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USING INDIRECT CALORIMETRY TO INVESTIGATE FEEDING VALUE OF BYPRODUCTS FOR LACTATING DAIRY CATTLE: CANOLA MEAL AND DRIED DISTILLERS GRAINS AND SOLUBLES

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by

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USING INDIRECT CALORIMETRY TO INVESTIGATE FEEDING VALUE OF BYPRODUCTS FOR LACTATING DAIRY CATTLE: CANOLA MEAL AND DRIED DISTILLERS GRAINS AND SOLUBLES

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Byproducts have played a major role in dairy nutrition by providing a cheaper protein and fiber source, while also utilizing a product that would otherwise be waste from the human perspective. Use of byproducts in the dairy industry should allow for continued and overall increases in production and efficiency of the dairy industry. Two of the more popular byproducts in the dairy industry today are dried distillers grains and solubles and canola meal.

In the first experiment, 12 multiparous lactating Jersey cows were used evaluate the feeding value of dried distillers grains and solubles (DDGS) or canola meal. A replicated 4 × 4 Latin square design was used to compare four different dietary treatments. Treatments were composed of a control (CON) containing no byproducts, a treatment diet containing 10% (DM basis) reduced fat DDGS (pDDGS), a 10% DDGS treatment with an alternative distillers grains source (aDDGS), and a 10% canola meal (CanM) treatment. Results suggest that milk production can be maintained when feeding these byproducts. However, energy utilization differences are observed, specifically in gross energy, digestible energy, metabolizable energy and energy balance (Mcal/kg of DM). The alternative source of DDGS contained the greatest amount of gross energy,

digestible energy, and metabolizable energy. The control and the alternative source of DDGS contained the greatest energy balance. Dry matter, organic matter, crude protein, and neutral detergent fiber digestibility differences were also observed between treatments, specifically the control and the DDGS treatments had the greatest digestibility.

In the second experiment, a comparison of sample preparation methods of urine to be analyzed for energy content by bomb calorimetry was conducted. The two methods to be tested included a lyophilization and oven drying method. Results of this study suggest that there were significant differences in gross energy content and total urine energy depending on which sample preparation method was used. The lyophilization method resulted in a greater gross energy and total urine energy compared to oven drying method, creating a negative method difference.

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INTRODUCTION

Ruminant production systems, including milk production, have become more efficient over time. When comparing dairying practices and resources needed in 1944 to 2007, Capper et al. (2009) reported that over this time dairy producers used 21% less animals, 23% less feedstuffs, 35% less water, and 10% less land to produce the same one billion kg of milk. Despite this increase in efficiency, increased pressure for land use and high commodity prices over the past decade have increased feed costs for dairy farmers, challenging them to consider less costly sources of protein and fiber (Bradford and Mullins, 2012). In doing so, feed byproducts have played a major role in dairy nutrition by providing a cheaper protein and fiber source, while also utilizing a product that would otherwise be waste from the human perspective. Therefore, extensive use of byproducts in the dairy industry should allow for continued and overall increases in production and efficiency (VandeHaar and St-Pierre, 2006). Two of the more popular byproducts in the dairy industry today are dried distillers grains and solubles and canola meal.

Dry distillers grains and solubles **(DDGS)** are byproducts of the ethanol industry and are often included in dairy rations (Foth et al., 2015). In 2016, the United States produced 23.2 million tons of DDGS (USDA, 2017) with the top ethanol producing states being Iowa, Nebraska, and Illinois (NEO, 2017). Corn DDGS contain approximately 39% NDF and 30% CP (DM basis) of which 51% of the protein is rumen undegradable protein (NRC, 2001). When DDGS are included in dairy rations, DMI and milk production have often been shown to increase (Benchaar et al., 2013, Castillo-Lopez et

al., 2017). Nitrogen utilization with cattle consuming DDGS has been varied between researchers. Feeding DDGS to beef cattle has resulted in increased nitrogen intakes and urinary nitrogen, and decreased fecal nitrogen excretion (Walter et al., 2012). In dairy cattle, Bechaar et al. (2013) reported increased nitrogen intake, fecal nitrogen, urinary nitrogen, and milk nitrogen, resulting in increased nitrogen balance when feeding increasing levels of DDGS. In this study the increased N intake was attributed to increased DMI and the greater concentration of CP when DDGS replaced flaked corn and soybean meal. Finally, Foth et al. (2015) reported similar nitrogen intake, fecal nitrogen, urinary nitrogen, and milk nitrogen with the addition of reduced-fat DDGS to the diet of lactating dairy cattle. Sampling error may play a role in estimating nitrogen partitioning from loss of feed through volatile loss of nitrogen from urine or drying fecal samples (Walter et al., 2012). Advancement in the ethanol industry has resulted in advancements enabling the use of raw starch hydrolyzing enzymes to increase the availability of starch in the ethanol production process (Wang, 2007). This technology allows DDGS production to proceed at lower temperatures (48°C), decreasing the use of heat, which may damage a considerable portion of the protein, making it unavailable for the animal (Kleinschmit et al., 2007). Recently, digestion of fiber differences have been observed in vitro between DDGS products that were produced at lower temperatures, and DDGS that were heated at higher temperatures (Dufour et al., 2017). Information is not currently available comparing the two products in vivo, thus research is needed to determine if differences in fiber digestibility translate into improvements in the supply of energy and milk production.

Canola meal is primarily produced in Canada and the northern United States and is a byproduct of the oil crushing industry. In 2017, Canola was harvested on 850,000 hectares in the United States, with top states including North Dakota, Oklahoma and Montana (USDA, 2017). Canola meal is the registered name for rapeseed and is a considered a better quality product because it contains lower concentrations of glucosinolates per gram of oil-free dry matter of the seed (30 μmoles vs. 50-100 μmoles, respectively) and erucic acid (<2%) (Bell, 1993). Glucosinolates may be problematic because they interfere with iodine metabolism, affecting the function of the thyroid and overall animal performance (Mawson et al., 1994). Canola meal contains approximately 30% NDF and 38% CP (DM Basis) of which 36% of the protein is RUP and has been considered to be of similar value to soybean meal (NRC, 2001). Investigation of inclusion of canola meal in dairy rations has demonstrated increased DMI and milk yield when compared to other protein sources (Hutanen et al., 2011; Martineau et al., 2013). For dairy cattle consuming canola meal, effects on whole animal energy and nitrogen utilization use are lacking, but observations on nitrogen efficiency (milk nitrogen/nitrogen intake) have been reported. In a meta-analysis conducted by Martineau et al. (2013), canola meal fed at 17.2% of the diet was predicted to increase apparent nitrogen efficiency by 12 g of milk nitrogen/kg of nitrogen intake. Furthermore, Gidlund et al. (2015) observed that apparent nitrogen efficiency was lower for cows fed soybean meal compared with cows fed canola meal. The RUP digestibility has been reported to be lower in canola meal when compared with soybean meal and DDGS in situ, in vitro and

through the mobile bag technique (NRC, 2001; Paz et al., 2014). However, information is lacking on understanding the feeding value of canola meal and DDGS.

Measuring solely milk production is not sufficient to determine feeding value of byproducts, as energy can be lost in feces, heat, urine, gas, and stored in tissue (Coppock, 1985; Moe et al., 1971). Therefore, the use of indirect calorimetry to measure carbon dioxide and methane production, oxygen consumption, and urea production can provide a more accurate measure of heat production and energy utilization of the animal (Nienaber et al., 2009). Total fecal and urine collections are also helpful in determining whole animal energy utilization, and do not present the same challenges as markers such as kinetic assumptions, incomplete recovery, and migration (Owens and Hanson, 1992).

Previously, a number of studies have tested the use of DDGS and canola meal in dairy diets (Maxin et al., 2013; Acharya et al., 2015; Mutsvangwa et al., 2016), however to the author's knowledge no comparison studies have been conducted on energy utilization as studied by indirect calorimetry. Therefore, the objectives of this of this work were to 1) evaluate chemical composition and nutrient digestibility utilization in dairy cattle consuming diets containing DDGS and canola meal, with focus on protein and fiber and 2) evaluate the nature of energy supply and utilization in dairy cattle consuming diets containing DDGS and canola meal.

CHAPTER 1
LITERATURE REVIEW

Byproducts

Distillers grains. Since 1981, when the first fuel-ethanol plant used grain as a source of livestock feed, byproducts such as distillers grains have been readily expanding as a source of feed for livestock (DeJong, 2011). Although multiple sources of grains can be used to produce ethanol, it is estimated that 69% is produced from corn, with a large portion of the remaining amount coming from wheat (Coad and Bristow, 2011). The demand for corn ethanol has risen due to an increase in the corn produced on the same acreage, leading to an increase in corn yield (Coad and Bristow, 2011). There are two main types of milling processes in order to achieve ethanol production, wet milling and dry milling. Each of these milling processes produces different byproducts that may be fed to livestock. Distillers grain are produced through the dry milling process, which this review will focus on.

