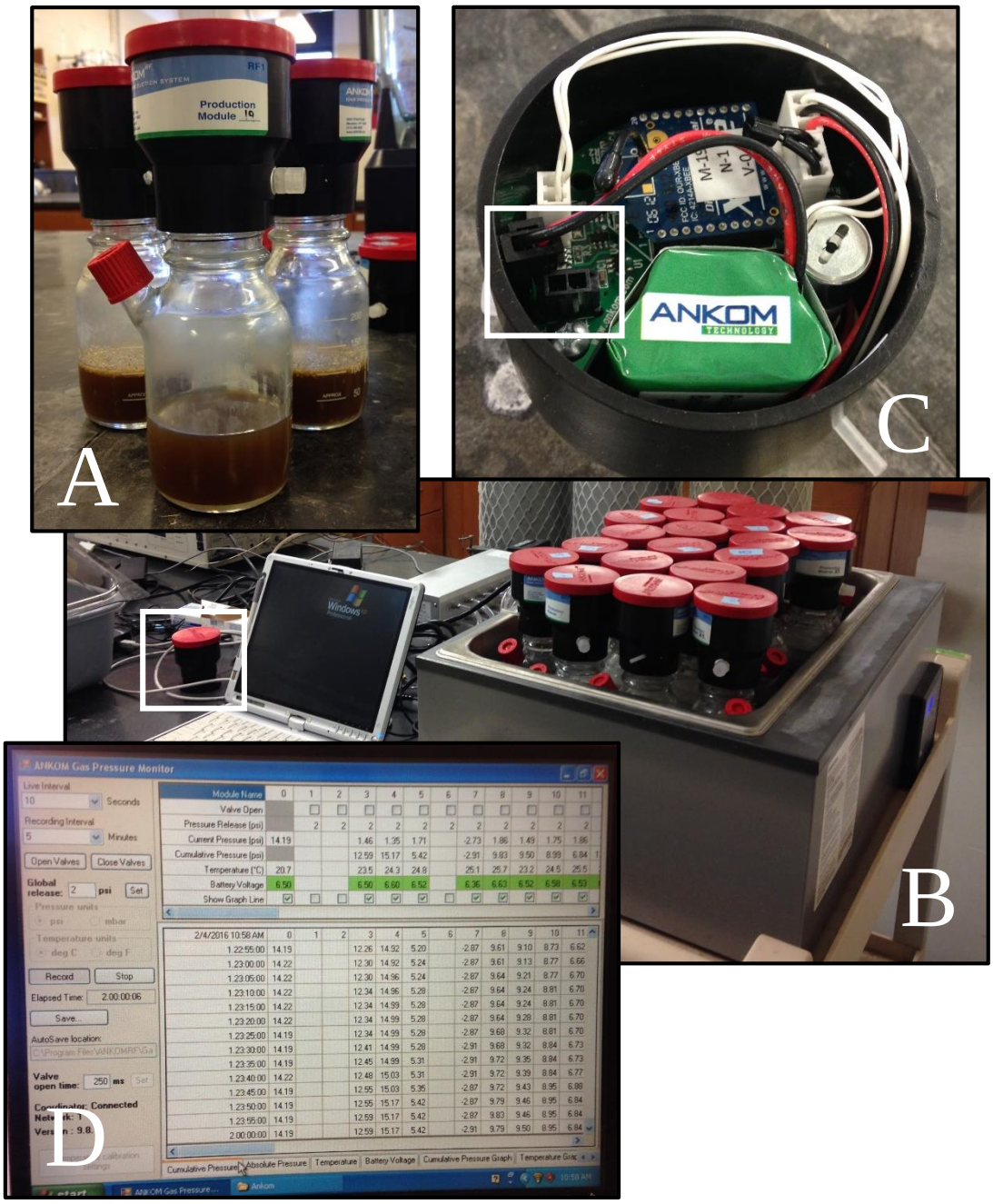

Figure 2.7. Center bottle: One hundred ml of rumen fluid and McDougall's buffer (blank) inoculum, and Left and right bottles: One hundred ml of rumen fluid and McDougall's buffer inoculum with treatment feed samples (A). All individual bottles include the gas production modules of the ANKOM^{RF} Gas Production System (Ankom Technology, Macedon, NY) placed on top of bottles (A). Bottles placed in the water bath for 48 hours (B). Battery plugs in at (square) (C) and communicates with module number zero (square) (B). Gas production monitor on computer where pressure release is set for 2 psi and records every 5 minutes (D). The whole system set up is illustrated in (B).

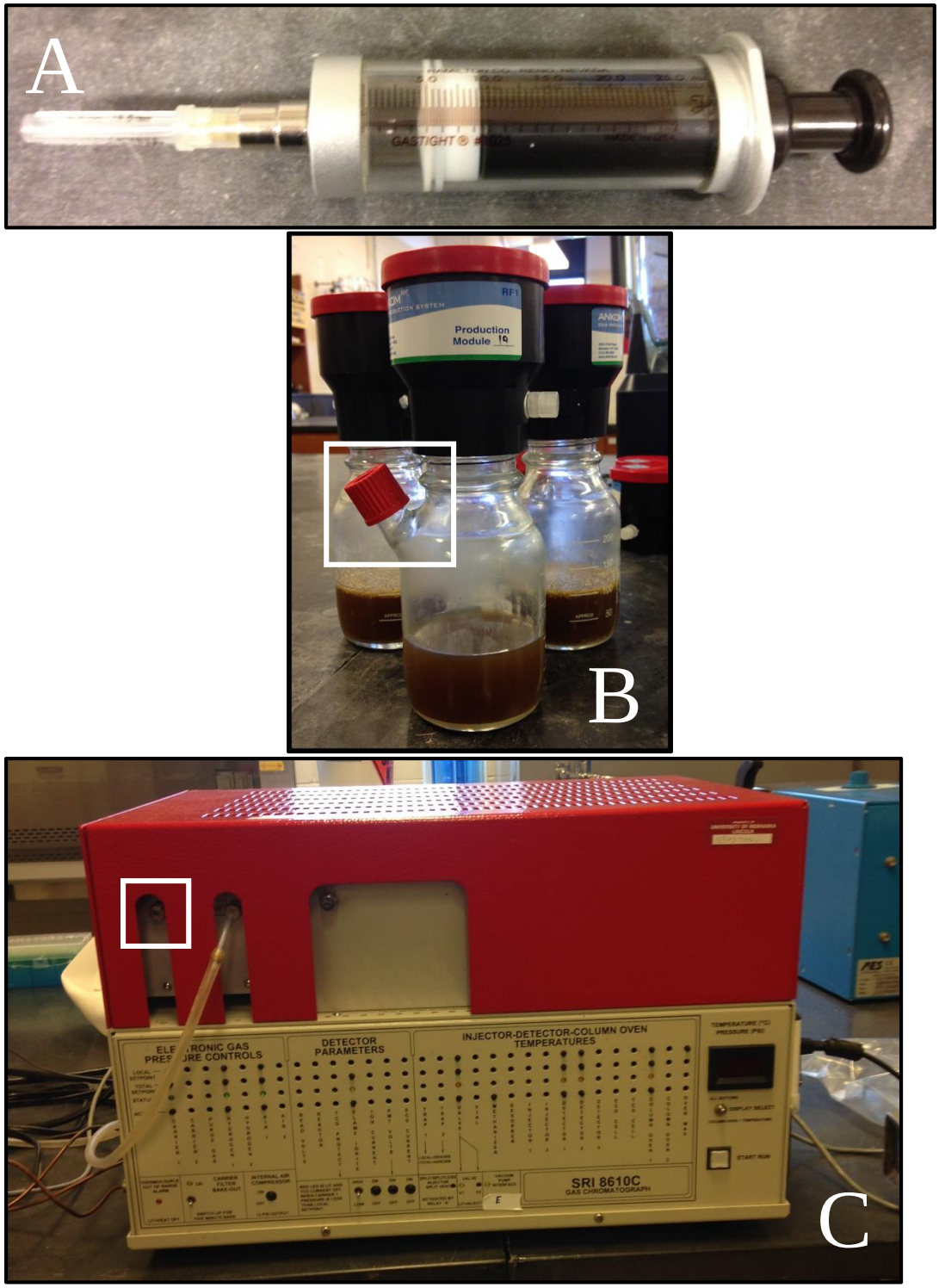

Figure 2.8. Five ml of gas removed from gas production bottles using a syringe (Hamilton Co, Reno, Nevada) (A) via the septum port on the bottle (square) (B) and pushed in the SRI 8610C Gas Chromatograph (GC) (SRI Instruments, Torrance, CA) (square) (C).

Figure 2.9. Set-up of the ANKOM^{RF} Gas Production System (Ankom Technology, Macedon, NY) to measure total gas production and SRI 8610C Gas Chromatograph (GC) (SRI Instruments, Torrance, CA) to measure methane concentration.

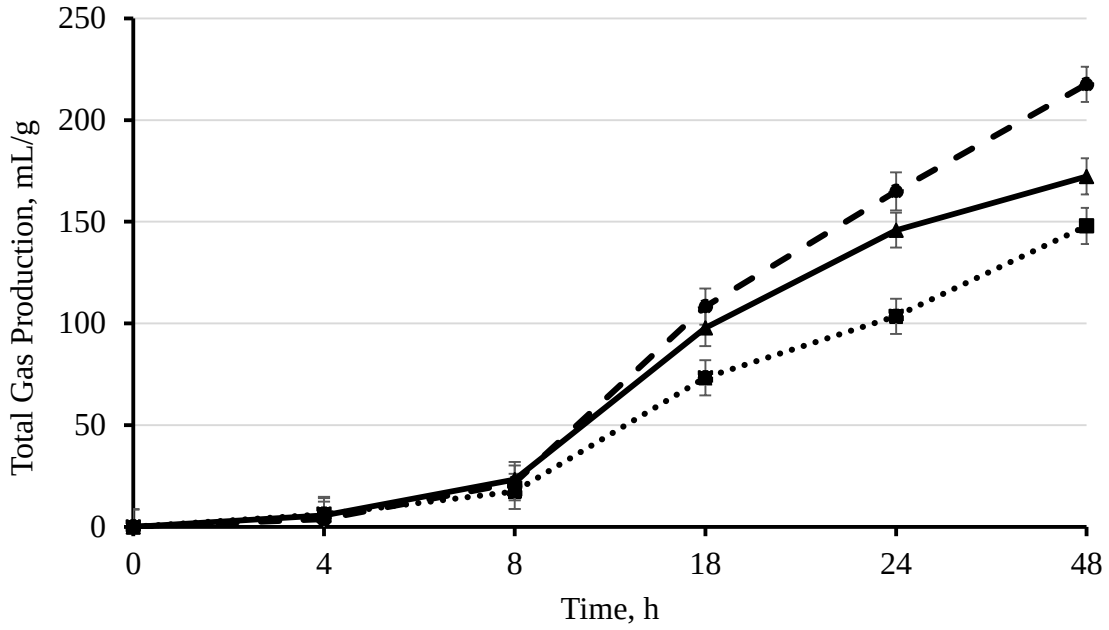

Figure 2.10. Total gas production from corn oil and cellulose added to NDF residue from dry distiller's grains and solubles over 48 hours. Mean values: control (only residue) (CRTL, solid line) = 74.0 mL/g, corn oil (residue plus corn oil) (CO, dotted line) = 58.0 mL/g and cellulose (residue plus cellulose) (CELL, dashed line) = 85.7 mg/L. *P* = 0.05 and SEM = 6.04.

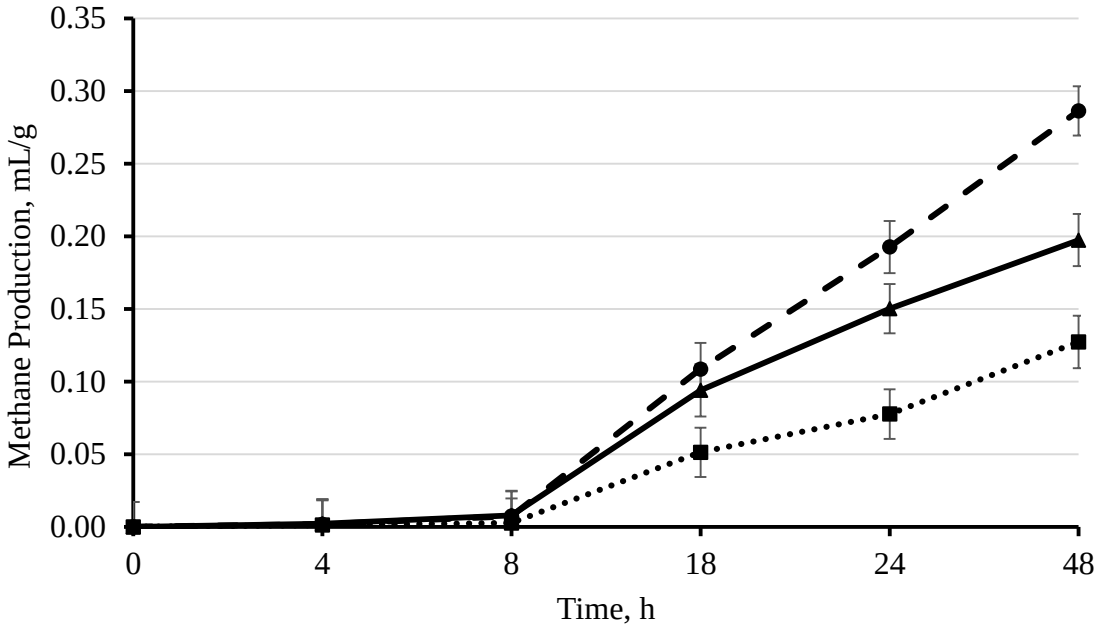

Figure 2.11. Methane production from corn oil and cellulose added to NDF residue from dry distiller's grains and solubles over 48 hours. Mean values: control (only NDF residue) (CRTL, solid line) = 0.08 mL/g, corn oil (NDF residue plus corn oil) (CO, dotted line) = 0.04 mL/g and cellulose (NDF residue plus cellulose) (CELL, dashed line) = 0.10 mg/L. *P* = 0.05 and SEM = 0.01.

APPENDIX A: EQUATIONS

Gas produced (volume) in mL = pressure in psi × 6.894757293 kPa × 0.000080952 L/ $L \cdot kPa \cdot K^{-1} \cdot mol^{-1}$ × K × 22.4 L/mol × 1000 mL/L [1]

Methane volume (mL) = CH_4 concentration (mg/kg)/1000000 × gas volume (mL) [2]

Total gas production (mL/g) = gas volume (mL)/sample amount (g) [3]

Methane production (mL/g) = (CH_4 concentration (mg/kg)/1000000)/sample amount (g) [4]

APPENDIX B: 2016 JOINT ANNUAL MEETING POSTER

Factors affecting methane production from ruminal fermentation of fiber isolated from dried distillers grains and solubles

O.R. Drehmel[1], S.C. Fernando[1], J.L. Gramkow[1], J.V. Judy[1], J.C. MacDonald[1], H.A. Paz[1] and P.J. Kononoff[1]

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Abstract # 17836

ABSTRACT

Ruminants produce more methane (CH_4) than any other livestock animal. Consequently focus has been placed on developing mitigation strategies for ruminants both in the dairy and beef industries. The objective of this study was to determine the effect of addition of fat or cellulose to fiber from dried distillers grains and solubles (DDGS) on ruminal CH_4 production. Three representative samples of DDGS were obtained from different commercial bio-refineries and NDF residue was isolated. The purified NDF residue was fermented 1) alone (control); 2) with feed grade corn oil; or 3) with cellulose powder microcrystalline using the in vitro gas production technique. Both cellulose and corn oil were added along with NDF residue at a 4:1 ratio (DM basis). Inoculum was obtained by collecting a mixture of rumen fluid from two steers (BW = 543.3 ± 20.6 kg) consuming a diet containing 30% concentrate and 70% roughage. For each treatment within each run, gas production was measured real time over a 48 hour period. Using a paired but separate bottle, the concentration of CH_4 gas produced was measured using a gas chromatograph at 0, 4, 8, 18, 24 and 48 h. Volume of methane produced at each time point was calculated by multiplying total gas produced by the concentration of CH_4. Three separate runs (n = 3) were conducted and data were analyzed as a randomized complete block in which run and source of DDGS was considered random effects while treatment was considered as a fixed effects. Compared to the control (74.0 ± 6.04 mL/g), addition of corn oil tended (P = 0.11) to reduce total gas production (58.0 ± 6.04 mL/g), whereas, addition of cellulose did not affect (P = 0.21) gas production (85.7 ± 6.04 mL/g). Cellulose increased (P = 0.02) total gas production compared to corn oil. Similarly, compared to the control (0.075 ± 0.0125 mL/g), the addition of corn oil tended (P = 0.12) to reduce CH_4 production (0.043 ± 0.0125 mL/g), whereas, addition of cellulose did not affect (P = 0.22) CH_4 production (0.099 ± 0.0125 mL/g). Cellulose increased (P = 0.02) CH_4 production compared to corn oil. In an in vitro setting, the addition of oil or cellulose to NDF resulted in the decrease or increase of methane production, which suggests that dietary components can be used to mitigate methane in ruminant livestock.