Dry milling process. Dry milling can be conducted using a variety of grains including corn, wheat, sorghum, and barley. The dry milling process is illustrated in Figure 1.1. The corn dry milling process begins by grinding the whole corn kernel through a hammer mill into a coarse flour, and hot water is added to create a slurry. Next, the pH is adjusted to approximately 6.0 and alpha amylase is added to begin breaking down the starch into dextrins. The slurry passes through a jet cooker at 100°C to help with the breakdown of starch molecules. After cooking, the now “mash” is cooled to 32°C and transferred to fermenters. Here, yeast is added to convert the sugars to ethanol

and carbon dioxide. The mash is fermented for 48-72 h and contains about 10-12 % ethanol. The ethanol is separated from the solids and water in the mash through a distillation column through heating. The solids and water remaining in the mash are now referred to as "whole stillage". Whole stillage contains the fiber, protein, and oil component of the grain along with any starch that was not fermented. The whole stillage can then be centrifuged resulting in "thin stillage" and solids. The thin stillage can be evaporated to create a thick syrup and is added back to the solids to create wet distillers grains and solubles (Bothast and Schindler, 2005).

Types of distillers grains. Wet distillers grains and solubles (WDGS) are typically 35 % DM and can be dried to 88 to 90 % DM to produce dried distillers grains and solubles (DDGS). WDGS can also be dried to 45-50 % DM to produce modified distillers grains and solubles (MDGS). Shelf life is typically about 1-2 weeks for WDGS and can provide some challenges with handling and storage. Drying WDGS to DDGS or MDGS can improve shelf life of the product and decrease issues with storage and transportation. Overall, improper grain storage, re-introduced stillage, moldy grain, air and faulty equipment can all play a role in the quality of the final distillers product (Bothast and Schindler, 2005). When comparing WDGS and DDGS on dairy cattle performance, Anderson et al. (2006) used 15 lactating dairy cattle to test the lactation performance of dairy cattle fed dried or wet distillers at 2 dietary concentrations (10 % or 20 % on a DM basis). Dry matter intake was similar between the distillers treatments. Milk yields were greater for cattle fed the distillers treatments vs the control treatment containing no distillers grains. Milk fat and milk protein were greater for cows fed WDGS than DDGS.

The researchers concluded that feeding distillers grains improved feeding efficiency by decreasing DMI and increasing milk yield, milk protein and milk fat.

Advances in Dry Milling. As mentioned previously, in a conventional dry milling operation, the ground corn is cooked at > 90 °C for 1 to 2 hours using liquefied enzyme to break down the starch molecules into dextrins. Recently, a new enzyme called raw starch hydrolyzing enzyme (RSH) was developed (Wang, 2007). This enzyme allows the starch to be converted to dextrins at lower temperatures (48°C) and therefore improves the energy used to produce the product (Wang, 2007). Also, cooking the product at lower temperatures can decrease the chance for heat damage, which may damage a considerable portion of the protein, making it unavailable for the animal (Kleinschmit et al., 2007). Recently, fiber digestion differences have been observed in vitro between DDGS products that were produced at ethanol plants utilizing the RSH enzyme at lower temperatures, and DDGS that were cooked at higher temperatures. Dufour et al. (2017) conducted an in vitro experiment evaluating DDGS produced at different ethanol plants. One of the ethanol plants involved in the study produced DDGS at lower temperatures. The results of this study indicated that DDGS produced at lower temperatures had an average total tract neutral detergent fiber digestibility (TTNDFD) of 66.4 % compared with DDGS produced at higher temperatures (60.0 % TTNDFD). This is important information for estimating in vivo fiber digestibility, however given that this is an in vitro assay this estimation does not account for selective retention of feed particles in the rumen (Huhtanen et al., 2007; Lopes et al., 2015). Another modification of dry milling has been to separate the corn kernel into the germ, bran, and endosperm before

fermentation (Kelzer et al., 2009). Cows fed separated germ have been reported to have an increased DMI and milk production (Kelzer et al., 2009).

Reduced fat DDGS. Several sources have determined the concentration of fat, representing corn oil, in DDGS to be between 10 to 12 % on a dry basis (Spiehs et al., 2002; Belyea et al., 2004; Liu, 2011). There has been concern that fat in DDGS will result in milk fat depression due to the high concentration of poly unsaturated fatty acids (Bauman and Griinari, 2003). Additionally, Abdelqader et al. (2009) reported that diets containing 30 % DDGS on a dry basis resulted in milk fat depression. Centrifugation and solvent extraction both represent methods to remove oil from DDGS (Berger and Singh, 2010; Mjoun et al., 2010). Reduced fat DDGS (RFDDGS) contain 50-60 % less crude fat than that of conventional DDGS depending on the technology of each ethanol plant (Ramirez-Ramirez et al., 2016) resulting in a product that is about 5.5 % fat (Castillo-Lopez et al., 2014). Although the loss of fat has the potential to decrease the energy in the product, Foth et al. (2015) reported a greater net gross energy content for RFDDGS (4.11 Mcal/kg of DM) compared to a control containing corn and soybean meal (3.96 Mcal/kg of DM). Furthermore, several studies have shown success in feeding RFDDGS without any negative effects on milk fat (Mjoun et al., 2010;, Foth et al., 2015; Ramirez-Ramirez et al., 2016).

Distillers in dairy diets. Corn DDGS contain approximately 30 % CP (DM basis) of which 51 % of the protein is rumen-undegradable protein (RUP) (NRC, 2001). The high RUP, along with ruminal microbial CP, and endogenous protein will all contribute to the amino acid requirements of the animal with absorption through the small intestine

(Dufour et al., 2017). In general, diets containing DDGS have been limited to 10 % of dietary dry matter because of the traditionally high fat content of 10 to 12 % (Janicek et al., 2008). High fat levels in the diet can contributed to milk fat depression and decrease fiber digestion (Pantoja et al., 1994; Van Soest, 1994). However, several studies have been conducted to test the effects of increased inclusion of DDGS into the diet due to the low cost of the feed. Janicek et al. (2008) studied the effects of feeding increasing concentrations of DDGS on lactation performance of dairy cows. Both DMI and milk production increased linearly with increasing amount of DDGS in the diet. Similarly, Benchaar et al. (2013) conducted a study using lactating Holstein dairy cattle to test the effects of feeding a TMR containing increasing concentrations of DDGS. Dry matter intake and milk yield increased and methane production decreased linearly with increasing levels of DDGS. Additionally, Castillo-Lopez et al. (2017) evaluated feeding lactating dairy cattle DDGS, reduced fat DDGS, or a mix of the two products. Dry matter intake and milk yield increased in the diets with distillers grains when compared to the control. Diets with distillers grains tended to decrease methanogenisis per unit of feed intake. With benefits such as increased DMI and reduced methane production, previous research suggest that dairy rations may be formulated to contain as much as 20 to 30 % DDGS (Janicek et al., 2008; Benchaar et al., 2013; Castillo-Lopez et al., 2017).

Canola meal. The term “canola” (Canadian oil) is used to differentiate the plant from rapeseed. Canola is primary produced in Canada, Australia, China, India, and the European Union (Canola Council, 2015). Canola meal is a derivative of *Brassica napus* and is a considered a better quality product due to the lower levels of glucosinolates per

gram of oil-free dry matter of the seed (30 μmoles vs. 50-100 μmoles, respectively) and erucic acid (< 2%) (Bell, 1993). Glucosinolates interfere with iodine metabolism, affecting the function of the thyroid and overall animal performance (Mawson et al., 1994). The low-glucosinolate trait was identified in 1969 in the Polish rapeseed spring variety "Bronowski" which inspired an international backcrossing breeding program to introduce the low glucosinolates trait into high yielding and euric acid free rapeseed varieties (Abbadi and Leckband, 2011). It has been reported that cows fed rations containing canola meal produce lower milk concentration of iodine than cows supplemented with other protein sources (Norouzian and Azizi, 2013) due to goitrogenic compounds, which inhibit the sodium-iodine transporter (Tripathi et al., 2004; De La Vieja et al., 2000). Additionally, Weiss et al. (2015) tested the concentration of iodine in milk when canola meal and iodine was supplemented to lactating dairy cattle. Results indicated that as canola meal inclusion increased in the ration, iodine concentrations in the milk decreased. However, milk iodine concentration was maintained in rations containing canola meal when 2 mg/kg of supplemental iodine was added to the diet.

Canola meal processing. The commercial process of extracting oil from canola and producing canola meal is illustrated in Figure 1.2. Canola meal is commonly produced using solvent extraction to separate the oil from the meal. In general, there are six steps in canola meal production: 1) seed cleaning 2) seed pre-conditioning and flaking 3) seed cooking 4) pressing the flake to mechanically remove a portion of the oil 5) solvent extraction to remove remaining oil and 6) desolventizing and toasting of the meal. (Canola Council, 2015). During cleaning, the seed is inspected for moisture content, seed

damage, and chlorophyll levels. If the seed passes inspection, it is then flaked to an optimum size of 0.3-0.38 mm by roller mills to rupture the seed coat to expel oil. Next, the seed is cooked in steam-heated drums to rupture the remaining oil cells that survived the flaking process. At the onset of cooking, the temperature is elevated to 80-90°C. This serves to inactivate the myrosinase enzyme in canola which can hydrolyze the glucosinolates that affect the meal quality. Cooking typically last 15-20 minutes at an optimum temperature of 88°C. Next the seed is pressed. Here, the objective is to remove as much oil as possible (50-60 % of the seed oil content) and produce a presscake for best solvent extraction. During the solvent extraction step, hexane is used and the end product of this step is hexane-saturated meal, which contains less than 1% oil. The final step of canola meal processing is desolventizing and toasting. The hexane is removed and the meal is toasted at 95-115°C. The meal is then cooled, with the finished product containing about 12 % moisture (Canola Council, 2015).

Canola meal in dairy diets. Canola meal contains approximately 30 % NDF and 38 % CP (DM Basis) of which 36 % of the protein is RUP and has been considered to be of similar value to soybean meal (NRC, 2001). The NRC (2001) includes RUP digestibility for various feed ingredients based on in vitro and mobile bag procedure studies and reports canola meal to have the lowest value (75 %) when compared to DDGS (80 %), soybean meal (93 %), and ground corn (90 %). Additionally, Paz et al. (2014) reported the greatest intestinal digestibility in situ for expeller soybean meal (98 %) followed by DDGS (90 %) and canola meal (72 %). Although in situ experiments are useful in giving researchers an idea of protein digestibility, this procedure does not

account for rate of degradation out of the rumen of other chemical and physical factors of the feedstuff (NRC, 2001).

Investigation of inclusion of canola meal in dairy rations to observe effects on lactation performance has been performed by multiple researchers. In a review and meta-analysis by Huhtanen et al., (2011) canola meal (CM) and heat treated canola meal (TCM) were evaluated as a protein replacement for soybean meal. The results showed that although all three protein sources increased DMI, the responses were greater for CM and TCM when compared with soybean meal. Milk yield and milk protein also increases with the inclusion of canola meal in the diets. Positive responses in milk protein may be a result of underestimated metabolizable protein in canola meal, as milk protein secretion in lactating dairy cows is often determined by the amount of metabolizable protein, from RUP or microbial protein (NRC, 2001). More recently, Martineau et al. (2013) conducted a meta-analysis with three objectives: 1) evaluate lactational performances when canola meal substituted other protein sources in dairy rations 2) to determine if the lactational responses were affected by experimental conditions or factors such as the type of forage, and 3) to evaluate if changes in milk protein yield were in line with changes in estimated supply of metabolizable protein. Responses for substituting canola meal were generally positive and increased DMI by on average by 0.24 kg/cow per 10% of CM inclusion. Researchers observed that for a cow fed 17.2 % CM in the diet, milk yield, 4 % FCM and ECM would increase by 1.07, 0.85, and 0.84 kg/cow per day, respectively. Milk protein and fat yields responses were generally positive in response to substitution of canola meal. The authors attributed the response in milk protein yield to a combination effect of

positive milk yields and positive milk protein percentage with canola meal substitution. Responses in apparent nitrogen efficiency (milk N/ N intake) was positive, and the author attributed this to a positive effect of milk protein secretion. Surprisingly, the researchers reported that despite the positive response in milk yield and milk protein secretion, changes in total metabolizable protein and metabolizable protein coming from RUP responded negatively with substitution of canola meal.

Energy Utilization

Energy Balance. Gross energy intake (GEI) is defined as the amount of energy an animal consumes (Equation 1) and is calculated by multiplying the gross energy of the feed by the animal's intake. When fecal outputs of energy are taken into account, digestible energy (DE) remains (Equation 2). Metabolizable energy (ME) is calculated when the urinary and gaseous outputs of the animal are subtracted from DE (Equation 3). Net energy for lactation (NE_L) is calculated by subtracting heat production (HP) from ME (Equation 4). The net energy for lactation is the energy required for maintenance, lactation, gestation, and growth and is considered to be the most accurate method for differentiating feeds when formulating rations (Weiss, 2007).

Heat production, and specifically heat increment, represents an energetic loss to the animal. Heat increment is the increase in heat production following consumption of food when the animal is in a thermoneutral environment, and it includes the heat of fermentation and the heat of nutrient metabolism (Flat and Moe, 1969). VandeHaar (1998) estimated that one-third of ME is lost as heat increment. The main components of HI were summarize by Bondi (1987); 1) the work of nutrient metabolism (inefficiencies

in conversion of nutrients to ATP); 2) the work associated with the digestion and mastication of food; 3) the heat of fermentation; 4) the work of excretion by the kidney; and 5) the increased muscular activity of various organs due to the metabolism of nutrients. Another process that contributes to HI is the movement of sodium and potassium ions, and other substances across membranes (McDonald et al., 2002). Coppock (1985) described that for a 600 kg cow producing 40kg of 4 % milk, as a percentage of energy intake, 35.3 % of energy was lost in feces, 31.1 % in heat, 25.5 % in milk, 5.3 % in gas, and 2.8 % in urine. Tissue energy **(TE)** is ME intake and heat production subtracted from lactation energy. It can be useful in analyzing energy balance, although it is considered to have the greater error (Moe et al., 1971) because it contains the collective error of ME, HP, and lactation energy.

$$\text{GEI (Mcal/d)} = \text{intake of feed} \times \text{GE of feed} \quad [1]$$

$$\text{DE (Mcal/d)} = \text{GEI} - \text{fecal energy} \quad [2]$$

$$\text{ME (Mcal/d)} = \text{DE} - \text{urinary energy} - \text{gaseous energy} \quad [3]$$

$$NE_L \text{ (Mcal/d)} = \text{ME} - \text{heat production} \quad [4]$$

The gross energy of a feed can be determined the use of bomb calorimetry. It is calculated by the increase in temperature of the water inside the bomb multiplied by the heat capacity of the water, this will provide an estimate for heat produced. (Blaxter, 1962).

Efficiencies. Energetic efficiency is also an important consideration when discussing energy utilization. Brody (1945) defined gross efficiency as the percentage of

the energy in the given feed category, inclusive of maintenance, recovered in the desired product. Similarly, Bauman et al. (1985) defined productive efficiency is as the yield of milk and milk components in ratio to the nutritional cost of maintenance, lactation and of returning the cow to level of body condition that existed before the onset of lactation.

Dilution of maintenance may be a reason for increased efficiency. Dilution of maintenance occurs when milk production is increased while maintenance requirements remain relatively constant and has been acknowledged in previous experiments (Freeman, 1975; Bauman et al, 1985). Freetly et al. (2006) reported a similar dilution in maintenance when comparing maintenance requirements of lactating beef cattle to previous research involving dairy cattle, although milk production of the beef cattle was lower.

Estimates of energy efficiency for use of milk production (60 to 64 %) are lower than earlier estimates (69 to 70 %) mainly because of lower maintenance requirements (Moe, 1981). The use of body tissue for milk production has an 82 % efficiency while the use of metabolizable energy for body gain is 75 % for lactating animals (Moe, 1981). Therefore, milk secretion is considered to be a more efficient process energetically when compared with body fat deposition (Brody, 1945; Blaxter, 1962 and Bauman et.al, 1985). There are three main reasons for this increase in efficiency. Firstly, amino acids are incorporated into the proteins of the milk, and as a result very little energy has to be expended in the synthesis of urea and no energy is lost in forming urea. Secondly, the fatty acids of milk generally have a shorter chain length then those of fat deposition. The energetic cost of increasing the fatty acid chain length is very expensive. Thirdly, the

synthesis of lactose from glucose is not energetically expensive if the starting point is glucose or lactic acid (Blaxter, 1962).

Maintenance. The portion of an animals intake used for maintenance will be used for life-sustaining functions such as circulation and respiration even when the cow is not producing milk, growing, working, or is pregnant, all in a thermoneutral environment (VandeHaar et al., 2016). Baldwin et al. (1985) developed three major classes for maintenance energy expenditure: work functions, synthesis of cell components, and membrane transport. The work functions account for 40 to 50 % of basal energy expenditure and include ion resorption in the kidney, heart and muscle and also integrative functions from the liver and nervous tissue. Synthesis of cell components make up 15 to 25 % of basal energy expenditure and include functions such as resynthesizing of protein and membrane lipids. Finally, membrane transport makes up the resulting 25 to 30 % of basal energy expenditure and is involved with maintaining membrane potential and the sodium-potassium ATPases. McNamara (2015) reported that variation in basal maintenance functions, such as iron pumping and protein turnover, could lead to 20 % variance in the maintenance NE requirement of cows producing similar levels of milk. The 2001 Dairy NRC reported that the average daily maintenance requirement as 0.080 Mcal of NE_L × metabolic body weight (Equation 5).

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

Recent evidence suggests that the maintenance requirement for lactating dairy cattle has increased over time and is now closer to 0.086 Mcal × MBW (Moraes et al., 2015). The reasons for the increased maintenance requirement are not clear but are most

likely due to increased digestive and metabolic activity (VandeHaar, 2016). Baldwin et al. (1985) observed liver weights increased 50 % during lactation. Changes in organ weights during lactation may account for a 9.5 % increase in energy expenditure, possibly resulting in increased maintenance needs (Baldwin et al., 1985). Other factors affecting the maintenance requirement include sex (Garrett, 1970; Ferrell and Jenkins, 1985), breed (Xue et al., 2011, Reynolds and Tyrrell, 2000; Ferrell and Jenkins, 1985; Laurenz et al., 1991; Tyrrell et al., 1990) body condition (Thompson et al., 1983), diet (Yan et al., 1997; Dong et. al, 2015; Flatt et al., 1967), age, and thermoneutral environment (West, 2003; Laurenz et al., 1991; Collier and Beede, 1985; Young 1983).