Keywords: Gas production, In vitro, Methane

ACKNOWLEDGEMENTS

Funding for study: Nebraska Corn Board (Lincoln, NE)

INTRODUCTION

Ruminants produce more CH_4 than any other livestock animal. If CH_4 emissions grow in direct proportion to projected increase in livestock numbers to feed an increasing world population, then global CH_4 emissions from livestock are expected to increase 60% by 2030 (Grainger and Beauchemin, 2011). So focus has been placed on developing mitigation strategies for both dairy and beef industries. There are multiple diet mitigation strategies that a producer could use such as increase the level of intake, influence of carbohydrate type, grinding and pelleting of forages, lipid additions, manipulation of ruminal microflora such as through the use of ionophores (Johnson and Johnson, 1995), feeding nonstructural or starchy carbohydrates, high quality forages at greater intake levels or optimally processing the forages (Knapp et al, 2014) and feeding higher concentrate diets. Also Foth et al (2015) suggest that the inclusion of DDGS may decrease CH_4 production. According to Knapp et al (2014) cows fed increasing amounts of DDGS that replace corn and soybean meal emitted less CH_4 (g/d) and CH_4/ECM. Furthermore, neutral detergent fiber is heterogeneous with respect to chemical composition, digestibility and potential to produce CH_4 (Knapp et al, 2014). Highly digestible NDF in distillers by-products produce half to one-third of CH_4 per kilogram of DM digested in vitro compared with forages with similar DM digestibilities (Knapp et al, 2014). This study specifically looked a nutritional mitigation through either adding fat or cellulose sources to NDF from DDGS plus look at what is responsible for reduction of CH_4 in DDGS in an in vitro setting.

OBJECTIVE

Determine the effect of addition of fat or cellulose to fiber from DDGS on ruminal CH_4 production. Therefore, the hypothesis is that corn oil will reduce CH_4 production and cellulose will increase CH_4 production plus DDGS will also reduce CH_4 production

MATERIALS AND METHODS

- 3 different DDGS with varying amounts of fat greater than 5%
  - Treatment CTRL: 100% neutral detergent fiber (NDF) residue
  - Treatment CO: 20% corn oil (Cargill Animal Nutrition, Blair, NE) plus 80% NDF residue
  - Treatment CEL: 20% cellulose powder microcrystalline (MP Biomedicals LLC, Solon, OH) plus 80% NDF residue

Factors affecting methane production from ruminal fermentation of fiber isolated from dried distillers grains and solubles

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DAIRY RESEARCH

Abstract # 17836

University of Nebraska Lincoln

MATERIALS AND METHODS

- NDF Isolation
  - ANKOM^{2000} Fiber Analyzer (Ankom Technology, Macedon, NY) utilized
  - Each DDGS used 48 bags with 1.25 g DDGS sample per bag plus 1.25 g sodium sulfite per bag
  - 24 bag were placed into on the Ankom at a time and ran for approximately 1 hour and 20 minutes
  - After NDF, bags soaked in acetone for 2 to 5 minutes after for lipid removal
  - Bags filtered with hot water after acetone soaking for excess acetone removal
  - Placed in 100 °C oven for 24 hours to dry and then composited by DDGS

MATERIALS AND METHODS

- In vitro gas production
  - Procedure was conducted through 3 runs
  - 1 g of total sample placed into twenty 250 ml gas production bottles
  - 1.5 g urea were combined with McDougall's buffer at a 1:1 ratio
  - 100 ml of rumen fluid and McDougall's buffer inoculum dispensed into 20 bottles and placed into a 39°C water bath
  - Gas production measured continuously for 48 hours and the pressure was recorded every 5 minutes in psi using the ANKOM^{RF} Gas Production System (Ankom Technology, Macedon, NY)
  - Simultaneously concentration of CH_4 was manually measured with SRI 8610C Gas Chromatograph (SRI Instruments, Torrance, CA) at 0, 4, 8, 18 and 24 hours in CH_4 ten gas production bottles by removing 5 ml of gas via septa port
  - Volume of produced at each time point was calculated by multiply total gas produced by concentration of CH_4
  - Total gas produced was calculated using the ideal gas law and Avogardo's law
    - Gas produced in ml = p in psi · 6.894757293 kPa · 0.000080952 L/ L·kPa·K^{-1}·mol^{-1} · K · 22.4 L/mol · 1000 ml/L

In vitro gas production set up

ANKOM^{RF} Gas Production System (Ankom Technology, Macedon, NY) Screen

Factors affecting methane production from ruminal fermentation of fiber isolated from dried distillers grains and solubles

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Abstract # 17836

RESULTS

Table 1. Chemical composition of DDGS[1] (n=3)

	DDGS	
Chemical, % DM	Mean	SD
DM	91.2	2.67
CP	30.0	1.69
Soluble Protein	3.33	0.929
ADICP	2.73	0.820
NDICP	3.40	0.369
ADF	10.1	2.35
NDF	32.2	1.22
Lignin	2.88	1.09
NFC	26.0	0.72
Sugar	4.43	1.56
Starch	6.33	0.378
Ether Extract	9.35	2.049
Ash	5.11	0.115
Ca, %	0.03	0.005
P, %	0.85	0.083
Mg, %	0.33	0.017
K, %	1.12	0.079
S, %	0.69	0.191
Na, %	0.14	0.030
Cl, %	0.14	0.011
Fe, mg/kg	87.0	7.93
Zn, mg/kg	63.7	4.04
Cu, mg/kg	3.00	1.000
Mn, mg/kg	17.00	1.000

[1] Values determined by Clumberland Valley Analytical Services, Hagerstown, MD

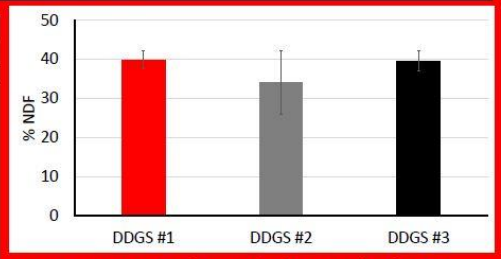

Figure 1. Proportion of NDF in the three samples of DDGS

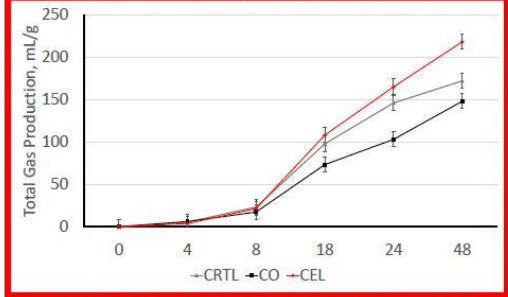

Figure 2. Total gas production of corn oil and cellulose to NDF residue over 48 hours. Mean values control (CRTL) = 74.0 mL/g, corn oil (CO) = 58.0 mL/g and cellulose (CEL) = 85.7 mg/L. *P* = 0.05 and SEM = 6.04

RESULTS

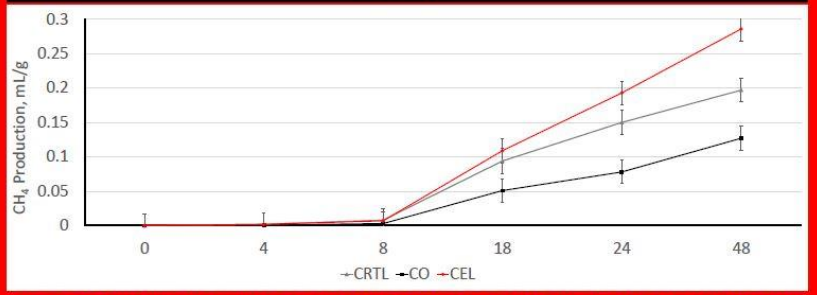

Figure 3. CH_4 production of corn oil and cellulose to NDF residue over 48 hours. Mean values control (CRTL) = 0.075 mL/g, corn oil (CO) = 0.043 mL/g and cellulose (CEL) = 0.099 mg/L. *P* = 0.05 and SEM = 0.0125

CONCLUSIONS

Results of this study it observed that in an in vitro setting, addition of corn oil to NDF residue resulted in decreased total gas and CH_4 production. The addition of cellulose to NDF residue resulted in increased total gas and CH_4 production compared to corn oil. These results suggest that dietary components can potentially be used to mitigate CH_4 in ruminant livestock.

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CHAPTER 3

INTERPRETIVE SUMMARY: Drehmel *et al. (2017).* "Increasing the diet concentrations of fat and hemicellulose on methane production and energy utilization in lactating Jersey cattle," illustrates the effect of adding fat and fiber in lactating dairy cow rations on methane production and energy utilization. This study shows that the reducing methane production will improve energy utilization in dairy cattle which will ultimately improve production.

Running Head: EFFECT OF FAT AND FIBER IN LACTATING DIARY COWS

Increasing the diet concentrations of fat and hemicellulose on methane production and energy utilization in lactating Jersey cattle

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ABSTRACT

Feeding fat to lactating dairy cows may reduce methane production. Relative to cellulose, fermentation of hemicellulose is believed to result in less methane; however, these factors have not been studied simultaneously. Eight multiparous, lactating Jersey cows averaging 98 ± 30.8 DIM and BW of 439.3 ± 56.7 kg were used in a twice replicated 4 × 4 Latin square to determine the effects of fat and hemicellulose on energy utilization and methane production using a headbox type indirect calorimetry method. To manipulate the concentration of fat, porcine tallow was included at either 0 or 2 % of the diet DM. The concentration of hemicellulose was adjusted by manipulating the inclusion rate of corn silage, alfalfa hay, and soybean hulls resulting in either 11.3 % or 12.7 % hemicellulose (DM basis). The resulting factorial arrangement of treatments were; Low Fat Low Hemicellulose (LFLH), Low Fat High Hemicellulose (LFHH), High Fat Low Hemicellulose (HFLH), and High Fat High Hemicellulose (HFHH). Neither fat nor hemicellulose affected DMI ($P \geq 0.25$) averaging 16.2 ± 1.18 kg/d across treatments. Likewise, treatments did not affect ($P \geq 0.51$) milk production averaging 23.0 ± 1.72 kg/d or energy corrected milk ($P \geq 0.15$), averaging 30.1 ± 2.41 kg/d. The inclusion of fat tended ($P = 0.12$) to reduce methane produced per kg of DMI from 24.9 to 23.1 ± 1.59 L/kg while hemicellulose had no effect ($P = 0.48$). Increasing hemicellulose increased ($P = 0.01$) NDF digestibility from 43.0 to 51.1 ± 2.35 %. Similarly, increasing hemicellulose concentration increased ($P = 0.02$) total intake of digestible NDF from 6.62 to 8.42 ± 0.89 kg/d while fat had no effect ($P = 0.62$). Methane per unit of digested NDF tended to decrease ($P = 0.12$) from 64.8 to 49.2 ± 9.60 L/kg with increasing hemicellulose while fat had no effect ($P = 0.80$). An interaction between hemicellulose and fat content on net

energy intake was observed. Specifically, increasing hemicellulose in low fat diets tended (P = 0.13) to increase net energy intake but this was not observed in high fat diets. These results confirm that methane production may be reduced with the inclusion of fat while energy utilization of lactating dairy cows is improved by increasing hemicellulose in low fat diets.

Keywords: energy utilization, fat, hemicellulose, indirect calorimetry, methane

INTRODUCTION

Methane is a potent greenhouse gas that contributes to global warming (Benchaar et al., 2001). Methanogenesis, the formation of methane, is a vital biological pathway in ruminants because it is the main hydrogen sink in the rumen, yet it is also characterized as an energetic loss for cattle that ranges from 2 to 12% gross energy intake (GEI) (Cabezas-Garcia et al., 2017). In the recent decades, an increasing focus on ruminants has developed because of their contribution to greenhouse gas emissions since they produce more methane than any other livestock animal. Thus, there is a need and focus to develop ways to reduce methane production in cattle. A world-wide focus has been placed on developing mitigation strategies for both dairy and beef industries such as the Paris Agreement which requires all countries to make a significant commitment to address climate change (NRDC, 2015). In 2009, the US dairy industry made a voluntary goal to reduce greenhouse gas emissions from fluid milk by 25 % by 2020 through the Innovation Center for US Dairy (Innovation Center, 2009).

There are many methods to reduce methane production. However, the most promise is through the nutrition of the cattle. One method to reduce methane production in dairy cattle is through manipulation of the ruminal microbial community via feed ingredients included in the diet. For example, the addition of fat is known to supply energy but also reduce methane production (Beauchemin et al., 2008). When consumed by cattle, fibrous byproducts are also believed to result in less methane per unit of digested DM compared to other forages (Johnson and Johnson, 1995). Knapp et al. (2014) has suggested this is because these feeds are high in hemicellulose and that the

digestion of hemicellulose produces 37% less methane than that of digested cellulose (Moe and Tyrrell, 1979). The hemicellulose fraction of dry distillers grains and solubles (DDGS) is approximately 19 % (NRC, 2001). The hemicellulose content of other by-products is generally less than the hemicellulose content of DDGS. For example, dried brewers grains has approximately 25 % hemicellulose, corn gluten meal is approximately 3 % and citrus pulp is approximately 2 %. When looking at the hemicellulose content of forages such as alfalfa hay which is approximately 9 %, grass hay which is approximately 25 %, and corn silage which is approximately 17 %, DDGS are generally higher in hemicellulose content compared to other forages (NRC, 2001). The hemicellulose values are calculated from the difference in ADF and NDF based on the dairy NRC values.

More recently Benchaar et al. (2013) and Foth et al. (2015) have observed that dairy cattle produce less methane, 14 g/d or 7 % respectively, when they consume diets containing 30 % DDGS. Thus, there is a need to study how methane production may be further reduced when cattle are consuming diets containing a high proportion of DDGS. The objective of this study was to determine the effects of feeding different concentrations of fat and hemicellulose on methane production and energy utilization in lactating Jersey cows consuming diets containing DDGS. It was hypothesized that the formulated DDGS diets that containing more fat and hemicellulose would result in a reduction of methane production and increase the supply of energy.

MATERIALS AND METHODS

Eight multiparous Jersey cows averaging 98 ± 30.8 DIM and a BW of 439.3 ± 56.7 kg at the beginning of the experiment were used for this study. 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 days pregnant so energy calculations could be taken. This was because energy to fetus is very minimal less than 90 days pregnant.

The experimental design was a twice replicated 4 × 4 Latin square. Cows were randomly assigned to 1 of the 4 dietary treatments: Low Fat Low Hemicellulose (LFLH), Low Fat High Hemicellulose (LFHH), High Fat Low Hemicellulose (HFLH) or High Fat High Hemicellulose (HFHH) according to Kononoff and Hanford (2006). Treatments were designed as a 2 × 2 factorial arrangement. Animals were blocked by milk production. Treatments alternated over 4 experimental periods and measurements were collected on each animal consuming each treatment. The study was conducted with a total of 4 experimental periods each being 35 days in duration. Each period included 28 days for ab libitum diet adaptation, targeting about 5% refusals during that time, followed by 7 days of collection with 4 days of 95% ad libitum feeding to reduce the amount of refusals as illustrated in Figure 3.1.

The 4 diets were formulated with treatments containing different concentrations of fat and hemicellulose. Manipulation of hemicellulose was done through varying the amounts of corn silage, alfalfa hay and ground soybean hulls. Ground corn also varied between treatments. The fat source used was porcine tallow which was added to the diet at approximately 2 % DM in 2 dietary treatments and the other 2 dietary treatments had none. High fat DDGS was added to all 4 dietary treatments at a constant amount of 20.1 %. Complete diet compositions and nutrient analysis for all treatments are presented in Table 3.1. All dietary treatments contained corn silage, alfalfa 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.

Individual feed ingredients were sampled (500 g) on the first day of each collection period and froze 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, MI), 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 (TMR) were sampled (500 g) on each day of each collection period and were froze 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. The TMR was used to determine particle size according to Heinrichs and Kononoff (2002) using the Penn State Particles 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 (AOAC international, 2000), N (Leco FP-528 N Combustion Analyzer, Leco Corp., St. Joseph, MI), 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 54’ × 30’ rubber mat was placed behind the cow to collect feces as illustrated in Figure 3.2. 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 as illustrated in Figure 3.2. The feces were subsampled (500 g) 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 sample were sent to Cumberland Valley Analytical Services Inc. (Hagerstown, MD) for nutrient analysis of DM (AOAC international, 2000), N (Leco FP-528 N Combustion Analyzer, Leco Corp., St. Joseph, MI), NDF with sodium sulfide (Van Soest et al., 1991), starch (Hall, 2009) and ash (943.05; AOAC international 2000). Furthermore, urine was collected using a catheter

with a clear tube attached to it that drained into a black plastic container behind the cow as illustrated in Figure 3.2. Using the funnel spout of the black plastic container, urine was deposited into a white 55-L plastic container 4 times a day as illustrated in Figure 3.2 and was acidified with 50 mL of HCl prior to subsampling (500 mL) and freezing at -20°C every day of the collection period. Prior to being lyophilized (VirTis Freezemobile 25ES, SP Scientific, Gardiner, NY), urine was thawed and boiled to remove the moisture. 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 as illustrated in Figure 3.3. The water bath was turned on in the morning and off in the afternoon, for approximately 6 hours each day, to reduce the chance of the sample being overheated and burned. After moisture was boiled away, the remaining dark brown paste was then composited by cow and period as illustrated in Figure 3.3. The brown paste was then lyophilized (VirTis Freezemobile 25ES, SP Scientific, Gardiner, NY) and analyzed. Once lyophilized (VirTis Freezemobile 25ES, SP Scientific, Gardiner, NY), the sample was hard and needed to be hammered within the bag to reduce the size to be used for analysis. Urine samples were analyzed at the University of Nebraska – Lincoln for lab 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 milkings for 5 consecutive days or days 29 to 33 of the collection 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 2 conical tubes 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 (100°C oven for 24 hr), N (Leco FP-528, Leco Corp) and GE (Parr 6400 Calorimeter & Parr 1281 Bomb Calorimeter, Moline, IL).