Methane Production and Mitigation Strategies

Methane Production. Methanogenisis, or methane production, is achieved by archaea in the rumen, often referred to as methanogens (Morgavi et al., 2010). Only 8 methanogens species have been identified (Kong et. al, 2013). These methanogens are found in the rumen and hindgut, although the population structure, ecology and microbial metabolism differ between the 2 compartments (Knapp et al, 2014). Sugars are fermented by rumen microbes to produce volatile fatty acids (VFA) and reducing equivalents such as metabolic hydrogen. The metabolic hydrogen is converted to H_2 by hydrogenase expressing bacteria and the H_2 is converted to methane and is represented by the following equation (Knapp et al., 2014):

$$CO_2 + 8H \rightarrow CH_4 + 2H_2O \text{ (methanogenisis)} \quad [6]$$

Removal of the H_2 in the rumen allows for fermentation and production of H^+ to continue. (McAllister and Newbold, 2008). Results from indirect calorimetry show that methane losses vary from approximately 2 to 12 % of GEI (Johnson and Johnson., 1993). Therefore, mitigation of methane would not only be beneficial from an environmental standpoint, but could lead to more productive and efficient animals. Greenhouse gases (GHG), including methane, enhance the effects of solar and thermal radiation on surface and atmospheric temperatures (Knapp et al., 2014). Methane has several natural (termites, wetlands, peat, bogs, ocean sediments, and wildlife) and man-made (natural gas production, coal mining, wastewater treatment, landfills, and agriculture) sources (Lassey, 2008). It has been predicted that 3.3 % of GHG are derived from ruminants (Knapp et al., 2014). By 2020, the Innovation Center for U.S. Dairy is striving to reduce the U.S. Dairy industry's total GHG emissions by 25 % (Innovation Center, 2013).

Methane Mitigation. In this review, nutritional approaches to methane mitigation will be summarized but it is important to note there are other strategies for methane mitigation being studied such as rumen modifiers and genetic approaches (Knapp et al., 2014).

Nutritional factors that have been studied to manipulate methane production include decreasing fiber digesting bacteria in the rumen which produce excess H_2 (Morgavi et al., 2010; Chaucheyras-Dunard et al., 2008).The addition of concentrate to a diet has been shown as an effective way to reduce methane because of the shift towards propionate production in the rumen, which is a consumer of reducing equivalents such as H_2. Therefore, an increase in propionate production will result in a decrease in methane

production per unit of feed fermented, although the opposite is true for acetate and butyrate (Van Nevel and Demeyer, 1996). The addition of nitrate to the diet have also plays a role in methane mitigation (Klop et al., 2016; Olijhoek et al. 2016; Van Zijderveld et al., 2010; Iwamoto et al., 1999). Conversion of nitrate to ammonia by bacteria in the rumen has the ability to utilize excessive hydrogens in a process that is more thermodynamically favorable then methanogenisis (Morgavi et al., 2010). Sulfate reducing bacteria have been shown to have a higher affinity for H_2 then methanogens (Weimer, 1998). Therefore studies adding sulfate to the diet have been carried out (Van Zijderveld et al., 2011; Van Zijderveld et al., 2010). Unsaturated fatty acids have the opportunity to be biohydrogenated in the rumen, thereby producing an alternative hydrogen sink to methanogens and reducing methane production (Beauchemin et al., 2006; Johnson and Johnson, 1995).

Distillers grains and methane production. Several studies have been done in both dairy and beef cattle using distiller's grains in an attempt to reduce methane production. McGinn et al. (2009) conducted a study involving Hereford steers where barley grain was replaced with distillers dried grains and solubles **(DDGS)** at 35 % of the dietary DM. The addition of DDGS to the diet reduced methane emissions by 19.9 % (16.4 % when corrected for DMI and 23.9 % when corrected for GEI). Additionally, Benchaar et al., (2013) fed four treatment diets to lactating dairy cattle containing 0, 10, 20, 30 % DDGS to test the effects on enteric methane production. Methane production decrease as the level of DDGS increased in the diet. As a percentage of GEI, the 30 % DDGS had the greatest methane reduction at 14 % when compared to 10 % DDGS (5 %

reduction) and 20 % DDGS (8 % reduction). Hünerberg et al. (2013) conducted a study with 16 cross-bred finishing beef cattle. The control diet contained mostly barley grain. The treatment diets replaced 40 % DM of barley grain with corn-based DDGS (CDDGS), wheat based DDGS (WDDGS), or corn oil supplemented WGGDS (WGGDS +oil). Methane emissions were measured using open circuit respirator chambers. Results showed that CDDGS and WDDGS + oil decreased methane emissions when compared to the WDDGS (4.0 %, 4.2 %, and 5.5 %, respectively) as a percentage of GEI. Furthermore, Hales et al. (2013) tested the effects of steam flaked corn based diets with increasing levels of WDGS (0, 15, 30, and 45 %) and reported that methane emissions increased with increasing concentrations of WDGS.

Foth et al. (2015) used headbox-type indirect calorimeters to measure methane from eight Holstein and eight Jersey lactating dairy cattle. The experimental diet replaced corn and soybean meal with 28.8 % reduced-fat DDGS. Methane was reduced from 504 L/d in the control diet to 472 L/d with the addition of DDGS. As a percentage of GEI, methane production was decreased in the DDGS diet when compared to the control (5.13 % and 5.72 %, respectively).

Canola meal and methane production. Canola is another popular byproduct in dairy rations and has been of interest in methane mitigation**.** Gidlund et al. (2015) evaluated the effects of soybean meal and heat-moisture-treated canola meal on milk and methane production of twenty-eight Swedish Red lactating cows. Seven treatments in total were offered: Control containing no soybean meal or canola meal, a low, medium, and high soybean treatment (50, 100, or 150 g/kg DM of soybean meal, respectively) and

a low, medium and high canola meal treatment (70, 140, or 210 g/kg of canola meal, respectively. Methane emissions were measured by a portable open-circuit head chamber system. Diet did not have a significant effect on methane emissions when expressed as g/day or g/kg of DMI. However, methane emissions per kg of ECM decreased when canola meal was used as the source rather than soybean meal, and as the dietary crude protein concentration increased. This research agrees with equations produced by Bannink et al. (2006) and Sveinbjörnsson et al. (2006), which calculated that protein fermentation produces 30 to 50 % less CH_4 than fermentation of carbohydrates.

Using an in vitro experiment, Paula et al. (2017) evaluated the effects of feeding canola meal with different concentrations of RUP versus soybean meal on methane production. Eight fermenters were assigned to one of three diets: (1) solvent-extracted soybean meal, (2) low-RUP extracted canola meal (38 % RUP as a percentage of crude protein) or (3) high-RUP extracted canola meal (50 % RUP as a percentage of crude protein). Cumulative pressure was recorded to determine methane production over a 48-h period. The soybean meal diet tended to increase methane production when compared both the low-RUP and high-RUP canola meal treatments. The soybean meal diet produced greater concentrations of butyrate than the canola meal treatments. This may be a possible explanation for the increase CH_4 production with the soybean meal diet, as for every 2 mol of butyrate produced, 1 mol of CH_4 is produced (Owens and Goetsch, 1988).

Calorimetry

Calorimetry, at its simplest form, is the measure of heat. More generally, animal calorimetry has been defined as the science of measurement of heat transferred between

an animal and its environment (Nienaber et al., 2009). Historically, calorimetry was first used by Lavoisier to define “oxygen” use in the combustion process (Nienaber et al., 2009). For modern nutritional calorimetry, the objective is to quantify the energy utilization of the animal and compare it with the energy supplied in their diet (Judy et al., 2017). Johnson et al. (2003) noted three main objectives for conducting animal energy research as to 1) measure relationship between heat production and gas exchange 2) evaluate feeding values for feeds that contribute to energy requirements and energy expenditure and 3) to predict sources of energy expenditure. In general, there are two types of calorimetry: direct calorimetry and indirect calorimetry. The main difference between the two is the type of heat they measure. Direct calorimetry measures heat loss, while indirect calorimetry measures heat production (Nienaber, 2009).

Direct Calorimetry. Direct calorimetry measures the sensible and evaporative heat losses of the animal (Nienaber et al., 2009). Lavoisier and Laplace discovered direct calorimeter by confining a guinea pig in a chamber which contained ice, and calculated heat production as the ice melted. They found that the amount of ice melted corresponds to a definite amount of carbon dioxide exhaled. (Brody, 1945). There are several types of direct calorimeters in use which include respiration calorimeters, gradient layer calorimeters, and spot or local calorimeters (Nienaber et al., 2009).

Respiration calorimeters measures the sensible heat loss of the animal by preventing the animal chamber from gaining or loosing heat to the outside environment (Nienaber et al., 2009). Respiration calorimeters can also be known as adiabatic or heat sink calorimeters. One of the earliest respiration calorimeters in the United States was the

Armsby Respiration Calorimeter at the Pennsylvania State University (University Park, PA). The air temperature in the air space between the walls was maintained at the same temperature to avoid the sensible heat from transferring through the walls (Nienaber et al., 2009).

Gradient layer calorimetry is a system that measures heat loss through the walls of the chamber. This type of calorimeter can also be known as a patitional calorimeter because of its ability to partition sensible heat into radiation and convention. Gradient layer calorimetry made an advancement in the 1940's when Benzinger and Kitzinger developed thermoelectric heat flow meters which were placed on the walls of the chamber to measure the total sensible heat lost through time. This calorimeter can measure heat loss in real-time which is usually the result of the animal's physical movement (Nienaber et al., 2009).