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 analyzed at the University of Nebraska – Lincoln for lab corrected DM (100°C oven for 24 hr) and GE (Parr 6400 Calorimeter & Parr 1281 Bomb Calorimeter, Moline, IL).

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 as illustrated in Figure 3.4. For each cow, a collection period of 2 consecutive 23-hr intervals measured oxygen, carbon dioxide, and methane. The design of the headboxes allowed for feed to be placed at the bottom and ad libitum access to water was available for the cows from a waterbowl placed inside the headbox. Within the headbox, the 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 minutes before the start of the collection, the doors were closed and motor was turned on. Line pressure was measured using a manometer (Item # 1221-8, United Instruments, Westbury, NY). Barometric pressure of the room was also recoded using a barometer (Chaney Instruments Co., Lake Geneva, WI) and uncorrected for sea level. Total volume of gas in the headbox was measured using a 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 2 locations: the University of Nebraska – Lincoln and at USDA meat animal research center (MARC) according to Nienaber and Maddy (1985). Measurements collected from the two days and both locations were averaged to obtain one combined value. Heat production was estimated through calculation of oxygen consumption, and carbon dioxide and methane production with correction for urinary N loss according to Brouwer (1965) (Equation 1). The gaseous products were reported in liters and the mass of urinary N in grams. Respiratory quotient was calculated using the ratio of carbon dioxide produced to the oxygen consumed. Volume of methane 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 × O_2 L + 1.200 × CO_2 L – 0.518 × CH_4 L– 1.431 x N g [1]

Metabolizable energy (ME) (Mcal/d) = intake energy 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) x (5.88 kg of protein/kg of N) x (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 shown in Figure 3.5. Tissue energy in protein describes the energy used for tissue protein synthesis (Equation 5).

Statistical Analysis

Data was analyzed using the MIXED procedure of SAS (SAS Institute, 2013, Cary, NC). Treatment was considered a fixed effect. Cow within square, was considered as a random effect. Using the LSMEANS option, the least square means of the treatments were found. The main effects of fat and hemicellulose and the interaction between these two factors were tested using the CONTRAST statement of SAS. Significance was declared at $P \leq 0.05$ and tendencies at $0.05 < P \leq 0.15$.

RESULTS AND DISCUSSION

Twenty-six of a possible 32 energy balances were completed. Below is a bulleted list of missing observations from the experiment, followed by a brief explanation:

- Square 2, Period 1, Cow 5108, was not on experiment yet. Was replacement cow for Cow 1125 that got E. coli mastitis shortly prior to the beginning of period 1 experimental collections and was removed from the remainder of the experiment.
- Square 2, Period 1, Cow 5124, was not on experiment yet. Was replacement cow for Cow 1213 that got mastitis also shortly prior to beginning of period 1 experimental collections and was removed from the remainder of the experiment.
- Square 1, Period 3, Cow 1270, removed from the experiment due to illness.
- Square 1, Period 3, Cow 6090, was not allowed to eat enough feed due to an error in calculation.
- Square 1, Period 4, Cow 1270, removed from the experiment due to illness.
- Square 1, Period 4, Cow 6090, was not allowed to eat enough feed due to an error in calculation.

Diet Composition

The chemical composition of individual feed ingredients and dietary treatments are listed in Tables 3.1, 3.2, 3.3 and 3.4. As estimated by the difference between ADF and NDF, hemicellulose concentration was adjusted by manipulating the inclusion rate of corn silage, alfalfa hay, and soybean hulls resulting in either 11.3 % DM for low hemicellulose diets or 12.7 % DM hemicellulose for high hemicellulose diets (Table 3.1). There was only a small change in hemicellulose concentration however this was the best we could do while maintaining energy and other nutrient requirements. Despite not being a direct measure, the difference between ADF and NDF is the most common way of determining hemicellulose for feed ingredients (Goering and Van Soest, 1970). Porcine tallow was included at approximately 2 % DM for high fat diets and 0 % DM for low fat diets (Table 3.1). Other than ground corn, diets were formulated to have ingredients included at similar inclusion rates (Table 3.1). All diets included DDGS at 20.1 % DM of the diet (Table 3.1) and contained 8.07 ± 0.62 % DM crude fat. The source of DDGS was E Energy Adams LLC., Adams, NE. The high fat diets were formulated to have the similar energy content (NE_L: 1.72 Mcal/kg) and the low fat diets were formulated to have the similar energy content (NE_L: 1. 68 Mcal/kg) as illustrated in Table 3.1. Neutral detergent fiber content of the high hemicellulose treatments was 35.0 ± 1.22 % which is higher than the low hemicellulose treatments at 33.7 ± 1.02 % (Table 3.4). This was expected because hemicellulose is a cell wall component and NDF is comprised of cellulose, hemicellulose and lignin. Therefore, more hemicellulose (12.7 % DM) would result in more NDF. Crude fat of the high fat treatments was 5.31 ± 0.53 % which was

higher than the low fat treatments at 4.34 ± 0.31 % (Table 3.4). This was also expected because tallow is a fat source and by design was only included in the high fat treatments.

Diet particle sizes were similar between the 2 low hemicellulose and 2 high hemicellulose diets as listed in Table 3.4. For the LFLH diets, 4.38, 20.6, 64.0, and 11.1 % remained on the > 19.0 mm, 8.0 mm, 1.18 mm and pan (< 1.18 mm), respectively and for the HFLH diets, 3.75, 19.5, 63.8 and 13.3 % remained. For the LFHH diets, 3.88, 34.3, 55.6 and 6.13 % remained on the > 19.0 mm, 8.0 mm, 1.18 mm and pan (< 1.18 mm), respectively and for the HFHH diets, 2.63, 34.6, 56.6 and 6.00 % remained. According to Heinrichs and Kononoff (2002), it is recommended that the distribution of particles in a ration should include 2 to 8% of particles remaining on the > 19.0 mm diameter sieve, 30 to 50% should be retained on the 8.0 and 1.18 mm sieve and ≤ 20% on the bottom pan. In the current study, the proportions of particles retained on the 8 mm sieve were lower than recommended, this is especially the case for the low hemicellulose diets. One possible consequence for the deviation from the recommended proportions is that cows may not consume enough effective fiber to maintain healthy rumen conditions and this may lead to rumen acidosis (Zebeli et al. 2010). It should however be noted that the recommendations of Heinrichs and Kononoff (2002) were developed before dairy diets contained large proportions of corn milling byproducts like DDGS used in the current study. In the current study DDGS were included at 20 % of the diet DM and starch content was low, approximately 20 % across treatments. This concentration of starch is substantially lower than what may be commonly fed in a commercial setting (Chase, 2007) and less likely to cause rumen acidosis (Bradford and Mullins, 2012).

Feed Intake, Milk Production and Composition, Water Intake

Feed intake, milk production and composition and water intake are listed in Table 3.5. No interactions ($P \geq 0.21$) were observed between fat and hemicellulose for any dependent variable tested. Neither fat nor hemicellulose affected DMI ($P \geq 0.25$) averaging 16.2 ± 1.18 kg/d across all treatments. The high fat diets were formulated to contain the similar energy content and the low fat diets were formulated to contain the same energy content. Similar DMI may be due to diets being formulated to have the same energy content. The addition of fat increased energy content and is likely what numerically reduced intake in the high fat diets. In a meta-analysis by Rabiee at al. (2012) inclusion of tallow in the diet tended to reduce DMI and is in contrast with the current study. In another study by Beauchemin et al (2007), DMI was not affected by inclusion of tallow and this agrees with the current study. In a study by Herrick et al (2012), a hemicellulose extract was fed and did not impact DMI. It is important to note that in this study a hemicellulose extract was added to the diet whereas in the current study the hemicellulose content was manipulated through feed formulation.

In the current study, treatments did not affect ($P \geq 0.51$) milk yield averaging 23.0 ± 1.72 kg/d. It is important to note that the numerical milk yields were low in this study. Inclusion of fat tended ($P = 0.15$) to reduce energy corrected milk (ECM) from 31.0 to 29.2 ± 2.41 kg/d while hemicellulose had no effect ($P = 0.80$). Rabiee et al. (2012) reported that the inclusion of tallow had no effect on milk yield. Herrick et al. (2012) also reported that inclusion of a hemicellulose extract had no effect on milk yield. Both of

these results agree with observations from in the current study. It is important to note that the effects of fat supplementation on milk production and milk components are variable and depend on many factors such as fat source, amount of fat, stage of lactation or composition of the diet (Knapp et al., 2014). Inclusion of fat tended ($P = 0.11$) to reduced milk fat from 5.91 to 5.56 ± 0.35 % while hemicellulose had no effect ($P = 0.31$). Milk fat yield tended ($P = 0.11$) to be reduced with the inclusion of fat from 1.36 to 1.26 ± 0.13 kg/d while hemicellulose had no effect ($P = 0.54$). As you increase the amount of unsaturated fatty acids, it is generally believed to also increase the chance of milk fat depression resulting in lower milk fat percentage. Higher formation of trans fatty acids might reduce milk fat production (NRC, 2001). In the literature recommendations for the amount of fat you can feed a lactating dairy cows varies. Palmquist and Jenkins (1980) observed that 3 to 5 % supplemental fat can be added to the diet to increase energy supply and reduce the amount of starch which increases the forage to concentrate ratio and prevents depression of milk fat. Coppock and Wilks (1991) observe that 6 % supplemental fat can be successfully added to diets. Rabiee at al. (2012) found that inclusion of tallow had no effect on milk fat percentage and milk fat yield. Herrick et al. (2012) observed that hemicellulose extract had a tendency to reduce milk fat percentage but no effect on milk fat yield. These observations agree and contrast with the current study. Inclusion of fat tended ($P = 0.15$) to reduce milk protein from 3.47 to 3.39 ± 0.13 % while hemicellulose had no effect ($P = 0.95$). Neither fat nor hemicellulose affected milk protein yield ($P \geq 0.30$) averaging 0.78 ± 0.06 kg/d across all treatments. In a recently conducted meta-analysis, milk protein percentage was significantly decreased with inclusion of tallow while milk protein yield was not affected by inclusion of tallow

(Rabiee et al., 2012). They further suggest the results were heterogenous and variable regarding the effect of fat on milk protein. It is difficult to explain why in the current study inclusion of fat affected milk protein but Raibee et al. (2012) hypothesized when fat supplementation negatively affects milk protein the response may be due to that glucose availability, insulin resistance, efficiency of milk production or even reduced plasma somatotropin. They also found that there is evidence of reduced amino acid supply in the mammary gland when fats are fed (Rabiee et al., 2012). When looking at the specific component of milk protein, the milk nitrogen fraction most depressed when fat are fed is Casein (NRC, 2001). Finally increasing hemicellulose concentration reduced ($P = 0.03$) milk urea nitrogen (MUN) from 21.9 to 20.4 ± 0.97 mg/dl while fat had no effect ($P = 0.22$). Although this difference is small it is likely due to the fact that diets that contained greater hemicellulose also contained more protein and as a result cows excreted more nitrogen in their milk.

For free water intake, fat had no effect ($P = 0.25$) while increasing hemicellulose concentration reduced ($P = 0.03$) free water intake from 77.4 to 66.7 ± 5.45 L/d. For water intake from feed, fat had no effect ($P = 0.33$) while increasing hemicellulose concentration increased ($P < 0.01$) water intake from feed from 5.61 to 7.59 ± 0.49 L/d. For total water intake, fat had no effect ($P = 0.24$) while increasing hemicellulose concentration tended to reduced ($P = 0.07$) total water intake from 83.0 to 74.3 ± 5.70 L/d. Diets with increasing hemicellulose concentration had a higher proportion (44.1% DM) of corn silage and lower diet DM (54.2 %) compared to the diets with decreasing hemicellulose concentration (24.7% DM of corn silage and diet DM 64.8 %). Therefore,

increasing hemicellulose concentration of diets lowered diet DM, reduced free water intake and total water intake and increased water intake from feed due to the higher inclusion of corn silage. A previous study done by Kume et al. (2010) observed that free water intake increased and feed water intake decreased as diet DM increased with cows eating higher forage diets, these suggestions agree with those observations of the current study.

Gas Consumption and Production

Gas consumption and production estimates are listed in Table 3.6. No interactions ($P \geq 0.40$) were observed between fat and hemicellulose for any dependent variable tested. Oxygen consumption was not affected ($P \geq 0.40$) by treatments averaging 4459.4 ± 232.4 L/d across all treatments. Carbon dioxide production was not affected ($P \geq 0.24$) by treatments averaging 4600.7 ± 254.6 L/d across all treatments. Methane production was not affected ($P \geq 0.20$) by treatments averaging 381.5 ± 26.8 L/d across all treatments. Johnson and Johnson (1995) suggested that cattle fed supplemental fat, such as tallow, had reduced methane production compared to control diets. It is also generally expected that the fermentation of fibrous carbohydrates results in greater methane production than non-fibrous carbohydrates (Ribeiro et al., 2014). Therefore, one would have expected both treatments to have an effect on methane production, yet this was not observed in the current study. The respiratory quotient (RQ), the ratio of CO_2 produced and O_2 consumed, was reduced ($P < 0.01$) with the inclusion of fat from 1.04 to 1.02 ± 0.01 L/L while increasing hemicellulose concentration tended ($P = 0.08$) to increase RQ

from 1.02 to 1.04 L/L. Although full explanation of these effects are not obvious it is well known that the that changes in pathways for ATP production may be associated with changes in RQ. For example when carbohydrate is the main fuel the RQ is close to 1.0. In comparison, when fat is the main fuel the RQ is 0.7 (Blaxter, 1967; Ketelaars and Tolkamp, 1996). This may explain why the addition of fat reduced RQ in the current study. Additionally, when used as the main fuel acetate results in an RQ of 1.0 followed by propionate (0.86) and butyrate (0.80) (Cherepanov and Agaphonov, 2010). Thus, the increase of hemicellulose on increasing RQ may be due differences in rumen fermentation and end products of fermentation.

Methane per unit of DMI tended ($P = 0.12$) to be reduced with the inclusion of fat from 24.9 to 23.1 ± 1.59 L/kg while hemicellulose had no effect ($P = 0.48$). In a study conducted by Beauchemin et al. (2007), when including tallow, methane produced per unit of DMI was significantly reduced by 11 %. In the current study tallow tended to reduce methane produced per unit of DMI by 9 %. Milk produced per unit of methane was increased ($P = 0.03$) with the inclusion of fat from 0.057 to 0.063 ± 0.004 kg/L while hemicellulose had no effect ($P = 0.17$). This means that approximately 0.06 kg of milk is produced per L of methane emitted. It is a beneficial that milk produced per unit of methane increased when fat was included and suggests that cows have a better efficiency of milk production and more energy was being partitioned towards milk production than methane production. Johnson et al (2002) observed that supplementation of oilseeds did not affect methane production but tended to increase milk produced per unit of methane. The fat source was different in the current study but followed the same trend. Heat

production was not affected ($P \geq 0.29$) by treatments averaging 22.1 ± 1.17 Mcal/d across all treatments. Heat produced per metabolic body weight was tended ($P = 0.06$) to be reduced with the inclusion of fat from 244.1 to 234.1 ± 10.1 d/MBW while hemicellulose had no effect ($P = 0.79$). This suggests that the heat increment fraction, specifically heat of fermentation and digestion is being reduced with the inclusion of fat. This reduction in heat increment or heat production due to fat is a common observation and can be seen in both ruminants and monogastrics (Moallem et al., 2010; Pettigrew and Moser, 1991). The high fat treatment also reduced DMI. Therefore cows on this diet would not be able to produce as much heat because of reduced feed intake. Very little research has been done looking at the effect of hemicellulose on gas ie oxygen, carbon dioxide or methane consumption and production and heat production in lactating dairy cattle.

Energy Partitioning

Energy partitioning estimates are listed in Table 3.7. Tendencies for interactions ($P \leq 0.12$) were observed between fat and hemicellulose for NE_L (Mcal/d), ME (Mcal/kg of DM), and NE_L (Mcal/kg of DM). The total intake of NE_L was lowest for LFLH diet (16.3 Mcal/d). This diet had the least digestible fiber and the least fat (Table 3.1). Both fat and fiber will supply energy but because these diets had the least amount of both less energy will be supplied compared to the other treatments. This treatment also had the most negative tissue energy and this is because the cows were losing body stores on this diet. For net energy for lactation (NE_L) Mcal/d, an interaction was observed. Increasing hemicellulose in low fat diets tended ($P = 0.12$) to increase NE_L but this was not observed

in high fat diets. Energy lost as feces was not affected ($P \geq 0.32$) by treatments averaging 21.9 ± 1.09 Mcal/d whereas energy lost as urine tended ($P = 0.08$) to be reduced from 3.58 to 3.31 ± 0.22 Mcal/d with increasing hemicellulose concentration while fat had no effect ($P = 0.28$). Energy lost as methane was not affected ($P \geq 0.16$) by treatments averaging 3.59 ± 0.25 Mcal/d. Total retained energy (RE) was found by adding milk and tissue energies together. An interaction was observed with RE. Increasing hemicellulose in low fat diets tended ($P = 0.12$) to increase RE but this was not observed in high fat diets. Milk energy was not affected ($P \geq 0.58$) by fat or hemicellulose. Tissue energy (TE) tended ($P = 0.13$) to increase with the inclusion of fat from -3.5 to -1.08 ± 2.95 Mcal/d while increasing hemicellulose concentration increased ($P = 0.04$) TE from -4.07 to -0.52 ± 2.95 Mcal/d. This may be because both fat and fiber are energy sources and therefore will result in higher TE and less mobilization of body stores.

Methane production when expressed as a percent of GE was reduced ($P = 0.04$) with the inclusion of fat from 5.35 to 4.85 ± 0.33 % while hemicellulose had no effect ($P = 0.59$). Beauchemin et al. (2007) observed that tallow reduced methane production as percent of GE by 15 %. In the current study, methane as percent of GE was reduced by 11 %. Net energy when expressed as percent of GE was improved ($P = 0.04$) with the inclusion of fat from 25.6 to 29.5 ± 2.94 % while increasing hemicellulose concentration also improve ($P = 0.05$) NE_L from 25.7 to 29.5 ± 2.94 %. Finally, when expressing NE_L as Mcal/kg of DM an interaction was observed. Specifically increasing hemicellulose in low fat diets tended ($P = 0.13$) to increase net energy intake but this was not observed in high fat diets. It is important to note that little research has been done looking at both fat

and fiber and consequently the interaction between them previously on energy partitioning in lactating dairy cattle. More research in this area may shed light on practical methods through ration formulation to reduce methane production in lactating dairy cattle.

Maintenance energy requirements were calculated through regression of ME and RE and solving for ME when RE equals zero as illustrated in Figure 3.5 (Foth et al., 2015). Maintenance was calculated to be 188 kcal/MBW with an efficiency of ME use for lactation (k_1) of 0.84. Observations of the current study are higher than some of the previous estimates of maintenance energy requirements and efficiencies of lactation for lactating dairy cows. Previous maintenance energy requirements mean is 134.1 ± 25.7 kcal/MBW (Birkelo et al., 2004; Foth, 2014; Moe and Tyrrell, 1971; Vermorel et al., 1982; Xue et al., 2011). Foth et al. (2015) reported a maintenance estimate of 208 Mcal/MBW and k_1 of 0.76. Comparably Yan et al. (1997) study reported maintenance estimates ranging from 146 to 179 kcal/MBW, with a mean of 160 kcal/MBW and found the k_1 to range from 0.61 to 0.68. Furthermore, Blaxter (1967) found k_1 to be about 0.70 and Blaxter (1989) also found the k_1 to be around 0.65. Over 7 lactation balance trials, Coppock et al. (1964) reported the efficiency of conversion of ME to milk estimates ranged from 63 to 107 %, with a mean of 75.5 %. The mean of the current study, 84 %, agrees with this study. In the current study, higher values for maintenance requirements and k_1 were determined than the Yan et al. (1997) study therefore suggesting greater maintenance energy requirements and higher efficiency of converting ME to milk. As compared to Foth et al. (2015), the maintenance requirements were lower while a greater

efficiency of ME use for lactation was observed in the current study. This potentially suggests that the higher conversion efficiency of ME use for lactation results in lower maintenance requirements because more energy is being partitioned towards lactation. Consequently, it is reasonable to accept the maintenance estimates of the current study (188 kcal/MBW). The previous studies were all a mix of Holsteins and Jerseys and in the current study Jerseys were used. This data suggests that maintenance requirements in Jersey is not lower than Holsteins.

Nitrogen Balance

Nitrogen partitioning estimates are listed in Table 3.8. Interactions ($P \leq 0.12$) were observed between fat and hemicellulose for urine N as percent of N intake and N balance as percent of N intake. Total N balance expressed as total mass or as a proportion of N intake was increased when increasing the fat and hemicellulose content of the diet. N balance was lowest in diets containing the lowest concentration of fat and hemicellulose and was in a negative balance (-56.5 g/d). This is likely because the LFLH treatment contained the lowest concentration of energy (0.98 Mcal/kg) and that when consuming this treatment cows mobilized large proportions of body stores to meet the energetic demands of lactation and excreted catabolized protein as urea (Maltz and Silanikove, 1996). Dietary factors can have an effect on the amount and route of N excretion (i.e. fecal or urinary N) (Weiss et al., 2009). In the current study, the LFLH diet resulted in the most total N excretion (g/d) and urine N (g/d and % of N intake). These

observations may suggest the cows were excreting the excess N mostly through urinary routes. Energy balance and nitrogen balance likely are connected.

Total urine nitrogen (g/d) was reduced ($P < 0.01$) with increasing hemicellulose from 277.8 to 229.2 ± 17.4 g/d. Milk urea nitrogen had similar results suggesting that there could be greater absorption of nitrogen in the hind gut from hemicellulose therefore less nitrogen is lost via the mammary glands and excreted as urine. When expressing urine nitrogen as a percent of N intake, an interaction was observed. Increasing fat in low hemicellulose diets reduced ($P = 0.03$) urine nitrogen but this was not observed in high hemicellulose diets. Total nitrogen balance (g/d) (intake nitrogen minus fecal, urinary, and milk nitrogen) was improved ($P = 0.02$) with inclusion of fat from -26.7 to 4.64 ± 21.0 g/d while increasing hemicellulose tended ($P = 0.06$) to improve nitrogen balance from -28.0 to 3.96 ± 21.0 g/d. Nitrogen balance expressed as % of N intake also observed an interaction. Increasing fat in low hemicellulose diets tended ($P = 0.12$) to improve nitrogen balance but this was not observed in high hemicellulose diets. From these observations it could be suggested that the inclusion of fat improves N utilization.

Nutrient Digestibility

Apparent digestibilities of the diets are listed in Table 3.9. No interactions ($P \geq 0.31$) were observed between fat and hemicellulose for any dependent variable tested. Dry matter digestibility increased ($P = 0.05$) with increasing hemicellulose concentrations from 68.0 to 69.9 ± 1.30 % while fat had no effect ($P = 0.18$). Comparably Herrick et al. (2012) observed no difference in DM digestibility. Organic matter

digestibility increased ($P = 0.05$) with increasing hemicellulose concentration from 70.0 to 71.9 ± 1.23 % while the inclusion of fat tended ($P = 0.10$) to increase digestibility from 70.2 to 71.7 ± 1.23 %. Crude protein (CP) digestibility increased ($P = 0.02$) with the inclusion of fat from 73.8 to 77.2 ± 1.62 % while hemicellulose had no effect ($P = 0.55$). Crude protein digestibility was significantly increased with the inclusion of fat which has been previously observed (Simas et al., 1997). The authors state the reasons for increased CP digestibility was not apparent. Herrick et al. (2012) observed no difference in CP digestibility when hemicellulose extract was feed. This agrees with the current study. Starch digestibility was not affect ($P \geq 0.60$) by treatments averaging 97.4 ± 0.43 % across all treatments. Herrick et al. (2012) observed no difference in starch digestibility when hemicellulose extract was fed.

Neutral detergent fiber digestibility increased ($P = 0.01$) with increasing hemicellulose concentration from 43.0 to 51.1 ± 2.35 % while fat had no effect ($P = 0.32$). The increase in NDF digestibility in increasing hemicellulose diets is due to the composition of NDF most likely. Hemicellulose is a component of NDF therefore it is expected that higher concentrations of hemicellulose with increase NDF digestibility. Herrick et al. (2012) observed a significant increase in NDF digestibility (48.1 %) when hemicellulose extract was feed. Although the addition of fat to the diet can reduce methane production, it can also reduce fiber digestibility by reducing the activity of the fibrolytic microbes (Beauchemin et al., 2007). Huhtanen et al. (2009) observed reduced fiber (NDF) digestibility with increasing concentrations of fat. In the current study, a reduction in fiber digestibility due to fat was not observed. This may be because the fat

supplementation was not high enough to have negative effects. Generally lactating cow rations include 4 to 5 % crude fat with fat supplementation of up to 5 to 7 % DM. Current recommendations in the dairy NRC 2001 for ration crude fat is not to exceed 6 to 7 % DM (Knapp et al., 2014).

Methane emissions can be related to nutrient digestibilities. Increasing hemicellulose concentrations tended ($P = 0.12$) to increase total intake of digestible DM from 10.9 to 11.7 ± 0.99 kg/d while fat had no effect ($P = 0.97$). Methane per unit of digested DM tended ($P = 0.11$) to decrease with the inclusion of fat from 36.8 to 33.3 ± 2.98 L/kg while hemicellulose had no effect ($P = 0.30$). When cattle consume fibrous byproducts it is believed to result in less methane per unit of digested DM, possibly because these feeds are high in hemicellulose (Johnson and Johnson, 1995; Knapp et al., 2014). We don't really know why hemicellulose reduces methane but biochemically there could be an effect. Hemicellulose is a 5 carbon sugar whereas cellulose is a 6 carbon sugar. Consequently, the fiber fractions likely have different metabolisms. Furthermore Knapp et al. (2014) suggests rather than chemical composition for the differences in methane production, differences appear to be a function of microbial species that degrade and ferment the substrate. Total intake of digestible NDF increased ($P = 0.02$) with increasing hemicellulose concentration from 6.62 to 8.42 ± 0.89 kg/d while fat had no effect ($P = 0.62$). Methane per unit of digested NDF tend to ($P = 0.12$) to decrease with increasing hemicellulose concentration from 64.8 to 49.2 ± 9.60 L/kg while fat had no effect ($P = 0.80$). The reduction in methane per unit of digested NDF is an important result because it shows that utilization of type and even maturity of forage may reduce

methane production and that it is possible to adjust the ingredients included in the diet in order to affect methane production.

CONCLUSIONS

Total volume of methane production was not affected by fat or hemicellulose but when expressed as volume per unit of DMI fat tended to decrease methane production. For digestibilities, increasing hemicellulose concentration tended to reduce methane per unit of digestible NDF while improving NDF digestibility. Net energy intake of lactating dairy cows is improved by increasing hemicellulose in diets containing lower amounts of fat. These results suggest that manipulations of dietary ingredients can improve energy utilizations in lactating dairy cattle but it is difficult to manipulate to total volume of methane produced.

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TABLES AND FIGURES

Table 3.1. Composition and analysis of treatments differing in fat and hemicellulose concentration with inclusion of dry distillers grains and solubles (DDGS)

	Treatment[1]			
	LFLH	LFHH	HFLH	HFHH
	% of DM			
Corn Silage	24.7	44.1	24.7	44.1
Alfalfa Hay	24.9	5.71	24.9	5.7
Ground Corn	16.7	5.94	15.1	4.6
Ground Soybean hulls	1.14	11.7	1.14	11.7
DDGS	20.1	20.1	20.1	20.1
Soybean Meal	5.94	5.94	5.94	5.94
Bypass Soy[2]	1.83	1.83	1.83	1.83
Bloodmeal	1.37	1.37	1.37	1.37
Porcine Tallow	--	--	1.60	1.33
Calcium Carbonate	1.60	1.60	1.60	1.60
Sodium Bicarbonate	0.59	0.59	0.59	0.59
Ca-salts LCFA[3]	0.69	0.69	0.69	0.69
Magnesium Oxide	0.18	0.18	0.18	0.18
Salt	0.21	0.21	0.21	0.21
Trace mineral premix[4]	0.05	0.05	0.05	0.05
Vitamin premix[5]	0.05	0.05	0.05	0.05
Chemical Composition[6]				
Hemicellulose[7], % DM	11.5 (0.76)	13.0 (0.78)	11.1 (1.52)	12.4 (0.90)
CP, % DM	18.3 (0.61)	18.0 (0.90)	18.5 (0.74)	17.7 (0.43)
Crude Fat, % DM	4.11 (0.29)	4.57 (0.33)	4.98 (0.47)	5.63 (0.59)
ADF, % DM	22.9 (1.14)	22.4 (1.77)	21.9 (1.50)	22.2 (0.91)
NDF, % DM	34.4 (0.91)	35.4 (1.36)	32.9 (1.12)	34.6 (1.08)
Lignin, % DM	4.29 (0.41)	3.15 (0.18)	4.44 (0.39)	3.34 (0.13)
Ash, % DM	7.37 (0.29)	7.12 (0.63)	7.74 (0.33)	6.88 (0.19)
Starch, % DM	20.2 (2.15)	21.4 (1.97)	19.3 (2.71)	20.7 (2.35)
NFC[8], % DM	37.5 (0.61)	36.5 (0.99)	37.6 (0.73)	36.8 (1.21)
Gross Energy, cal/g[9]	4410.7 (51.2)	4394.0 (59.4)	4502.0 (68.0)	4452.5 (76.1)
ME, Mcal/kg[10]	2.60	2.60	2.67	2.67
NE_L, Mcal/kg[10]	1.68	1.68	1.72	1.72

[1]Treatments: LFLH = Low Fat Low Hemicellulose; LFHH = Low Fat High Hemicellulose; HFLH = High Fat Low Hemicellulose; HFHH = High Fat High Hemicellulose.
[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]Hemicellulose = NDF – ADF.
[8]NFC = Nonfiber carbohydrate calculated by difference 100 – (% NDF + % CP + % Fat + % Ash).
[9]Determined from composite samples from experiment and analyzed at the University of Nebraska – Lincoln, mean (SD).
[10]Values formulated from CPM diets.

Table 3.2. Chemical composition for individual ingredients of corn silage, alfalfa hay and concentrate mixes (DM basis)[1,2]

	Corn Silage		Alfalfa Hay		LFLH Concentrate		LFHH Concentrate		HFLH Concentrate		HFHH Concentrate	
Chemical	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
DM	35.9	1.22	89.9	0.54	89.9	0.48	91.1	0.74	90.6	0.88	91.1	0.42
CP, % DM	8.15	0.24	18.8	1.26	26.3	0.91	27.1	0.66	26.9	1.14	27.1	0.68
Soluble protein, % DM	4.10	0.41	6.38	0.95	4.25	2.09	4.38	0.69	4.70	0.82	4.38	0.79
ADICP[3], % DM	0.76	0.18	1.70	0.29	1.25	0.33	1.48	0.32	0.88	0.52	1.50	0.36
NDICP[4], % DM	0.94	0.15	2.83	0.22	2.09	0.38	2.72	0.24	2.02	0.08	2.59	0.21
ADF, % DM	24.3	1.50	39.5	1.92	11.5	5.14	19.0	1.30	8.78	1.52	18.4	2.38
NDF, % DM	37.3	1.28	46.1	2.94	23.9	4.94	34.0	0.91	20.6	2.17	31.6	1.93
Lignin, % DM	3.38	0.27	8.58	0.92	2.10	1.27	2.22	0.84	2.23	1.18	1.85	0.83
NFC[5], % DM	46.5	1.89	25.8	1.54	38.5	5.24	27.3	1.60	34.8	6.00	27.3	2.28
Sugar, % DM	0.75	0.51	4.25	0.65	4.78	0.58	4.83	0.82	4.65	0.88	4.53	0.81
Starch, % DM	35.2	2.77	1.55	0.70	20.5	7.19	11.7	1.38	22.7	1.72	11.3	1.35
Crude Fat, % DM	3.77	0.51	1.49	0.67	5.09	0.64	5.03	0.65	7.65	0.66	6.82	0.81
Ash, % DM	5.15	0.21	10.7	0.73	8.36	0.38	9.32	1.07	8.48	0.48	9.85	0.67
Ca, % DM	0.17	0.02	1.24	0.13	1.48	0.15	1.74	0.30	1.74	0.13	1.86	0.31
P, % DM	0.24	0.03	0.36	0.03	0.65	0.03	0.63	0.05	0.65	0.06	0.61	0.04
Mg, % DM	0.12	0.02	0.24	0.02	0.44	0.02	0.47	0.01	0.46	0.03	0.48	0.04
K, % DM	0.98	0.13	3.32	0.29	1.14	0.04	1.43	0.04	1.23	0.17	1.40	0.06
S, % DM	0.15	0.02	0.25	0.03	0.48	0.02	0.47	0.01	0.47	0.02	0.49	0.03
Na, % DM	0.01	0.01	0.03	0.01	0.54	0.06	0.57	0.09	0.61	0.04	0.60	0.03
Cl, % DM	0.11	0.03	0.11	0.01	0.33	0.03	0.38	0.10	0.39	0.05	0.37	0.04
Fe, mg/kg	152.8	34.4	271.3	89.9	290.5	38.0	393.5	35.1	319.3	46.9	395.8	15.0
Zn, mg/kg	23.8	0.96	25.8	1.50	217.8	25.1	211.5	26.6	272.3	61.4	227.3	26.4
Cu, mg/kg	5.75	0.96	8.00	1.15	33.8	5.12	34.5	8.70	37.3	2.99	61.8	47.6
Mn, mg/kg	25.5	5.07	37.5	4.43	137.3	6.60	147.5	36.8	146.3	11.0	107.3	44.2

[1]Values determined by Cumberland Valley Analytical Services, Hagerstown, MD.