Spot calorimetry, or a portable calorimeter, is another form of a direct calorimeter. It was designed by Hillman et al. (2001) at Cornell University to measure the temperature and relative humidity of air passing over a defined area on the body of a cow. During the measurements, the calorimeter is held against the animal's body, usually the dorsal surface. Data is collected for 10 minutes at 10 second intervals and is read on a laptop connected to the portable calorimeter (Nienaber et al, 2009). Portable calorimetry is not widely used for lactating dairy cattle, as only few studies reference using this method (Hillman et al., 2001; Gebremedhin et al., 2008)

Indirect Calorimetry. Indirect calorimetry is based on the relationship between the amount of heat produced for oxidation of food or body components, and the amount

of oxygen consumed, carbon dioxide and methane produced, and nitrogen excreted in the urine (Agnew and Yan, 2005, Nienaber et al., 2009). The major advantage of indirect calorimetry over direct calorimetry is that indirect calorimetry allows the research to test various environmental conditions such as cooling or heating the animal, cyclic temperatures vs. constant temperatures, floor heating or cooling, and infrared heating (Nienaber et al., 2009). Several equations have been used to estimate heat production in indirect calorimetry shown in Equation 7 (Brouwer, 1965) (Nienaber et al., 2009):

$$HP\ (Mcal/d) = 3.866 \times O_2 + 1.200 \times CO_2 - 0.518 \times CH_4 - 1.431 \times N \quad [7]$$

Where HP = metabolic heat production rate

O_2= oxygen consumption rate, mL/s, STPD[1]

CO_2 = carbon dioxide production rate, mL/s, STPD

CH_4 = methane production rate, mL/s, STPD

N= urine nitrogen excretion rate g/s

[1]STPD = Standard pressure (760 mm Hg) and temperature and dry air

The ratio of CO_2 produced to O_2 consumed is commonly known as the respiratory quotient (RQ; Equation 8) and is a predictor of the body substance being oxidized (Neinaber, 2009). Measuring RQ is especially important in calorimetry experiments, as it can give researchers an idea of what metabolic substrate is being utilized by the animal for energy, and therefore energy utilization can be predicted more accurately. When carbohydrates are being oxidized, the RQ is close to 1.0 because 6 mol of CO_2 are

produced and 6 mols of O_2 are consumed (Equation 9). The RQ for mixed fat (long-chain and short-chain fatty acids) is 0.71, however each distinct fat has its own RQ value. Short-chain fatty acids have an RQ closer to 0.80, whereas long-chain fatty acids are closer to 0.70. The RQ for mixed protein is 0.81, although similar to fats, each amino acid has its particular RQ (Brody, 1945).

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

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

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

Previous estimations of RQ for lactating dairy cattle are fairly similar despite small differences in forage and concentrate quantities in rations. For example, in a meta-analyses by Aubry and Yan (2015), gaseous data collected by indirect open circuit calorimeters from 987 cattle were summarized. Of the 987 cattle involved in the study, 811 were lactating, and were fed diets averaging 59 % forage and 41 % concentrate. For this study, the average RQ was reported to be 1.00. Additionally, in a study validating a respiration chamber (Derno et al., 2009), four dairy cattle were fed a total of 74 % forage and 26 % concentrate, and the RQ for each individual cow ranged from 0.99 to 1.01. In another validation study of a respiratory system containing four climate controlled chambers (Machado et al., 2016), twelve lactating dairy cattle were used and RQ was reported. The diets in this study contained 52 % forage and 48 % concentrate. Interesting, the RQ range for these animals was higher than previous reported (Aubry and Yan, 2015; Derno et al., 2009) with values ranging from 1.05 to 1.17. Increased CO_2 production

leads to an increase in RQ, therefore higher RQ values may be a result of CO_2 produced from other sources besides the animal such as manure storage (Madsen et al., 2010; Pendersen et al., 2008). In contrast, lower RQ values have been observed with increased hours of fasting (Yan et al., 1997b) displayed in Figure 1.3.

Indirect Calorimetry Methods

There are two types of indirect calorimetry systems, closed and open-circuit. Closed-circuit indirect calorimeter was designed by Regnault and Reiset in 1849. In this type of calorimetry system, air is circulated continuously through absorbents which are designed to remove carbon dioxide and water vapor (Blaxter, 1962). The absorbents are weighed at the end of the experiment to directly measure the amount of carbon dioxide produced, and the amount of oxygen can be measured by weight or volume. The closed-circuit system checks the air composition at the beginning and end of the experiment to ensure the oxygen and carbon dioxide concentration are the same. One issue with closed-circuit calorimeters is they are very sensitive to changes in temperature or pressure. An increase in barometric pressure or a decrease in temperature could cause excess admission of oxygen into the system. Also, when these calorimeters are used with ruminants, methane gas produced by the animal needs to be removed as it will depress the amount of oxygen admitted (Blaxter, 1962).

Open circuit calorimetry systems measure changes in oxygen, methane, and carbon dioxide of outside air that has been passed through the system. The original calorimeters were designed by Pettenkofer and Voit (University of Munich) and did not measure oxygen consumption. Tigerstedt was the first to measure oxygen in these

systems (Blaxter, 1962). Open-circuit systems require very precise measurements of) the volume of air passing through the system, a true sample of the ingoing and outgoing air, and the oxygen, carbon dioxide, and methane content of the air passing through the system. Ventilation rates can provide difficulties for an open-circuit system. With low ventilation rates, the greater precision in the changes in oxygen, carbon dioxide, and methane of the in- and outgoing air. However, low ventilation rates also if accumulation of carbon dioxide occurs, respiration will be stimulated in the animal and water vapor will increase in the chamber. This would result in inaccurate gas exchange and calculation of HP (Blaxter, 1962).

Carbon Dioxide Entry Rate Technique. The carbon dioxide entry rate technique (CERT) was developed as a method to calculate HP through measuring CO_2 production in the body. It has primarily been used in grazing animals (Herselman et al., 1998). The technique used a ^{14}C isotope which can be lost through CO_2 from the lungs, CO_2 or CH_4 from fermentation in the rumen, or in the feces and urine. The isotope is infused as ^{14}C-bicarbonate and equilibrates within the body pool of CO_2. Once this equilibrium is reached, saliva from the parotid gland is collected and tested for the ^{14}C isotope, from which CO_2 can be calculated. Sahlu et al. (1988) conducted a validation study of CERT comparing this method against indirect respiration calorimeters. The authors reported daily CO_2 production did not differ between the two methods (20.6 vs 20.3 L/kg MBW). They also reported heat production and RQ did not differ, suggesting that the CERT is a suitable method for calculating CO_2 production.

Sulfur Hexafluoride (SF6). Another indirect calorimetry method is the sulfur hexafluoride (SF_6) method. The method measures methane emissions by a known emission rate of tracer gas in the rumen. The method is performed by filling permeation tubes with SF_6. The rate of diffusion of SF_6 out of the permeation tubes is calculated by placing the tubes in a 39 °C water bath and measuring daily weight loss until stable. Once stable, the permeation tube is placed in the rumen of the animal and sampling begins. Sample is collected through capillary tubing into a canister. The concentration of SF_6 and CH_4 in the canister are measured through gas chromatography. (Storm et al., 2012).This sampling method is shown in Figure 1.4. One advantage of this method is its availability to be used on grazing animals and does not involve the use of chambers. However, it has been reported that the SF_6 technique provides more variable methane emissions when compared to chamber measurements, partly because the CV within and between animal is greater. (Storm et al., 2012).

Comparative Slaughter Technique. Comparative slaughter calculates heat production based on the difference in metabolizable energy and retained energy shown in Equation 10 below.

$$HP = ME + RE \quad [10]$$

This is in contrast to calorimetry, which measures metabolizable energy and heat production and uses these variables to calculate retained energy. In this technique, animals on the feeding experiment are split into representative groups. One group of animals are slaughtered at the beginning of the experiment and body composition is determined. The remaining groups of cattle on the feeding trail are slaughtered later at

predetermined times and body composition is also determined. From these series of body compositions, RE can be calculated (Neinaber et al., 2009). The comparative slaughter technique allows an animal to only be used once, therefore would not be suitable for a lactating dairy cattle operation (Neinaber et al., 2009).

Whole-animal Chambers. Whole-animal chambers have been used for the last 100 years and were the most common way to study energy metabolism of animals historically (Storm et al., 2012). The chamber needs to be well sealed and have a slight negative pressure. The negative pressure inside the chamber insures that any leaks in the walls of the chamber will flow inward, avoiding any gas loss. The chambers should also promote natural behavior from the animal through feces and urine disposal, air conditioning, dehumidifiers, feed, and water. One significant disadvantage to whole-animal chambers is the initial expense to build them, especially for large animals such as dairy cattle (Johnson and Johnson, 1995). Whole-animal chambers have been widely perceived as the most accurate because of their ability to potentially capture all gases derived from the animal such as flatulence or gas eructated. Numerous comparison studies have been done with whole-animal chambers and other gas collection methods to determine accuracies (Young et al., 1975; Sahlu et al., 1988; Boadi and Wittenberg, 2002; Grainger et al., 2007).

Headboxes. A headbox style indirect calorimeter can also be used for gas collection. This technique can be less expensive to construct than a whole-animal chamber given that the headbox is only surrounding the animals head versus the whole body (Johnson and Johnson, 1995). The box is equipped with feed and water and is large

enough that the animal can stand up, lie down, and move its head. A vinyl hood is placed around the animal's neck and tied to provide a seal between the box and animal. (Freetly et al., 2006). Use of a headbox for gas collection is displayed in Figure 1.5. Much like the whole-animal chambers, headboxes have a slightly negative pressure inside the box. Proportions of O_2, CO_2 and CH_4 are collected into gas bags to provide a composite gas sample for each box (Freetly et al., 2006). Headboxes are advantageous to lactating dairy cattle research because the cattle can be milked without any disruption to gas collection. A disadvantage to headboxes is they do not account for hind gut fermentation losses of gases. However, 89% of hindgut methane is absorbed in the blood expired through the lungs, which can be collected (Boadi and Wittenberg, 2002).

SUMMARY OF LITERATURE

Byproducts have become a popular protein supplement in the dairy industry by providing a cheaper source of protein and fiber. Two of the more popular byproducts today are DDGS and canola meal. Dried distillers grains and solubles are produced at ethanol plant using a dry milling process. Types of distillers include dried distillers, wet distillers, and modified distillers, which vary in their DM content. Advances in dry milling include the use of a raw starch hydrolyzing enzyme (RSH) which allows DDGS to be cooked at a lower temperature, potentially improving the value of the product to the animal. In vitro studies have shown improvements in fiber digestion with this product, but this has not yet been tested in vivo. Concentrations of fat in DDGS have been reported to induce milk fat depression in lactating dairy cattle. Fat can be

removed through centrifugation or solvent extraction and reduced fat DDGS (RFDDGS) can be produced. Although reducing fat content may reduce energy content, studies have shown RFDDGS to maintain gross energy content and milk fat in dairy cattle. In general, diets containing DDGS have been reported to increase DMI and milk production.

Canola meal is a byproduct of the oil crushing industry and is a derivative of *Brassica napus*, but is considered a better quality product due to its lower concentration of glucosinolates, which interfere with iodine metabolism. Canola meal is produced in a six step process which includes 1) seed cleaning, 2) seed pre-conditioning and flaking, 3) seed cooking, 4) pressing the flake to mechanically remove a portion of the oil, 5) solvent extraction to remove the remaining oil, and 6) desolventizing and cooking the meal. The final product, canola meal, is about 30 % NDF and 38 % CP. A large portion of the CP in canola meal is RDP (> 50%). The remaining RUP portion of CP in canola meal has been reported be less digestible than corn, soybean meal, and DDGS in vitro, but has yet to be tested in vivo. Responses to substituting canola meal for other protein source have been reported as generally positive, with increased DMI and milk yield.

Energy utilization is a complicated process in dairy cattle. Researchers have simplified this process by partitioning energy into gross energy, digestible energy, metabolizable energy, and net energy. Losses of energy can be excreted by the animal in the form of feces, heat, milk, gas and urine. Energetic efficiency can be useful in determining the energy utilization of an animal. In dairy cattle, efficiency can be increased due to dilution of maintenance energy, as a result of increase milk production.

Maintenance requirements for dairy cattle may be increasing over time, possibly as a result of increased digestive and metabolic activity.

Methane production is the result of removal of hydrogen ions in the rumen by methanogens. Mitigation of methane is not only beneficial from an environmental standpoint, but it also has the potential to increase efficiency in dairy cattle, seeing as methane is an energetic loss to the animal. A few nutritional strategies for reducing methane include decreasing fiber digesting bacteria, increasing concentrate inclusion, and the addition of nitrate, sulfate, or unsaturated fatty acids to rations. The addition of DDGS meal to rations has been reported to decreased methane production. Similarly, methane production has been reported in vivo and in vitro to decrease with the addition of canola meal to the ration.

Calorimetry, by simple definition, is the measure of heat. In general, two types of calorimetry exist: direct calorimetry and indirect calorimetry. Direct calorimetry measures heat loss while indirect calorimetry measures heat production. Open circuit indirect calorimetry is the most common way to measure heat production in lactating dairy cattle. Headboxes are advantageous for lactating dairy cattle, as gas collection does not infer with milking. Hind gut fermentation is not accounted for in a headbox system. However, 89 % of methane produced post rumen is absorbed in the blood and expired through the lungs. Therefore gas collection with headboxes is deemed to be accurate.

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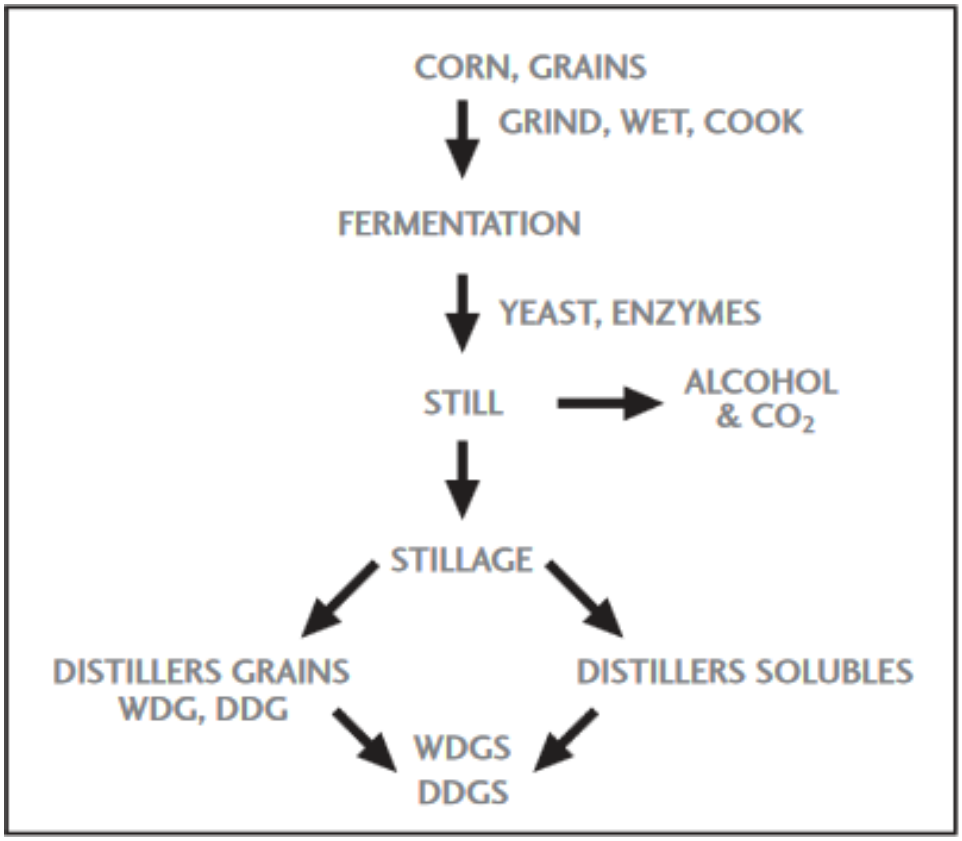

Figure 1.1. Dry milling process for production of DDGS (Erickson, 2005).