[2]Treatments: LFLH = Low Fat Low Hemicellulose; LFHH = Low Fat High Hemicellulose; HFLH = High Fat Low Hemicellulose; HFHH = High Fat High Hemicellulose.

[3]ADICP = Acid detergent insoluble crude protein.

[4]NDICP = Neutral detergent insoluble crude protein.

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

Table 3.3. Calculated chemical composition of treatments differing in fat and hemicellulose concentration based on individual ingredients[1,2]

	LFLH		LFHH		HFLH		HFHH	
Chemical	Mean	SD	Mean	SD	Mean	SD	Mean	SD
DM	64.3	1.75	53.9	2.06	65.3	2.04	54.5	1.11
CP, % DM	20.0	0.55	18.3	0.23	20.3	0.90	18.3	0.45
Soluble Protein, % DM	4.75	1.33	4.37	0.38	4.97	0.69	4.37	0.43
ADICP[3], % DM	1.24	0.11	1.17	0.16	1.05	0.33	1.19	0.21
NDICP[4], % DM	1.99	0.20	1.94	0.15	1.96	0.09	1.88	0.18
ADF, % DM	21.6	2.99	22.5	0.57	20.3	0.35	22.2	1.09
NDF, % DM	32.7	3.13	36.2	0.30	31.1	0.63	35.0	0.54
Lignin, % DM	4.03	0.70	3.09	0.39	4.10	0.52	2.91	0.29
NFC[5], % DM	37.3	2.94	35.7	0.60	35.5	3.43	35.7	1.14
Sugar, % DM	3.65	0.54	3.00	0.62	3.59	0.71	2.85	0.63
Starch, % DM	19.4	4.25	21.5	1.85	20.5	1.48	21.3	1.85
Crude Fat, % DM	3.87	0.60	4.27	0.58	5.16	0.62	5.18	0.66
Ash, % DM	8.14	0.19	7.57	0.58	8.20	0.38	7.83	0.23
Ca, % DM	1.10	0.08	1.02	0.15	1.23	0.07	1.08	0.15
P, % DM	0.47	0.03	0.44	0.03	0.47	0.04	0.43	0.03
Mg, % DM	0.31	0.01	0.30	0.01	0.32	0.02	0.31	0.02
K, % DM	1.64	0.08	1.34	0.05	1.69	0.10	1.33	0.08
S, % DM	0.34	0.01	0.31	0.01	0.34	0.02	0.33	0.02
Na, % DM	0.28	0.03	0.30	0.04	0.32	0.02	0.31	0.01
Cl, % DM	0.22	0.02	0.24	0.05	0.25	0.02	0.24	0.03
Fe, mg/kg	252.0	46.3	280.8	23.9	266.5	43.1	281.9	18.3
Zn, mg/kg	122.2	12.6	118.3	13.4	149.8	31.2	126.3	12.9
Cu, mg/kg	20.46	2.92	20.4	4.07	22.2	1.75	34.1	23.7
Mn, mg/kg	85.0	1.82	87.6	20.0	89.5	6.23	67.3	23.4

[1]Values determined by Cumberland Valley Analytical Services, Hagerstown, MD.

[2]Treatments: LFLH = Low Fat Low Hemicellulose; LFHH = Low Fat High Hemicellulose; HFLH = High Fat Low Hemicellulose; HFHH = High Fat High Hemicellulose.

[3]ADICP = Acid detergent insoluble crude protein.

[4]NDICP = Neutral detergent insoluble crude protein.

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

Table 3.4. Chemical composition and particle distribution of treatments differing in fat and hemicellulose concentration based on the total mixed ration[1,2]

	LFLH		LFHH		HFLH		HFHH	
Chemical	Mean	SD	Mean	SD	Mean	SD	Mean	SD
DM	64.3	1.75	53.9	2.06	65.3	2.04	54.5	1.11
CP, % DM	18.3	0.61	18.0	0.90	18.5	0.74	17.7	0.43
Soluble Protein, % DM	5.20	0.82	5.50	0.71	5.18	0.53	4.88	1.06
ADICP[3], % DM	1.22	0.20	1.13	0.11	1.18	0.18	1.13	0.15
NDICP[4], % DM	1.68	0.11	1.50	0.06	1.66	0.16	1.57	0.12
ADF, % DM	22.9	1.14	22.4	1.77	21.9	1.50	22.2	0.91
NDF, % DM	34.4	0.91	35.4	1.36	32.9	1.12	34.6	1.08
Lignin, % DM	4.29	0.41	3.15	0.18	4.44	0.39	3.34	0.13
NFC[5], % DM	37.5	0.61	36.5	0.99	37.6	0.73	36.8	1.21
Sugar, % DM	4.58	2.00	3.18	1.12	4.30	0.64	3.90	1.54
Starch, % DM	20.2	2.15	21.4	1.97	19.3	2.71	20.7	2.35
Crude Fat, % DM	4.11	0.29	4.57	0.33	4.98	0.47	5.63	0.59
Ash, % DM	7.37	0.29	7.12	0.63	7.74	0.33	6.88	0.19
Ca, % DM	0.88	0.08	0.78	0.07	0.98	0.16	0.83	0.07
P, % DM	0.47	0.01	0.45	0.03	0.46	0.02	0.43	0.02
Mg, % DM	0.29	0.01	0.31	0.02	0.31	0.02	0.31	0.02
K, % DM	1.78	0.06	1.38	0.04	1.77	0.13	1.38	0.02
S, % DM	0.31	0.02	0.31	0.01	0.34	0.07	0.31	0.02
Na, % DM	0.31	0.02	0.32	0.02	0.32	0.03	0.31	0.01
Cl, % DM	0.24	0.02	0.25	0.03	0.30	0.13	0.24	0.01
Fe, mg/kg	241.0	22.5	265.5	17.2	254.3	30.1	282.8	22.2
Zn, mg/kg	126.0	16.2	129.0	17.5	127.8	15.6	131.8	18.6
Cu, mg/kg	21.0	1.41	23.0	1.41	20.8	1.50	23.0	0.82
Mn, mg/kg	108.8	5.06	119.8	4.03	114.3	8.62	120.0	6.48
Particle Size[6]					%			
> 19.0 mm	4.38	1.92	3.88	0.83	3.75	2.19	2.63	1.30
8.0 – 19.0 mm	20.6	2.88	34.3	10.6	19.5	1.77	34.6	9.41
1.18 – 8.0 mm	64.0	2.14	55.6	6.97	63.8	2.82	56.6	7.48
< 1.18 mm	11.1	5.22	6.13	3.76	13.3	3.54	6.00	3.78

[1]Values determined by Cumberland Valley Analytical Services, Hagerstown, MD.

[2]Treatments: LFLH = Low Fat Low Hemicellulose; LFHH = Low Fat High Hemicellulose; HFLH = High Fat Low Hemicellulose; HFHH = High Fat High Hemicellulose.

[3]ADICP = Acid detergent insoluble crude protein.

[4]NDICP = Neutral detergent insoluble crude protein.

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

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

Table 3.5. DMI, milk production and components, body weight, BCS and water intake of treatments differing in fat and hemicellulose concentration

	Treatment[1]					*P*-value[3]		
	LFLH	LFHH	HFLH	HFHH	SEM[2]	F	H	I
DMI, kg/d	15.7	17.0	16.0	16.1	1.18	0.63	0.25	0.27
Milk yield, kg/d	23.0	23.4	23.1	22.3	1.72	0.51	0.78	0.40
ECM[4], kg/d	30.4	31.5	29.5	28.9	2.41	0.15	0.80	0.46
Fat, %	5.78	6.04	5.48	5.64	0.35	0.11	0.31	0.81
Fat yield, kg/d	1.32	1.40	1.26	1.26	0.13	0.11	0.54	0.50
Protein, %	3.46	3.47	3.39	3.38	0.13	0.15	0.95	0.83
Protein yield, kg/d	0.79	0.80	0.77	0.75	0.06	0.30	0.82	0.63
Lactose, %	4.81	4.80	4.80	4.82	0.04	0.90	0.91	0.64
MUN[5], mg/dl	22.1	20.8	21.6	20.0	0.97	0.22	0.03	0.77
SCC[6], cells/ml	74.9	90.5	288.9	63.1	86.4	0.31	0.30	0.21
Body weight, kg	442.1	447.2	447.0	447.5	19.9	0.68	0.67	0.71
BCS[7]	3.30	3.31	3.40	3.38	0.14	0.28	0.97	0.82
Free water intake, L/d	79.2	69.8	75.5	63.5	5.45	0.25	0.03	0.75
Water intake from feed, L/d	5.60	7.85	5.61	7.33	0.49	0.33	< 0.01	0.30
Total water intake, L/d	84.8	77.7	81.1	70.9	5.70	0.24	0.07	0.71

[1]Treatments: LFLH = Low Fat Low Hemicellulose; LFHH = Low Fat High Hemicellulose; HFLH = High Fat Low Hemicellulose; HFHH = High Fat High Hemicellulose.
[2]Lowest standard error of treatment means is shown.
[3]F = Main effect of fat inclusion, H = main effect of hemicellulose concentration, I = Interaction between fat and hemicellulose.
[4]Energy corrected milk = 0.327 × milk yield (kg) + 12.95 × fat (kg) + 7.20 × protein (kg) adjusted for 3.5% fat and 3.2% total protein (DHI Glossary, 2014).
[5]MUN = Milk urea nitrogen.
[6]SCC = Somatic cell count.
[7]BCS = Body condition score, 1-5 according to Wildman et al. (1982).

Table 3.6. Daily consumption of oxygen and production of carbon dioxide and methane for treatments differing in fat and hemicellulose concentration

	Treatment[1]					*P*- value[3]		
	LFLH	LFHH	HFLH	HFHH	SEM[2]	F	H	I
O_2 consumption, L/d	4518.1	4509.1	4441.6	4368.6	232.4	0.40	0.75	0.79
CO_2 production, L/d	4663.6	4717.4	4529.2	4492.7	254.6	0.24	0.95	0.75
CH_4 production, L/d	393.0	396.4	364.7	371.9	26.8	0.20	0.79	0.92
RQ[4], L/L	1.03	1.04	1.01	1.03	0.01	< 0.01	0.08	0.72
Milk produced/CH_4, kg/L	0.059	0.055	0.065	0.061	0.004	0.03	0.17	0.88
CH_4/ECM, L/kg	13.2	13.0	12.5	12.8	0.60	0.33	0.99	0.55
CH_4 /DMI, L/kg	25.7	24.0	23.0	23.1	1.59	0.12	0.48	0.40
Heat production[5], Mcal/d	22.5	22.5	21.8	21.7	1.17	0.29	0.92	0.90
Heat production[6], d/MBW	244.1	244.0	235.3	232.8	10.1	0.06	0.79	0.78

[1]Treatments: LFLH = Low Fat Low Hemicellulose; LFHH = Low Fat High Hemicellulose; HFLH = High Fat Low Hemicellulose; HFHH = High Fat High Hemicellulose.

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

[3]F = Main effect of fat inclusion, H = main effect of hemicellulose concentration, I = Interaction between fat and hemicellulose.

[4]Respiratory quotient, CO_2 production/O_2 consumption.

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

[6]Heat production, kcal/day/$BW^{0.75}$.

Table 3.7. Energy partitioning of treatments differing in fat and hemicellulose concentration

	Treatment[2]					*P*- value[4]		
Item[1]	LFLH	LFHH	HFLH	HFHH	SEM[3]	F	H	I
	Mcal/d							
GE intake	68.8	74.6	71.7	70.5	5.03	0.82	0.39	0.18
DE	46.2	52.0	49.7	49.9	4.18	0.75	0.18	0.19
ME	38.8	44.8	42.8	43.2	3.89	0.55	0.12	0.16
Component								
Feces	22.3	22.4	22.0	20.7	1.09	0.32	0.52	0.47
Urine	3.65	3.40	3.51	3.22	0.22	0.28	0.08	0.90
Methane	3.72	3.73	3.40	3.50	0.25	0.16	0.78	0.82
Heat	22.5	22.5	21.8	21.7	1.17	0.29	0.92	0.90
Retained (NE_L)	16.3	22.3	20.9	21.5	3.26	0.27	0.08	0.12
Milk	22.6	22.8	22.8	22.0	1.46	0.78	0.77	0.58
Tissue	-6.34	-0.66	-1.79	-0.37	2.95	0.13	0.04	0.16
	% of GE							
Feces	33.2	30.8	31.0	29.5	1.27	0.07	0.06	0.58
Urine	5.40	4.62	4.91	4.53	0.19	0.07	< 0.01	0.20
Methane	5.55	5.15	4.77	4.93	0.33	0.04	0.59	0.20
Milk	33.3	31.2	31.8	31.2	1.52	0.34	0.11	0.37
DE	66.8	69.2	69.0	70.5	1.27	0.07	0.06	0.58
ME	55.8	59.4	59.4	61.0	1.64	0.03	0.03	0.35
NE_L	22.5	28.7	28.8	30.2	2.94	0.04	0.05	0.17
	Mcal/kg of DM							
GE	4.38	4.37	4.47	4.38	0.03	0.06	0.05	0.09
DE	2.93	3.03	3.09	3.09	0.05	0.01	0.18	0.20
ME	2.45	2.60	2.66	2.68	0.07	< 0.01	0.08	0.14
NE_L	0.99	1.25	1.29	1.32	0.13	0.03	0.06	0.13

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

[2]Treatments: LFLH = Low Fat Low Hemicellulose; LFHH = Low Fat High Hemicellulose; HFLH = High Fat Low Hemicellulose; HFHH = High Fat High Hemicellulose.

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

[4]F = Main effect of fat inclusion, H = main effect of hemicellulose concentration, I = Interaction between fat and hemicellulose.

Table 3.8. Nitrogen partitioning of treatments differing in fat and hemicellulose concentration

	Treatment[1]					*P*- value[3]		
Item	LFLH	LFHH	HFLH	HFHH	SEM[2]	F	H	I
Mass	g/d							
N intake	519.7	527.8	546.9	495.1	39.9	0.90	0.33	0.17
Fecal N	133.5	133.3	127.5	118.4	6.44	0.08	0.42	0.42
Urine N	285.0	233.9	270.5	224.5	17.4	0.37	< 0.01	0.84
Total N excretion[4]	419.0	367.7	398.0	342.7	22.8	0.20	0.01	0.91
Milk N	153.9	160.2	149.4	142.7	11.8	0.21	0.98	0.44
N balance[5]	-56.5	-2.78	-1.43	10.7	21.0	0.02	0.06	0.20
TE in protein[6]	-1.89	-0.09	-0.05	0.36	0.70	0.02	0.03	0.13
N intake	% of N intake							
Fecal N	26.7	25.7	23.5	24.3	1.31	0.06	0.96	0.44
Urine N	56.1	45.3	49.4	45.3	2.41	0.03	< 0.01	0.03
Milk N	30.4	30.8	27.5	29.2	1.66	0.07	0.36	0.56
N balance	-13.0	-1.78	-0.41	1.27	4.26	0.02	0.05	0.12

[1]Treatments: LFLH = Low Fat Low Hemicellulose; LFHH = Low Fat High Hemicellulose; HFLH = High Fat Low Hemicellulose; HFHH = High Fat High Hemicellulose.
[2]Lowest standard error of treatment means is shown.
[3]F = Main effect of fat inclusion, H = main effect of hemicellulose concentration, I = Interaction between fat and hemicellulose.
[4]Fecal N + Urine N.
[5]Nitrogen balance = intake N – fecal N – urine N – milk N.
[6]TE = Tissue Energy.

Table 3.9. Apparent digestibilities of treatments differing in fat and hemicellulose concentration

	Treatment[1]					*P*-value[3]		
Component	LFLH	LFHH	HFLH	HFHH	SEM[2]	F	H	I
DM, %	67.2	69.4	68.7	70.4	1.30	0.18	0.05	0.81
OM, %	69.2	71.2	70.8	72.6	1.23	0.10	0.05	0.89
Ash, %	45.1	48.7	46.1	47.1	3.24	0.92	0.48	0.67
CP, %	74.0	73.5	77.7	76.7	1.62	0.02	0.55	0.84
Starch, %	97.0	97.8	97.4	97.2	0.43	0.78	0.60	0.32
NDF, %	44.4	52.2	41.5	49.9	2.35	0.32	0.01	0.90
Total intake of digestible DM, kg/d	10.6	11.9	11.1	11.4	0.99	0.97	0.12	0.31
CH_4 per unit of digested DM, L/kg	38.6	34.9	33.6	32.9	2.98	0.11	0.30	0.46
Total intake of digestible NDF, kg/d	6.58	8.77	6.66	8.07	0.89	0.62	0.02	0.54
CH_4 per unit of digested NDF, L/kg	66.7	49.4	62.9	48.9	9.60	0.80	0.12	0.85

[1]Treatments: LFLH = Low Fat Low Hemicellulose; LFHH = Low Fat High Hemicellulose; HFLH = High Fat Low Hemicellulose; HFHH = High Fat High Hemicellulose.

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

[3]F = Main effect of fat inclusion, H = main effect of hemicellulose concentration, I = Interaction between fat and hemicellulose.

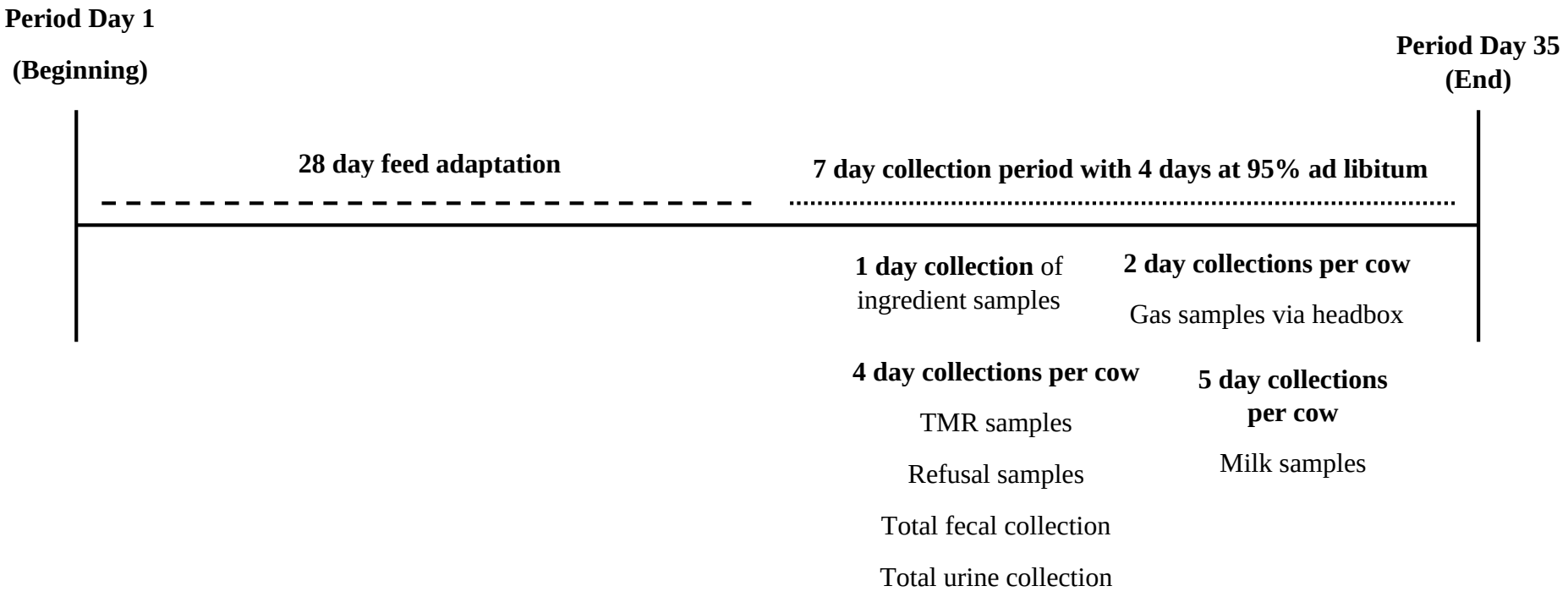

Figure 3.1. Timeline for each period, which includes a 28 day feed adaptation period and 7 days of collection and sampling.

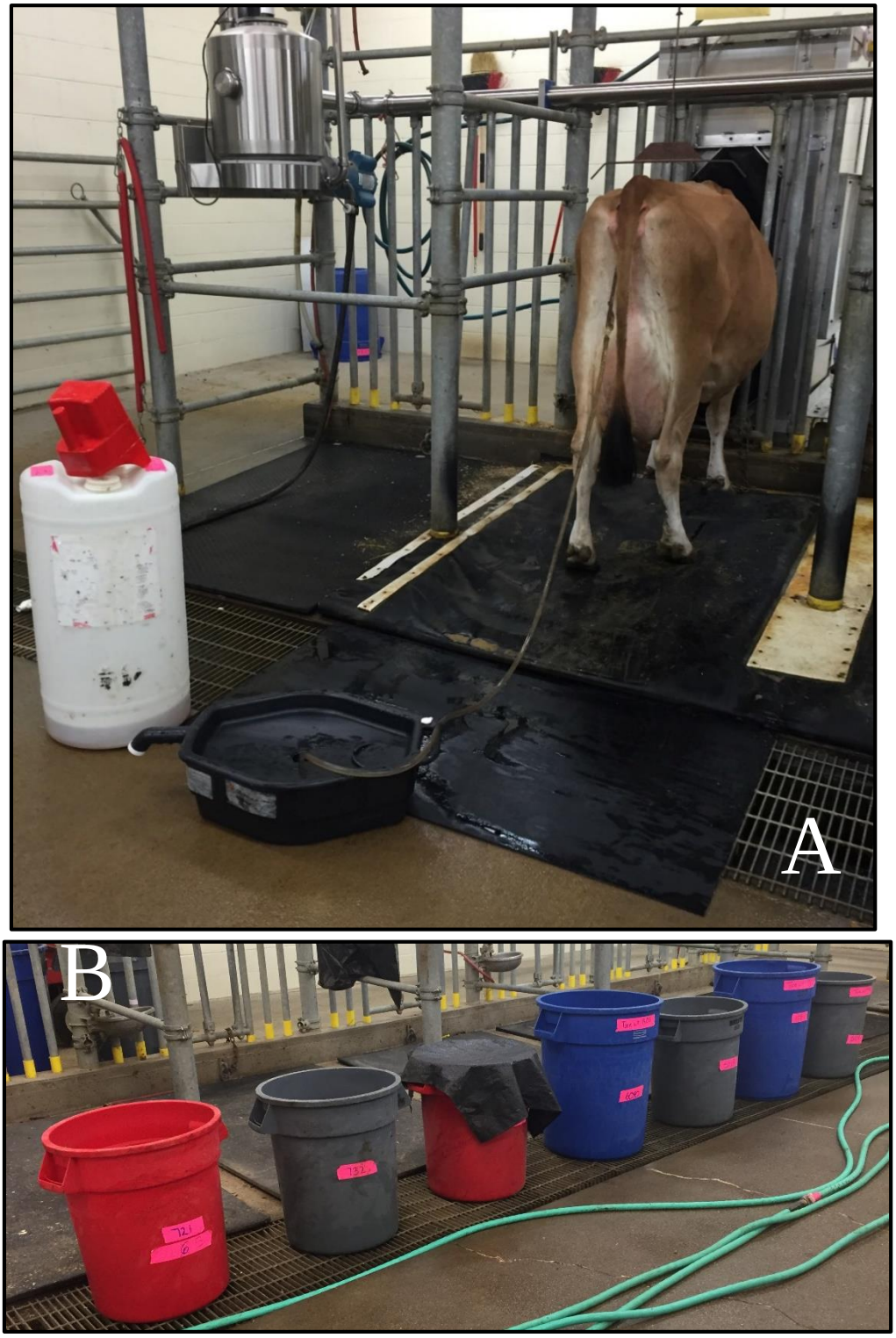

Figure 3.2. Urine collection system comprising of the catheter, clear tubing, black plastic container and white 55 L plastic container (A), Fecal collection system comprising of rubber mat and large garbage container (A & B).

Figure 3.3. Beakers filled with urine and placed in a water bath underneath a hood (A), Moisture has been removed and resulted in the dark brown paste (B).

Figure 3.4. Collection of gases from a Jersey cow using an indirect calorimeter headbox system.

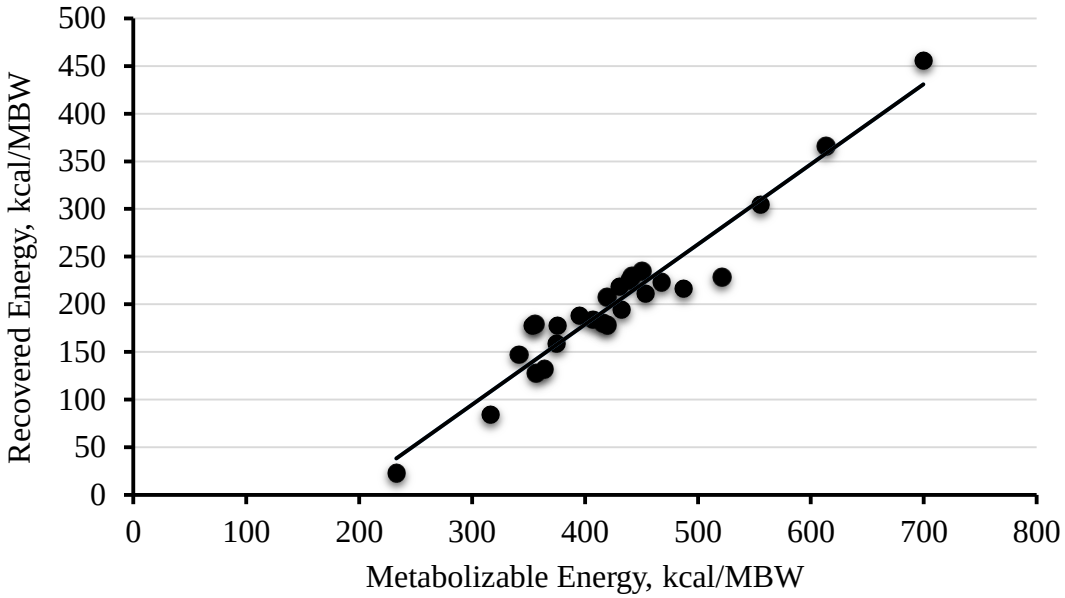

Figure 3.5. Regression of recovered energy on metabolizable energy in kilocalories per metabolic body weight (kcal/MBW; $y = 0.8413x - 157.8$; $R^2 = 0.93$). Recovered energy = 0 at 188 kcal/MBW and efficiency of converting ME to lactation energy is 84 %.

GENERAL SUMMARY AND CONCLUSIONS

Use of the in-vitro gas production technique (IVGPT) to test the effects of the chemical composition of feed on rumen methane production appeared to be a useful and robust method. The major limitation of this IVGPT is that it only measures stimulated ruminal gas production. For this study, we were only interested in total gas and methane production in the rumen. So, this system was useful for what was tested. With this method, we tested the effect of the addition of fat or cellulose to fiber from dried distillers grains and solubles (DDGS) on ruminal methane production. It was concluded that the addition of fat to NDF residue of DDGS resulted in reduced total gas and methane production while the addition of cellulose had no effect on total gas and methane production. Based on previous research we expected that fat would reduce methane production while cellulose would increase methane production. However not all of these expectations were observed in the current study.

Future research with this method would be to have both continuous methane and total gas production. In the current study, we had continuous total gas production and manual sampling of methane from the bottle. We did attempt to do both continuous total gas and methane production however had issues with the system. We used two different systems. The limitation of using the two systems were fighting with each other since they are taking the sample from the same place (headspace of the bottle) and there is really no other place to take the gas sample from on the bottle. So it would be best if one system could be used to do both however I am unaware of a system that would do that.

Utilizing the headbox-style indirect calorimeter to determine gas exchange and measure methane production and energy utilization in lactating dairy cattle is an accurate and robust tool for animal studies. Over the whole duration of the study the headboxes held up well. They allowed for easy use and maintenance during this study. The cows appeared to exhibit normal behavior and seemed comfortable in the headboxes. During the first few times in the headboxes cows were reluctant however with time cows learned they could lie down. In the current study, only 8 cows were utilized. For some measures of dependent variables, the statistical tests appeared to lack power and I would recommend that in future studies 12 cows are used as a minimum.

With this system, we tested effects of feeding different concentrations of fat and hemicellulose on methane production and energy utilization in lactating Jersey cows consuming diets containing high proportions of DDGS. It was concluded that total volume of methane produced was not affected by fat or hemicellulose. When expressed as volume per unit of DMI inclusion of fat tended to reduce methane production. Also, when expressed as volume per unit of digested NDF increasing hemicellulose concentration tended to reduce methane production. Net energy intake of lactating dairy cows was improved by increasing hemicellulose in low fat diets. Based on previous research we expected that the formulated diets that contained more fat and hemicellulose in diets containing DDGS would result in reduction of methane production and increase the supply of energy. However not all of these expectations were observed in the current study.

Total fecal and urine collection was laborious and suffers from some experimental error. Practically some feces are lost when upon being defecated they are not immediately collected and cows step on them. Additionally, in some cases the moisture content of the feces is high and when they defecated it splattered and cannot be completely collected. Even with the difficultly of collecting the feces we are still confident our observations. Urine was collected using a urinary catheter. This method was effective and appeared to collect most of the urine however care should be taken when transferring collected material to ensure that little is lost. Prior to lyophilizing the urine sample was boiled. This is a new step in the procedures for our group and was done to reduce the extent of freeze drying needed to process the sample for analysis. Although effective some samples retracted moisture and this addition of moisture resulted in some difficulties in handling the sample after. Subsequent lyophilization the sample was difficult and needed to be broken into small particles for subsampling and lab analysis.

Another idea for future research, reducing the feed offered from 95% ad libitum to 90% ad libitum. The 4 day feed intakes prior to collections were used for the 95% calculation. Over the experiment 2 cows during the last 2 periods actually consumed all their feed and that would be 14% of the cows. Over the whole experiment 95% ad libitum was 26.5 ± 10.8 kg. The 90% ad libitum would have been 25.1 ± 10.1 kg. By reducing to 90% there isn't too much of a difference. A reduction the amount of feed refusal would still be expected therefore reducing lab sample analysis. However, a downside could be the cows aren't being allowed enough feed. This could cause less energy to be consumed

resulting in lower energy balance numbers, lower milk production and component numbers and lower gas exchange numbers.

APPENDIX A: EQUATIONS

HP (Mcal/d) = 3.866 × O_2 L + 1.200 × CO_2 L – 0.518 × CH_4 L – 1.431 × N g [1]

Metabolizable energy (ME) (Mcal/d) = intake energy 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) x (5.88 kg of protein/kg of N) x (5.7 Mcal/kg of protein)/1000 [5]

APPENDIX B: LFLH, LFHH, HFLH AND HFHH DIETS ACCORDING TO THE CPM DAIRY RATION ANALYZER (2000)

Low Fat Low Hemicellulose (LFLH) Diet:

CPM-Dairy

CNCPS Evaluation

Farm: UNL Dairy Research Unit
Ration: 1603DA Low HemiCellulose Low Fat
Ration By: Kononoff & Judy & Drehmel
Organization: University of Nebraska-Lincoln

BW: 920 lb
BCS: 3.00
Growth: 0.00 lb/d
Lact#: 3

DIM: 120
Milk: 65.00 lb
Fat: 5.50 %
TP: 3.60 %

						Ingredient	DM (lb/d)
Cost ($)	1.98	IOF ($)	-1.98				
DMI (lb/d)	43.8	Model	41.0	% Model	106.7	Corn silage	10.800
ME Bal (mCal)	-0.4	CP (%)	19.9	NDF (%)	32.2	Alfalfa hay	10.900
MP Bal (g)	201.4	RUP (% CP)	43.5	ForageNDF (% NDF)	66.8	TimHay11Cp61Ndf6LNdf	0.000
NP / MP (%)	57.9	LCFA (%)	3.4	ForageNDF (% DM)	21.5	High Fat DDGS	8.800
BactMP (% MP)	44.6	EE (%)	4.2	peNDF (%)	21.6	SoybeanML47.5Solv	2.600
Rumen N Balance				Lignin (%)	3.9	Ground Corn	7.300
Pept (g)	64	Pept & NH3 (g)	122	NFC (%)	38.0	SoybeanHullsGrnd	0.500
% rqd	139	% rqd	140	Sil Acids (%)	2.0	Soy Pass	0.800
Amino Acid Balance				Sugar (%)	3.9	FatTallowPorcine	0.000
Met (g)	4.0	Lys (g)	20.4	Starch (%)	22.9	CalciumCarbonate	0.700
Met (% rqd)	110	Lys (% rqd)	115	Sol Fiber (%)	9.3	BloodMeal	0.600
Met (% mp)	1.82	Lys (% mp)	6.22	Lys:Met	3.41:1	SodiumBicarbonate	0.260
Possible production due to ME and MP						Megalac	0.300

	Milk(lb)	Fat (%)	TP (%)	Milk(lb)	Fat (%)	TP (%)	SaltNaCl	0.090
Trg:	65.0	5.50	3.60	65.0	5.50	3.60	MagOx	0.080
		Yield Constant			Composition Constant		Trace Premix	0.020
ME:	65.0	n/a	n/a	64.3	5.50	n/a	Vitamin Premix	0.022
MP:	65.0	n/a	4.04	73.0	5.50	3.60	FatTallowBeef	0.000
Adjustments based on Rulquin AA Ratios:							BrmdHy10Cp70Ndf9LNdf	0.000
	65.0	n/a	-0.18	-3.3	5.50	3.60	Total	43.772

n/a - Equations not available

Ration DM (%) 65.25 Forage (% DM) 49.58

Diet Summary:

Ingredient	Cost $ / T	DM %	AF lb/d	DM lb/d	% AF	% DM
Corn silage	33.00	35.50	30.42	10.80	45.35	24.67
Alfalfa hay	110.00	89.90	12.12	10.90	18.07	24.90
TimHay11Cp61Ndf6LNdf	0.00	89.00	0.00	0.00	0.00	0.00
High Fat DDGS	0.00	90.19	9.76	8.80	14.55	20.10
SoybeanML47.5Solv	0.00	90.00	2.89	2.60	4.31	5.94
Ground Corn	182.00	88.00	8.30	7.30	12.37	16.68
SoybeanHullsGrnd	0.00	91.00	0.55	0.50	0.82	1.14
Soy Pass	0.00	90.14	0.89	0.80	1.32	1.83
FatTallowPorcine	0.00	99.00	0.00	0.00	0.00	0.00
CalciumCarbonate	0.00	99.50	0.70	0.70	1.05	1.60
BloodMeal	0.00	90.00	0.67	0.60	0.99	1.37
SodiumBicarbonate	0.00	99.50	0.26	0.26	0.39	0.59
Megalac	0.00	97.00	0.31	0.30	0.46	0.69
SaltNaCl	185.00	99.50	0.09	0.09	0.13	0.21
MagOx	330.00	99.50	0.08	0.08	0.12	0.18
Trace Premix	848.00	95.97	0.02	0.02	0.03	0.05
Vitamin Premix	2316.00	95.75	0.02	0.02	0.03	0.05
FatTallowBeef	0.00	99.00	0.00	0.00	0.00	0.00
BrmdHy10Cp70Ndf9LNdf	0.00	89.93	0.00	0.00	0.00	0.00
Total			67.08	43.77		

Macro Nutrients		
Nutrient	DM	AF
Dry Matter (%)	100.00	65.25
Forage (%)	49.58	28.76
Crude Prot (%)	19.87	12.97
RUP (%CP)	43.51	43.51
RDP (%CP)	56.49	56.49
RDP (%)	11.23	7.32
Sol Prot (%CP)	27.52	27.52
ME (mCal/lb)	1.18	0.77
NEl (mCal/lb)	0.76	0.50
Nem (mCal/lb)	0.76	0.50
NEg (mCal/lb)	0.49	0.32
ADF (%)	20.94	13.66
NDF (%)	32.19	21.00
For NDF (%NDF)	66.83	43.61
Forage NDF (%)	21.51	14.04
peNDF (%)	21.64	14.12
Lignin (%)	3.85	2.52
NFC (%)	38.01	24.80
Sil Acids (%)	1.97	1.29
Sugar (%)	3.86	2.52
Starch (%)	22.87	14.92
Sol Fiber (%)	9.30	6.07
EE Total (%)	4.17	2.72
EE 1 (%)	3.56	2.32
EE 2 (%)	0.03	0.02
EE 3 (%)	0.58	0.38
LCFA Total (%)	3.42	2.23
Ash (%)	8.45	5.51
Cost ($/d)	1.98	1.98
Cost ($T)	90.50	59.06

Minerals and Vitamins		
Nutrient	DM	AF
Dry Matter (%)	100.00	65.25
Calcium (%)	1.07	0.70
Phosphorus (%)	0.47	0.31
Magnesium (%)	0.32	0.21
Potassium (%)	1.54	1.00
Sulfur (%)	0.33	0.22
Sodium (%)	0.33	0.21
Chlorine (%)	0.24	0.16
Iron (ppm)	185.06	120.76
Zinc (ppm)	56.36	36.78
Copper (ppm)	14.49	9.46
Manganese (ppm)	44.60	29.10
Selenium (ppm)	0.23	0.15
Cobalt (ppm)	0.25	0.16
Iodine (ppm)	0.40	0.26
Vitamin A (KIU/lb)	1.31	0.86
Vitamin D (KIU/lb)	0.33	0.22
Vitamin E (IU/lb)	10.57	6.90
DCAD1 (meq/100g)	25.96	16.94
DCAD2 (meq/100g)	32.44	21.17
Cost ($/d)	1.98	1.98
Cost ($T)	90.50	59.06

Low Fat High Hemicellulose (LFHH) Diet:

CPM-Dairy

CNCPS Evaluation

Farm: UNL Dairy Research Unit
Ration: 1603DA High HemiCellulose Low Fat
Ration By: Kononoff & Judy & Drehmel
Organization: University of Nebraska-Lincoln

BW: 920 lb
BCS: 3.00
Growth: 0.00 lb/d
Lact#: 3

DIM: 120
Milk: 65.00 lb
Fat: 5.50 %
TP: 3.60 %

						Ingredient	DM (lb/d)
Cost ($)	1.38	IOF ($)	-1.38				
DMI (lb/d)	43.8	Model	41.0	% Model	106.7	Corn silage	19.300
ME Bal (mCal)	-0.1	CP (%)	18.3	NDF (%)	36.2	Alfalfa hay	2.500
MP Bal (g)	69.2	RUP (% CP)	43.7	ForageNDF (% NDF)	53.9	TimHay11Cp61Ndf6LNdf	0.000
NP / MP (%)	62.4	LCFA (%)	3.5	ForageNDF (% DM)	19.5	High Fat DDGS	8.800
BactMP (% MP)	46.2	EE (%)	4.4	peNDF (%)	21.3	SoybeanML47.5Solv	2.600
Rumen N Balance				Lignin (%)	2.8	Ground Corn	2.600
Pept (g)	43	Pept & NH3 (g)	89	NFC (%)	35.9	SoybeanHullsGrnd	5.100
% rqd	129	% rqd	130	Sil Acids (%)	3.5	Soy Pass	0.800
Amino Acid Balance				Sugar (%)	3.0	FatTallowPorcine	0.000
Met (g)	2.7	Lys (g)	13.6	Starch (%)	22.1	CalciumCarbonate	0.700
Met (% rqd)	106	Lys (% rqd)	110	Sol Fiber (%)	7.3	BloodMeal	0.600
Met (% mp)	1.87	Lys (% mp)	6.28	Lys:Met	3.37:1	SodiumBicarbonate	0.260
Possible production due to ME and MP						Megalac	0.300

	Milk(lb)	Fat (%)	TP (%)	Milk(lb)	Fat (%)	TP (%)	
Trg:	65.0	5.50	3.60	65.0	5.50	3.60	SaltNaCl 0.090
		Yield Constant			Composition Constant		MagOx 0.080
ME:	65.0	n/a	n/a	64.8	5.50	n/a	Trace Premix 0.020
MP:	65.0	n/a	3.75	67.8	5.50	3.60	Vitamin Premix 0.022
							FatTallowBeef 0.000
Adjustments based on Rulquin AA Ratios:							BrmdHy10Cp70Ndf9LNdf 0.000
	65.0	n/a	-0.16	-2.8	5.50	3.60	Total 43.772

n/a - Equations not available

Ration DM (%)	53.78	Forage (% DM)	49.80

Diet Summary:

Ingredient	Cost $ / T	DM %	AF lb/d	DM lb/d	% AF	% DM
Corn silage	33.00	35.50	54.37	19.30	66.79	44.09
Alfalfa hay	110.00	89.90	2.78	2.50	3.42	5.71
TimHay11Cp61Ndf6LNdf	0.00	89.00	0.00	0.00	0.00	0.00
High Fat DDGS	0.00	90.19	9.76	8.80	11.99	20.10
SoybeanML47.5Solv	0.00	90.00	2.89	2.60	3.55	5.94
Ground Corn	182.00	88.00	2.95	2.60	3.63	5.94
SoybeanHullsGrnd	0.00	91.00	5.60	5.10	6.89	11.65
Soy Pass	0.00	90.14	0.89	0.80	1.09	1.83
FatTallowPorcine	0.00	99.00	0.00	0.00	0.00	0.00
CalciumCarbonate	0.00	99.50	0.70	0.70	0.86	1.60
BloodMeal	0.00	90.00	0.67	0.60	0.82	1.37
SodiumBicarbonate	0.00	99.50	0.26	0.26	0.32	0.59
Megalac	0.00	97.00	0.31	0.30	0.38	0.69
SaltNaCl	185.00	99.50	0.09	0.09	0.11	0.21
MagOx	330.00	99.50	0.08	0.08	0.10	0.18
Trace Premix	848.00	95.97	0.02	0.02	0.03	0.05
Vitamin Premix	2316.00	95.75	0.02	0.02	0.03	0.05
FatTallowBeef	0.00	99.00	0.00	0.00	0.00	0.00
BrmdHy10Cp70Ndf9LNdf	0.00	89.93	0.00	0.00	0.00	0.00
Total			81.40	43.77		

Macro Nutrients		
Nutrient	DM	AF
Dry Matter (%)	100.00	53.78
Forage (%)	49.80	31.84
Crude Prot (%)	18.25	9.82
RUP (%CP)	43.71	43.71
RDP (%CP)	56.29	56.29
RDP (%)	10.27	5.53
Sol Prot (%CP)	27.90	27.90
ME (mCal/lb)	1.18	0.64
NEl (mCal/lb)	0.76	0.41
Nem (mCal/lb)	0.76	0.41
NEg (mCal/lb)	0.49	0.26
ADF (%)	22.05	11.86
NDF (%)	36.17	19.45
For NDF (%NDF)	53.89	28.98
Forage NDF (%)	19.49	10.48
peNDF (%)	21.31	11.46
Lignin (%)	2.76	1.48
NFC (%)	35.90	19.31
Sil Acids (%)	3.53	1.90
Sugar (%)	3.03	1.63
Starch (%)	22.07	11.87
Sol Fiber (%)	7.28	3.91
EE Total (%)	4.41	2.37
EE 1 (%)	3.81	2.05
EE 2 (%)	0.03	0.01
EE 3 (%)	0.58	0.31
LCFA Total (%)	3.46	1.86
Ash (%)	7.92	4.26
Cost ($/d)	1.38	1.38
Cost ($T)	62.87	33.81

Minerals and Vitamins		
Nutrient	DM	AF
Dry Matter (%)	100.00	53.78
Calcium (%)	0.95	0.51
Phosphorus (%)	0.44	0.24
Magnesium (%)	0.31	0.17
Potassium (%)	1.27	0.68
Sulfur (%)	0.29	0.16
Sodium (%)	0.32	0.17
Chlorine (%)	0.24	0.13
Iron (ppm)	202.11	108.69
Zinc (ppm)	59.58	32.04
Copper (ppm)	16.01	8.61
Manganese (ppm)	43.17	23.22
Selenium (ppm)	0.23	0.12
Cobalt (ppm)	0.26	0.14
Iodine (ppm)	0.40	0.21
Vitamin A (KIU/lb)	1.31	0.71
Vitamin D (KIU/lb)	0.33	0.18
Vitamin E (IU/lb)	10.57	5.69
DCAD1 (meq/100g)	21.46	11.54
DCAD2 (meq/100g)	26.74	14.38
Cost ($/d)	1.38	1.38
Cost ($T)	62.87	33.81

High Fat Low Hemicellulose (HFLH) Diet:

CPM-Dairy

CNCPS Evaluation

Farm: UNL Dairy Research Unit
Ration: 1603DA Low HemiCellulose High Fat
Ration By: Kononoff & Judy & Drehmel
Organization: University of Nebraska-Lincoln

BW: 920 lb
BCS: 3.00
Growth: 0.00 lb/d
Lact#: 3

DIM: 120
Milk: 65.00 lb
Fat: 5.50 %
TP: 3.60 %

Cost ($)	1.91	IOF ($)	-1.91			Ingredient	DM (lb/d)
DMI (lb/d)	43.8	Model	41.0	% Model	106.7	Corn silage	10.800
ME Bal (mCal)	1.0	CP (%)	19.7	NDF (%)	32.0	Alfalfa hay	10.900
MP Bal (g)	162.4	RUP (% CP)	43.5	ForageNDF (% NDF)	67.1	TimHay11Cp61Ndf6LNdf	0.000
NP / MP (%)	59.1	LCFA (%)	4.8	ForageNDF (% DM)	21.5	High Fat DDGS	8.800
BactMP (% MP)	44.1	EE (%)	5.7	peNDF (%)	21.6	SoybeanML47.5Solv	2.600
Rumen N Balance				Lignin (%)	3.9	Ground Corn	6.600
Pept (g)	67	Pept & NH3 (g)	126	NFC (%)	36.8	SoybeanHullsGrnd	0.500
% rqd	143	% rqd	143	Sil Acids (%)	2.0	Soy Pass	0.800
Amino Acid Balance				Sugar (%)	3.8	FatTallowPorcine	0.700
Met (g)	3.1	Lys (g)	17.9	Starch (%)	21.7	CalciumCarbonate	0.700
Met (% rqd)	107	Lys (% rqd)	113	Sol Fiber (%)	9.3	BloodMeal	0.600
Met (% mp)	1.82	Lys (% mp)	6.22	Lys:Met	3.43:1	SodiumBicarbonate	0.260
Possible production due to ME and MP						Megalac	0.300

	Milk(lb)	Fat (%)	TP (%)	Milk(lb)	Fat (%)	TP (%)	
Trg:	65.0	5.50	3.60	65.0	5.50	3.60	SaltNaCl 0.090
		Yield Constant			Composition Constant		MagOx 0.080
ME:	65.0	n/a	n/a	66.7	5.50	n/a	Trace Premix 0.020
MP:	65.0	n/a	3.96	71.5	5.50	3.60	Vitamin Premix 0.022
Adjustments based on Rulquin AA Ratios:							FatTallowBeef 0.000
	65.0	n/a	-0.19	-3.4	5.50	3.60	BrmdHy10Cp70Ndf9LNdf 0.000
							Total 43.772

n/a - Equations not available

Ration DM (%)	65.34	Forage (% DM)	49.58

Diet Summary:

Ingredient	Cost $ / T	DM %	AF lb/d	DM lb/d	% AF	% DM
Corn silage	33.00	35.50	30.42	10.80	45.41	24.67
Alfalfa hay	110.00	89.90	12.12	10.90	18.10	24.90
TimHay11Cp61Ndf6LNdf	0.00	89.00	0.00	0.00	0.00	0.00
High Fat DDGS	0.00	90.19	9.76	8.80	14.56	20.10
SoybeanML47.5Solv	0.00	90.00	2.89	2.60	4.31	5.94
Ground Corn	182.00	88.00	7.50	6.60	11.20	15.08
SoybeanHullsGrnd	0.00	91.00	0.55	0.50	0.82	1.14
Soy Pass	0.00	90.14	0.89	0.80	1.32	1.83
FatTallowPorcine	0.00	99.00	0.71	0.70	1.06	1.60
CalciumCarbonate	0.00	99.50	0.70	0.70	1.05	1.60
BloodMeal	0.00	90.00	0.67	0.60	1.00	1.37
SodiumBicarbonate	0.00	99.50	0.26	0.26	0.39	0.59
Megalac	0.00	97.00	0.31	0.30	0.46	0.69
SaltNaCl	185.00	99.50	0.09	0.09	0.14	0.21
MagOx	330.00	99.50	0.08	0.08	0.12	0.18
Trace Premix	848.00	95.97	0.02	0.02	0.03	0.05
Vitamin Premix	2316.00	95.75	0.02	0.02	0.03	0.05
FatTallowBeef	0.00	99.00	0.00	0.00	0.00	0.00
BrmdHy10Cp70Ndf9LNdf	0.00	89.93	0.00	0.00	0.00	0.00
Total			66.99	43.77		

Macro Nutrients		
Nutrient	DM	AF
Dry Matter (%)	100.00	65.34
Forage (%)	49.58	28.80
Crude Prot (%)	19.73	12.89
RUP (%CP)	43.52	43.52
RDP (%CP)	56.48	56.48
RDP (%)	11.14	7.28
Sol Prot (%CP)	27.58	27.58
ME (mCal/lb)	1.21	0.79
NEl (mCal/lb)	0.78	0.51
Nem (mCal/lb)	0.78	0.51
NEg (mCal/lb)	0.52	0.34
ADF (%)	20.88	13.64
NDF (%)	32.05	20.94
For NDF (%NDF)	67.13	43.86
Forage NDF (%)	21.51	14.06
peNDF (%)	21.60	14.11
Lignin (%)	3.85	2.52
NFC (%)	36.78	24.03
Sil Acids (%)	1.97	1.29
Sugar (%)	3.84	2.51
Starch (%)	21.68	14.16
Sol Fiber (%)	9.29	6.07
EE Total (%)	5.70	3.72
EE 1 (%)	3.49	2.28
EE 2 (%)	1.63	1.06
EE 3 (%)	0.58	0.38
LCFA Total (%)	4.76	3.11
Ash (%)	8.42	5.50
Cost ($/d)	1.91	1.91
Cost ($T)	87.20	56.97

Minerals and Vitamins		
Nutrient	DM	AF
Dry Matter (%)	100.00	65.34
Calcium (%)	1.07	0.70
Phosphorus (%)	0.47	0.30
Magnesium (%)	0.32	0.21
Potassium (%)	1.53	1.00
Sulfur (%)	0.33	0.22
Sodium (%)	0.33	0.21
Chlorine (%)	0.24	0.16
Iron (ppm)	185.06	120.92
Zinc (ppm)	56.36	36.83
Copper (ppm)	14.49	9.47
Manganese (ppm)	44.60	29.14
Selenium (ppm)	0.23	0.15
Cobalt (ppm)	0.25	0.16
Iodine (ppm)	0.40	0.26
Vitamin A (KIU/lb)	1.31	0.86
Vitamin D (KIU/lb)	0.33	0.22
Vitamin E (IU/lb)	10.57	6.91
DCAD1 (meq/100g)	25.95	16.95
DCAD2 (meq/100g)	32.48	21.22
Cost ($/d)	1.91	1.91
Cost ($T)	87.20	56.97