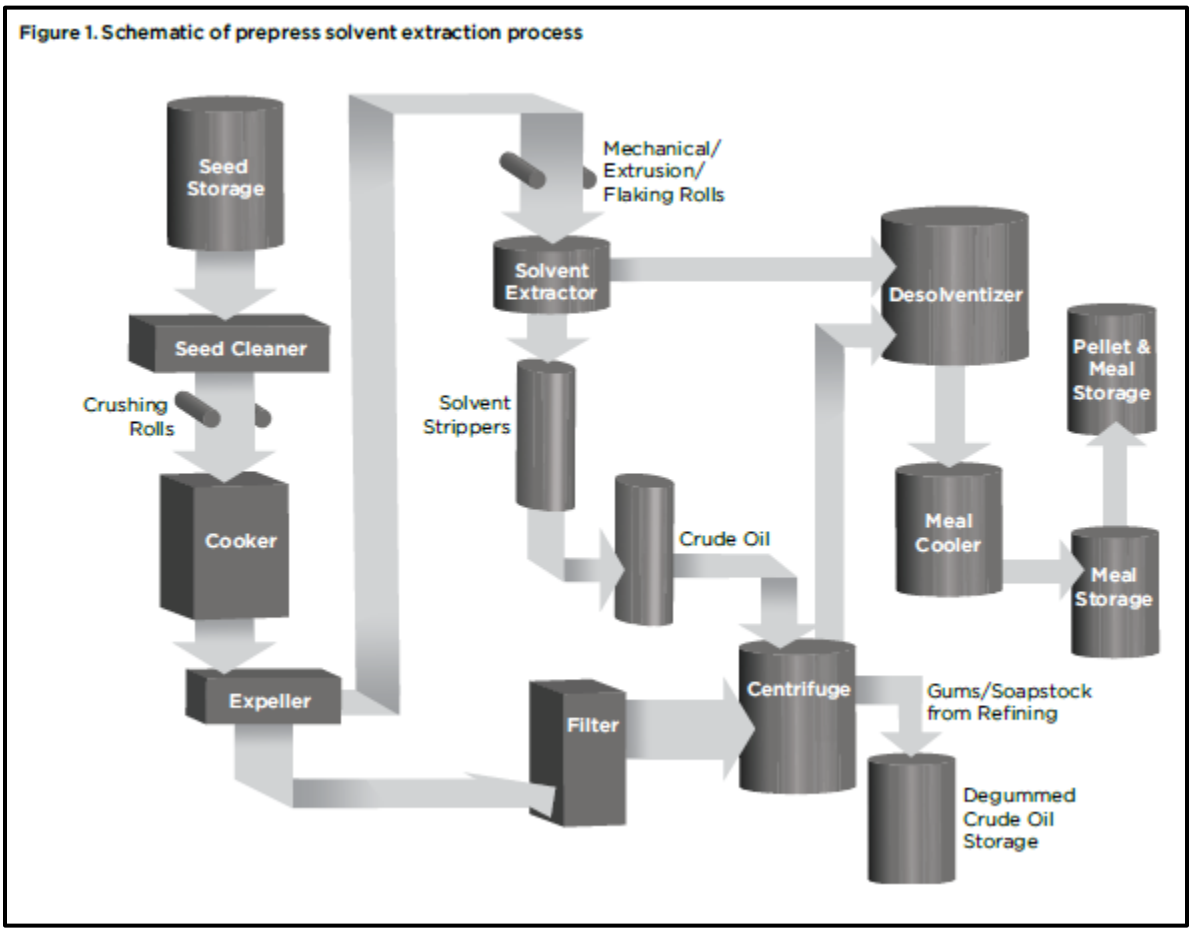

Figure 1.2. Canola meal processing. Canola Council, 2015.

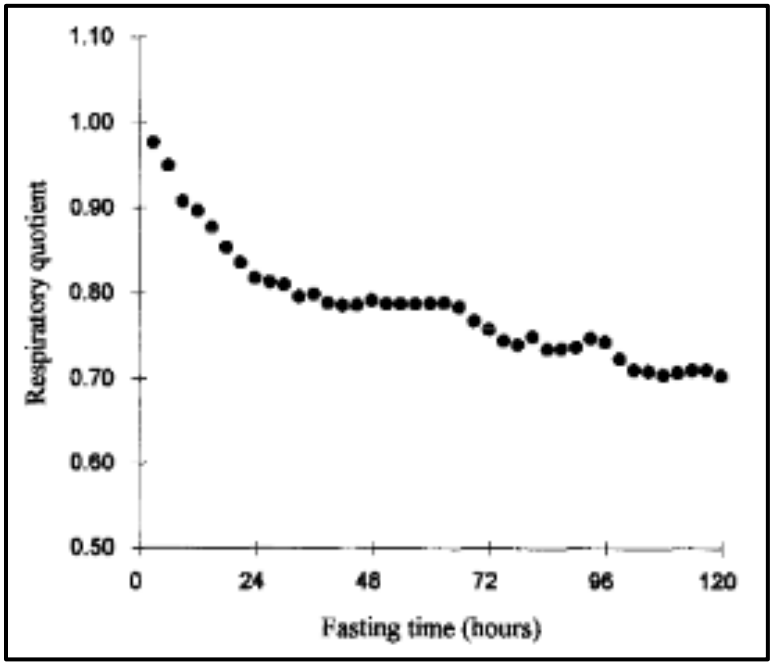

Figure 1.3. Decline of respiratory quotient (RQ) with increased fasting time (Yan et al., 1997b)

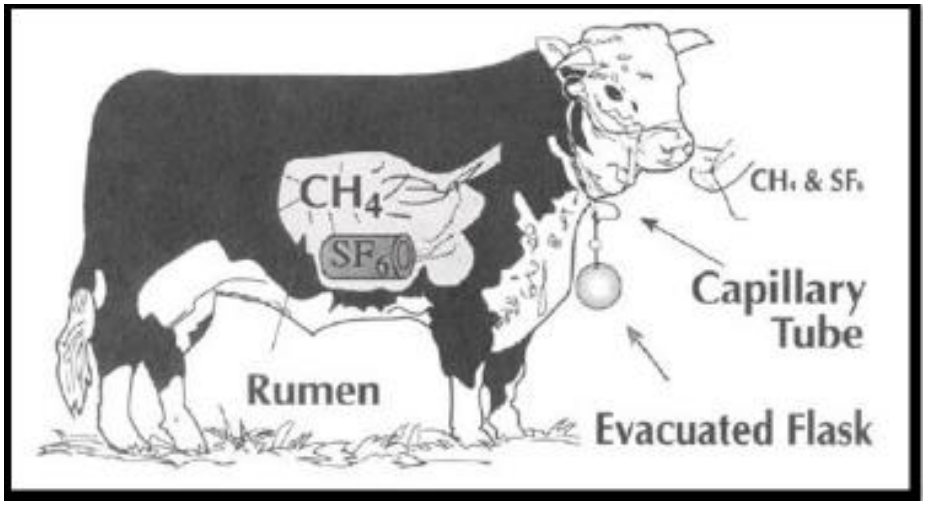

Figure 1.4. Sulfur hexafluoride (SF6) method for indirect calculation of methane production (Storm et al., 2012).

Figure 1.5. Use of a headbox for gas collection from a Holstein cow (Place et al, 2012).

CHAPTER 2

INTERPRETIVE SUMMARY: Myers *et al.* (2018). "Use of indirect calorimetry to evaluate utilization of energy in lactating Jersey dairy cattle consuming distillers dried grains or canola meal" describes the effect of adding two sources of DDGS, which differed in the concentration of fat, or canola meal in lactating dairy cow rations on milk production and energy utilization. This study shows that milk production can be maintained when feeding these byproducts. However, differences in energy utilization exist. The alternative source of DDGS, which contained the greatest fat content, also contained the greatest amount of gross energy, digestible energy, and metabolizable energy. The control and the alternative source of DDGS contained the greatest energy balance. Differences in digestibility also existed specifically the control and treatments containing DDGS had the greatest digestibility of dry matter, organic matter, crude protein, and neutral detergent fiber.

Running Head: DDGS AND CANOLA MEAL FOR LACTATING DAIRY COWS

Use of indirect calorimetry to evaluate utilization of energy in lactating Jersey dairy cattle consuming distillers dried grains or canola meal

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ABSTRACT

The use of byproducts as an alternative feed source is a common practice when formulating dairy rations. A study using 12 multiparous (79 ± 16 DIM) (mean ± SD) lactating Jersey cows, was conducted over 5 months to evaluate the effects of dried distillers grains and solubles (DDGS) or canola meal on milk and gas production. A replicated 4 × 4 Latin square design was used to compare four different dietary treatments. Treatments were composed of a control (CON) containing no byproducts, a treatment diet containing 10% (DM basis) DDGS supplied by POET LLC (Sioux Falls, SD) (pDDGS), 10% DDGS treatment with an alternative distillers grains source (aDDGS), and a 10% canola meal (CanM) treatment. The crude fat content of the pDDGS, aDDGS, and CanM were 6.05 ± 0.379 %, 10.0 ± 0.134 %, and 3.46 ± 0.085 %. Byproducts were included in partial replacement for corn and soybean meal. Indirect headbox-style calorimeters were used to estimate heat production. Dry matter intake and milk yield were similar ($P > 0.44$) between all treatments averaging 17.4 ± 0.56 kg/d and 24.0 ± 0.80 kg. Milk urea nitrogen (MUN) was also affected by treatment ($P < 0.01$) and was highest in CON (20.6, 18.0, 19.9, and 18.1 ± 0.62 mg/dl, pDDGS, CanM, and aDDGS, respectively). Heat production per unit of metabolic body weight tended ($P = 0.058$) to be affected by treatment and was lowest for CON and diets containing byproducts were not different (192, 200, 215, and 204 ± 5.91 kcal per kg of metabolic body weight for CON, pDDGS, CanM, and aDDGS respectively). The ME concentration of the diet was affected ($P = 0.034$) by dietary treatment specifically, aDDGS did not differ from CON, but was greater than pDDGS and CanM (2.58, 2.46, 2.29, and 2.27 ±

0.09 Mcal/kg for aDDGS, CON, pDDGS and CanM respectively). Lastly, the energy balance concentration of the diet tended to be affected ($P = 0.062$) by dietary treatment. Although aDDGS did not differ from CON and pDDGS, it was higher than CanM (1.38, 1.36, 1.14, and 1.06 ± 0.11 Mcal/kg for aDDGS, CON, pDDGS and CanM, respectively). Results of this study indicate milk production and dry matter intake are not adversely affected when feeding common byproducts replacing both corn and soybean meal.

Key Words: dairy cow, dried distillers grains and solubles, energy utilization, canola meal, indirect calorimetry

INTRODUCTION

Feed byproducts are defined as secondary products that are produced in addition to a principle product (AAFCO, 2016). A wide array of feed byproducts are produced from the food, fuel, and beverage industries and these are widely available and used by the dairy industry as feed (Crawshaw, 2004). Although they may contain a high concentration of nutrients and improve palatability of dairy rations, their chemical composition and nutrient availability varies, and are affected by the origin of the feed, changes in the primary industry, and production process. Two of the more popular byproducts in the United States are canola meal and dried distillers grains and solubles (DDGS). Canola is largely an imported commodity into the United States as only 850,000 hectares are harvested in the United States (USDA, 2017). However, much of the canola meal fed to dairy cattle in the United States is imported from Canada. Recent estimates suggest that over 3.5 million metric tons of canola meal are imported annually, while less than 1 million metric tons are produced in the United States (USDA, 2016). Production of DDGS from ethanol plants in the United States is much greater than canola meal, with 23.2 million metric tons produced in 2016 (USDA, 2017b).