High Fat High Hemicellulose (HFHH) Diet:

CPM-Dairy

CNCPS Evaluation

Farm: UNL Dairy Research Unit	BW: 920 lb	DIM: 120
Ration: 1603DA High HemiCellulose High Fat	BCS: 3.00	Milk: 65.00 lb
Ration By: Kononoff & Judy & Drehmel	Growth: 0.00 lb/d	Fat: 5.50 %
Organization: University of Nebraska-Lincoln	Lact#: 3	TP: 3.60 %

Cost ($)	1.32	IOF ($)	-1.32			Ingredient	DM (lb/d)
DMI (lb/d)	43.8	Model	41.0	% Model	106.7	Corn silage	19.300
ME Bal (mCal)	1.1	CP (%)	18.1	NDF (%)	36.0	Alfalfa hay	2.500
MP Bal (g)	36.7	RUP (% CP)	43.7	ForageNDF (% NDF)	54.1	TimHay11Cp61Ndf6LNdf	0.000
NP / MP (%)	63.6	LCFA (%)	4.6	ForageNDF (% DM)	19.5	High Fat DDGS	8.800
BactMP (% MP)	45.8	EE (%)	5.7	peNDF (%)	21.3	SoybeanML47.5Solv	2.600
Rumen N Balance				Lignin (%)	2.8	Ground Corn	2.020
Pept (g)	46	Pept & NH3 (g)	94	NFC (%)	34.9	SoybeanHullsGrnd	5.100
% rqd	132	% rqd	132	Sil Acids (%)	3.5	Soy Pass	0.800
Amino Acid Balance				Sugar (%)	3.0	FatTallowPorcine	0.580
Met (g)	1.9	Lys (g)	11.5	Starch (%)	21.1	CalciumCarbonate	0.700
Met (% rqd)	105	Lys (% rqd)	108	Sol Fiber (%)	7.3	BloodMeal	0.600
Met (% mp)	1.86	Lys (% mp)	6.28	Lys:Met	3.38:1	SodiumBicarbonate	0.260
Possible production due to ME and MP						Megalac	0.300

	Milk(lb)	Fat (%)	TP (%)	Milk(lb)	Fat (%)	TP (%)	
Trg:	65.0	5.50	3.60	65.0	5.50	3.60	SaltNaCl 0.090
		Yield Constant			Composition Constant		MagOx 0.080
							Trace Premix 0.020
ME:	65.0	n/a	n/a	66.8	5.50	n/a	Vitamin Premix 0.022
MP:	65.0	n/a	3.68	66.5	5.50	3.60	FatTallowBeef 0.000
Adjustments based on Rulquin AA Ratios:							BrmdHy10Cp70Ndf9LNdf 0.000
	65.0	n/a	-0.16	-2.8	5.50	3.60	Total 43.772

n/a - Equations not available

Ration DM (%)	53.83	Forage (% DM)	49.80

Diet Summary:

Ingredient	Cost $ / T	DM %	AF lb/d	DM lb/d	% AF	% DM
Corn silage	33.00	35.50	54.37	19.30	66.85	44.09
Alfalfa hay	110.00	89.90	2.78	2.50	3.42	5.71
TimHay11Cp61Ndf6LNdf	0.00	89.00	0.00	0.00	0.00	0.00
High Fat DDGS	0.00	90.19	9.76	8.80	12.00	20.10
SoybeanML47.5Solv	0.00	90.00	2.89	2.60	3.55	5.94
Ground Corn	182.00	88.00	2.30	2.02	2.82	4.61
SoybeanHullsGrnd	0.00	91.00	5.60	5.10	6.89	11.65
Soy Pass	0.00	90.14	0.89	0.80	1.09	1.83
FatTallowPorcine	0.00	99.00	0.59	0.58	0.72	1.33
CalciumCarbonate	0.00	99.50	0.70	0.70	0.87	1.60
BloodMeal	0.00	90.00	0.67	0.60	0.82	1.37
SodiumBicarbonate	0.00	99.50	0.26	0.26	0.32	0.59
Megalac	0.00	97.00	0.31	0.30	0.38	0.69
SaltNaCl	185.00	99.50	0.09	0.09	0.11	0.21
MagOx	330.00	99.50	0.08	0.08	0.10	0.18
Trace Premix	848.00	95.97	0.02	0.02	0.03	0.05
Vitamin Premix	2316.00	95.75	0.02	0.02	0.03	0.05
FatTallowBeef	0.00	99.00	0.00	0.00	0.00	0.00
BrmdHy10Cp70Ndf9LNdf	0.00	89.93	0.00	0.00	0.00	0.00
Total			81.32	43.77		

Macro Nutrients		
Nutrient	DM	AF
Dry Matter (%)	100.00	53.83
Forage (%)	49.80	31.87
Crude Prot (%)	18.13	9.76
RUP (%CP)	43.71	43.71
RDP (%CP)	56.29	56.29
RDP (%)	10.21	5.49
Sol Prot (%CP)	27.96	27.96
ME (mCal/lb)	1.21	0.65
NEl (mCal/lb)	0.78	0.42
Nem (mCal/lb)	0.78	0.42
NEg (mCal/lb)	0.51	0.28
ADF (%)	22.00	11.84
NDF (%)	36.05	19.40
For NDF (%NDF)	54.06	29.10
Forage NDF (%)	19.49	10.49
peNDF (%)	21.28	11.46
Lignin (%)	2.76	1.48
NFC (%)	34.88	18.78
Sil Acids (%)	3.53	1.90
Sugar (%)	3.01	1.62
Starch (%)	21.08	11.35
Sol Fiber (%)	7.27	3.91
EE Total (%)	5.68	3.06
EE 1 (%)	3.75	2.02
EE 2 (%)	1.35	0.73
EE 3 (%)	0.58	0.31
LCFA Total (%)	4.57	2.46
Ash (%)	7.89	4.25
Cost ($/d)	1.32	1.32
Cost ($T)	60.13	32.36

Minerals and Vitamins		
Nutrient	DM	AF
Dry Matter (%)	100.00	53.83
Calcium (%)	0.95	0.51
Phosphorus (%)	0.44	0.24
Magnesium (%)	0.31	0.17
Potassium (%)	1.26	0.68
Sulfur (%)	0.29	0.16
Sodium (%)	0.32	0.17
Chlorine (%)	0.24	0.13
Iron (ppm)	202.11	108.79
Zinc (ppm)	59.58	32.07
Copper (ppm)	16.01	8.62
Manganese (ppm)	43.17	23.24
Selenium (ppm)	0.23	0.12
Cobalt (ppm)	0.26	0.14
Iodine (ppm)	0.40	0.21
Vitamin A (KIU/lb)	1.31	0.71
Vitamin D (KIU/lb)	0.33	0.18
Vitamin E (IU/lb)	10.57	5.69
DCAD1 (meq/100g)	21.45	11.55
DCAD2 (meq/100g)	26.78	14.41
Cost ($/d)	1.32	1.32
Cost ($T)	60.13	32.36

APPENDIX C: 2017 ADSA ANNUAL MEETING POSTER

Abstract M296: Increasing the diet concentrations of fat and hemicellulose on energy utilization and methane production in lactating Jersey cattle

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INTRODUCTION

- Methane (CH_4) is a potent greenhouse gas (GHG) that can have negative effects on the environment.
- Dairy cattle consuming grain and forage diets produce approximately 500 to 600 l/d of CH_4 (Beauchemin et al., 2008).
- Dairy cattle produce 24.8% of enteric CH_4 emissions which is 0.54% of the total US GHG emissions (Chase, 2014).
- One method to reduce CH_4 production may be through manipulation of feed ingredients included in the diet. The addition of fat is known to supply energy but also reduce CH_4 production (Beauchemin et al., 2008).
- Fibrous byproducts when consumed by cattle are believed to result in less CH_4 per unit of digested DM than forages (Johnson and Johnson, 1995). Knapp et al. (2014) suggested it is because these feeds are high in hemicellulose and the digestion of hemicellulose produces 37% less CH_4 than that of digested cellulose (Moe and Tyrrell, 1979).
- Benchaar et al. (2013) and Foth et al. (2015) observed that dairy cattle produce less CH_4 when consuming diets containing distillers grains and solubles (DDGS).

OBJECTIVE

- Determine the effects of feeding fat and hemicellulose on methane production and energy utilization in lactating Jersey cows consuming diets containing DDGS

HYPOTHESES

- Formulated diets that contain more fat and hemicellulose in diets containing DDGS will result in reduction on methane production and increase the supply of energy

MATERIALS AND METHODS

- 8 multiparous lactating Jersey cows (98 ± 30.8 DIM and BW of 436.3 ± 56.7 kg) housed in tie stall barn
- Twice replicated 4 × 4 Latin square
- Factorial arrangement of 4 dietary treatments differing in fat and hemicellulose concentrations, formulated with CPM Model
- 35-d periods, last 7 d of each period for data collection
  - Daily feed intake (fed once a day, restricted (95%) intake for 4 d)
  - Daily milk production (2× milking for 5 d)
  - Milk composition
  - Urine and feces collection (4 days)
  - Methane production (2 d of each period) collected via the headbox type indirect calorimetry method
- Data analyzed using MIXED procedure of SAS
  - Fixed effects: Period, Treatment and Square
  - Random effect: Cow

RESULTS

Table 1. Composition and analysis of treatments differing in fat and hemicellulose concentration

	Treatment[1]			
Item	LFLH	LFHH	HFLH	HFHH
	% of DM			
Corn Silage	24.7	44.1	24.7	44.1
Alfalfa Hay	24.9	5.71	24.9	5.71
Ground Corn	16.7	5.94	15.1	4.61
Ground Soybean hulls	1.14	11.7	1.14	11.7
Tallow	--	--	1.60	1.33
Grain Mix[2]	32.5	32.5	32.5	32.5
Trace mineral premix/Vitamin premix[3]	0.1	0.1	0.1	0.1
Chemical Composition	% DM			
Hemicellulose[4]	11.5	13.0	11.1	12.4
CP	18.3	18.0	18.5	17.7
Ether Extract	4.11	4.57	4.98	5.63
ADF	22.9	22.4	21.9	22.2
NDF	34.4	35.4	32.9	34.6
Ash	7.37	7.12	7.74	6.88
Starch	20.2	21.4	19.3	20.7

[1]Treatments: LFLH = Low Fat Low Hemicellulose; LFHH = Low Fat High Hemicellulose; HFLH = High Fat Low Hemicellulose; HFHH = High Fat High Hemicellulose.
[2]Grain Mix (% of DM): 20.10% high fat DDGS, 5.94% soybean meal, 1.83% soypass, 1.37% bloodmeal, 1.60% Ca Carbonate, 0.59% Na Bicarbonate, 0.69% Ca-salts LCFA, 0.18% Mg Oxide, 0.21% Salt.
[3]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, approximately 148,500 IU/d vitamin A, 38,500 IU/d vitamin D and 902 IU/d vitamin E in total rations.
[4]Hemicellulose = NDF – ADF.

Table 2. DMI, milk production and components, gas production of treatments differing in fat and hemicellulose concentration

	Treatment[1]					P-value[3]		
Item	LFLH	LFHH	HFLH	HFHH	SEM[2]	F	H	I
DMI, kg/d	15.7	17.0	16.0	16.1	1.18	0.63	0.25	0.27
Milk Yield, kg/d	23.0	23.4	23.1	22.3	1.72	0.51	0.78	0.40
ECM[4], kg/d	30.4	31.5	29.5	28.9	2.41	0.15	0.80	0.46
Milk Fat, %	5.78	6.04	5.48	5.64	0.35	0.11	0.31	0.81
Milk Fat yield, kg/d	1.32	1.40	1.26	1.26	0.13	0.11	0.54	0.50
Milk Protein, %	3.46	3.47	3.39	3.38	0.13	0.15	0.95	0.83
Milk Protein yield, kg/d	0.79	0.80	0.77	0.75	0.06	0.30	0.82	0.63
Lactose, %	4.81	4.80	4.80	4.82	0.04	0.90	0.91	0.64
MUN, mg/dl	22.1	20.8	21.6	20.0	0.97	0.22	0.03	0.77
O_2, L/d	4536.3	4467.1	4395.7	4260.5	232.8	0.19	0.43	0.77
CO_2, L/d	4683.9	4671.6	4478.9	4373.8	256.6	0.11	0.69	0.73
CH_4, L/d	394.1	392.6	361.1	363.4	27.2	0.17	0.99	0.92
Milk/CH_4, kg/L	0.08	0.08	0.08	0.08	0.00	0.10	0.92	0.51
CH_4/DMI, L/kg	25.8	23.8	22.7	22.6	1.61	0.10	0.37	0.41

[1]Treatments: LFLH = Low Fat Low Hemicellulose; LFHH = Low Fat High Hemicellulose; HFLH = High Fat Low Hemicellulose; HFHH = High Fat High Hemicellulose.
[2]Lowest standard error of treatment means is shown.
[3]F = Fat, H = Hemicellulose, I = Interaction.
[4]Energy correct milk (ECM)= 0.327 × milk yield (kg) + 12.95 × fat (kg) + 7.20 × protein (kg) adjusted for 3.5% fat and 3.2% total protein (DHI Glossary, 2014).

Table 3. Energy partitioning of treatments differing in fat and hemicellulose concentration

	Treatment[2]					P- value[4]		
Item[1]	LFLH	LFHH	HFLH	HFHH	SEM[3]	F	H	I
	% of GE							
Feces	33.3	31.0	30.3	29.7	1.35	0.05	0.17	0.38
Urine	5.42	4.66	4.79	4.55	0.22	0.05	0.01	0.14
Methane	5.59	5.14	4.61	5.84	0.34	0.02	0.67	0.14
Milk	33.5	31.4	31.1	31.4	1.59	0.25	0.38	0.25
DE	66.7	69.0	69.7	70.3	1.35	0.05	0.17	0.38
ME	55.6	59.4	60.6	61.4	1.80	0.02	0.10	0.22
NE_L	22.0	28.7	31.0	31.1	3.21	0.02	0.12	0.11
	Mcal/kg of DM							
GE	5.36	6.14	6.63	6.01	0.61	0.37	0.90	0.29
DE	3.57	4.28	4.60	4.24	0.42	0.27	0.71	0.26
ME	2.97	3.73	3.98	3.75	0.37	0.21	0.55	0.23
NE_L	1.17	1.82	2.04	1.91	0.22	0.04	0.25	0.08

[1]GE = gross energy; DE = digestible energy, ME = metabolizable energy; NE_L = net energy lactation.
[2]Treatments: LFLH = Low Fat Low Hemicellulose; LFHH = Low Fat High Hemicellulose; HFLH = High Fat Low Hemicellulose; HFHH = High Fat High Hemicellulose.
[3]Lowest standard error of treatment means is shown.
[4]F = Fat, H = Hemicellulose, I = Interaction.

Table 4. Apparent digestibilities of treatments differing in fat and hemicellulose concentration

	Treatments[1]					P-value[3]		
Item	LFLH	LFHH	HFLH	HFHH	SEM[2]	F	H	I
	%							
DM	67.2	69.4	68.7	70.4	1.30	0.18	0.05	0.81
OM	69.2	71.2	70.8	72.6	1.23	0.10	0.05	0.89
Ash	45.1	48.7	46.1	47.1	3.24	0.92	0.48	0.67
CP	74.0	73.5	77.7	76.7	1.62	0.02	0.55	0.84
Starch	97.0	97.8	97.4	97.2	0.43	0.78	0.60	0.32
NDF	44.4	52.2	41.5	49.9	2.35	0.32	0.01	0.90
Total intake of digestible NDF, kg/d	6.58	8.77	6.66	8.07	0.89	0.62	0.02	0.54
CH_4 per unit of digested NDF, L/kg	65.2	47.9	61.3	46.0	8.86	0.73	0.10	0.90
Total intake of digestible DM, kg/d	10.6	11.9	11.1	11.4	0.99	0.97	0.12	0.31
CH_4 per unit of digested DM, L/kg	38.8	34.4	33.1	31.7	3.02	0.08	0.21	0.46

[1]Treatments: LFLH = Low Fat Low Hemicellulose; LFHH = Low Fat High Hemicellulose; HFLH = High Fat Low Hemicellulose; HFHH = High Fat High Hemicellulose.
[2]Lowest standard error of treatment means is shown.
[3]F = Fat, H = Hemicellulose, I = Interaction.

CONCLUSIONS

- Results confirm that CH_4 production per unit of DMI may be decreased with the inclusion of fat while energy intake of lactating dairy cows is improved by increasing hemicellulose in low fat diets
- Increasing hemicellulose in low fat diets tended to increase net energy intake but, this was not observed in high fat diets
- Increasing hemicellulose tended to decreased CH_4 per unit of digestible NDF

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