In general, soybean meal is the preferred protein supplement for dairy cattle. This is because it is widely available and high in CP content (Huhtanen et al., 2011). Solvent extracted soybean meal contains approximately 54 % CP and 10 % NDF (DM basis). The rumen undegradable protein (RUP) content is approximately 43 % and this bypass protein is highly digestible (93 %) (NRC, 2001). In comparison, the RUP content and intestinal digestibility of RUP (dRUP) of canola meal is lower (36, and 75%,

respectively). Interestingly, despite these differences, recent meta-analysis' have suggested that that milk production and composition may frequently respond positively when canola meal is added to the diet (Huhtanen et al., 2011; Martineau et al., 2013). In contrast, the RUP content and dRUP in DDGS (51, and 85%, respectively) is higher than either soybean meal or canola meal (NRC, 2001). In a study in which canola meal or DDGS replaced soybean meal, yield of fat corrected milk and protein was maintained, however a reduction in milk fat yield in cattle consuming DDGS was observed and may have been due to an increased intake of polyunsaturated fatty acids and in turn, increased rumen outflow of CLA isomers than suppressed milk fat synthesis (Christen et al., 2010; Ramirez-Ramirez et al., 2016).

While the concentration and digestibility of protein may vary in feedstuffs, the concentration of energy is also different. Although energy concentration can be laborious to measure the Dairy NRC (2001) estimated that energy concentration for soybean meal, DDGS, and canola meal is 2.38, 1.97 and 1.76 Mcal/kg, respectively. In general, nutrient availability of ruminant feeds can be determined by lab-scale in vitro or in situ procedures or by in vivo feeding studies. Each of these provides an informative way to evaluate the feeding value of particular feeds (Flatt et al., 1969). To date, very few energy balance experiments have been carried out on modern byproducts with lactating dairy cattle (Birkelo et al., 2004; Foth et al., 2015). Without such studies, it is difficult to know if observed differences in milk production or composition are a result of differences in digestibility or nutrient utilization. The objective of this study was to test the effects of feeding canola meal or DDGS on feed intake, milk production and composition, total

tract digestibility and energy utilization in lactating dairy cows. We hypothesized that byproduct containing rations will maintain milk production without altering energy utilization, however we predict that canola meal will have less total tract digestibility of protein and that this may have a negative affect on milk production and composition.

MATERIALS AND METHODS

All animal care and experimental procedures were approved by the University of Nebraska-Lincoln Animal Care and Use Committee. Twelve multiparous Jersey (n = 12) cows averaging 79 ± 16 DIM and weighing 450 ± 11.5 kg were used in this study. Cows were housed at the temperature-controlled barn at the Dairy Metabolism Facility in the Animal Science Complex of the University of Nebraska-Lincoln. Each stall was equipped with rubber mats and a water bowl. Cows were milked twice daily at 0700 h and 1800 h and fed once daily at 0900h.

The experimental design was a replicated 4 × 4 Latin square where each cow was randomly assigned to 1 of 4 dietary treatments which alternated over four periods and cows were assigned a treatment structure according to Kononoff and Hanford (2006). Each experimental period was 28 days in length with 23 d for ad libitum diet adaptation, followed by 5 d of urine, fecal, milk and gas collections, during which time animals were fed 95% ad libitum intake. Animals were blocked into squares by milk production and DIM. Treatments were composed of a zero control (CON), not containing feed byproducts, a treatment diet containing 10% (DM basis) reduced fat DDGS (RFDDGS)

originating from POET LLC (Sioux Falls, SD) (pDDGS), a 10% canola meal (CanM) treatment, and a 10% DDGS treatment with an alternative high fat distillers grains (HFDDGS) source (aDDGS) originating from Golden Grain Energy LLC (Mason City, Iowa). The DDGS treatments differed in method of production; specifically the pDDGS were produced by a method which used lower temperatures for starch hydrolysis. In the production of pDDGS, centrifugation was also used to remove a portion of corn oil resulting in a RFDDGS. Complete diet compositions and nutrient analysis of the TMR and individual ingredients are presented in Table 2.1. through Table 2.4. Byproducts were included in partial replacement for corn and soybean meal. All diets contained corn silage, alfalfa hay, brome hay, and a concentrate mix specific to that diet which were mixed into a TMR. Diets were mixed using a Calan Data Ranger (American Calan, Inc., Northwood, NH).

Laboratory Analysis

During the five-day collection period, milk production was recorded and milk samples were collected from each cow at each AM and PM milking. During milking, three milk samples were collected. Two 50 mL conical tube (Model 430829, Corning Centristar, Corning, NY) samples were frozen at -20° C. The third sample was preserved using 2-bromo-2-nitropropane-1,3 doil and sent off to Heart of American DHIA (Kansas City, MO). These samples were analyzed for protein, fat, lactose, SNF, MUN and SCC using a Bentley FTS/FCM Infrared Analyzer (Bentley Instuments, Chaska, MN). One of the two 50 mL conical tubes was stored at -20° C. The other sample was lyophilized and composited by cow number and period. Milk samples were then analyzed at the

University of Nebraska-Lincoln for lab-corrected DM (100° C oven for 24 hr), gross energy (GE) (Parr 6400 Calorimeter, Moline, IL), and N (FlashSmart N/Protein Analyzer CE Elantech, Inc. Lakewood, NJ).

Total fecal and urine output was collected from each cow during the 5 d collection period (d 23 through d 28 of experimental period). A 137 × 76 cm rubber mat (Snake River Supply, Idaho Falls, ID) was placed behind each cow to aid in fecal collections. During this time personnel were present at all times to collect feces which were deposited into rubber trashcans (87.1 L, Rubbermaid, Wooster, OH). A garbage bag was placed over the trashcan to prevent nitrogen loss before sampling. Feces were subsampled consecutively every day of the 5 day collection period at 1000 h and immediately dried at 60° C in a forced air oven for 48 hours then composited by cow number and period. Samples were then ground through a 1 mm screen (Wiley Mill, Arthur H. Thomas Co., Philadelphia, PA). The ground feces were analyzed at the University of Nebraska-Lincoln for DM (100° C oven for 24 hr), N (FlashSmart N/Protein Analyzer CE Elantech, Inc. Lakewood, NJ), ash corrected NDF (with sodium sulfite and alpha amylase) (Van Soest et al., 1991), GE (Parr 6400 Calorimeter, Moline, IL), starch (Megazyme), and ash.

Urine was collected by inserting a 30 cc French foley catheter (Bard Catheters, Covington, GA, REF 0166L30) into each cow's bladder with a stylus (Tamura et. al, 2014). The balloon was inflated with 50 mL of physiological saline to keep the catheter in place for the duration of the 5 d collection. Urine drained from the catheter into a plastic carboy (14.2 L, Midwest Can Company, Melrose Park, IL) behind the cow using

tygon tubing. A funnel spout on the plastic carboy, urine was deposited in a 55-L plastic container 4 times daily and acidified with 50 mL of HCl at 1730 h. Urine was then subsampled at 1000h every day of the 5 day collection period using two 100 mL bottles. One bottle was dried at 60° in a force air oven to determine DM. The other was frozen at -20° C until analysis which included thawing, and boiling prior to lyophilization. To decrease water content of the urine and improve the ability to detect gross energy, the urine was boiled. To boil, five thawed 100-mL bottles were poured into a 600mL beaker and placed into a heated water bath (Ankom Technology, Macedon, NY) located underneath a fume hood. The water bath was turned on in the morning and off in the afternoon, approximately 6 hr each day, to avoid overheating and burning of the sample. After much of water was removed from each sample, the remaining residue was composited by cow number and period and lyophilized (VisTis Freezemobile 25ES, SP Scientific, Gardiner, NY). Urine samples were then analyzed for lab corrected DM (100° C oven for 24 hr), N (FlashSmart N/Protein Analyzer CE Elantech, Inc. Lakewood, NJ), and GE (Parr 6400 Calorimeter, Moline, IL).

Total mixed rations were sampled (500 g) on the first day of each collection period and frozen at -20°C. Sample were analyzed at the University of Nebraska-Lincoln for of DM (100° C oven for 24 hr), N (FlashSmart N/Protein Analyzer CE Elantech, Inc. Lakewood, NJ), ash corrected NDF (with sodium sulfite and alpha amylase) (Van Soest et al., 1991, Whatman filter papers CAT No. 1541-125), starch (Megazyme), and ash. Feed ingredients were sampled (500 g) on each day of each collection period and were frozen at -20°C. The samples were then composited by period and treatment. A

subsample was sent to Cumberland Valley Analytical Services Inc. (Waynesboro, PA) for complete nutrient analysis of DM (AOAC International, 2000), N (Leco FP-528 N Combustion Analyzer, Leco Corp., St. Joseph, MO), ash corrected NDF (with sodium sulfite and alpha amylase) (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). In addition to the assays previously described, byproducts were also analyzed at Cumberland Valley Analytical Services Inc. (Waynesboro, PA) for rumen and intestinal protein degradability (Ross et al., 2013).

Heat production by cattle was determined through the headbox-type indirect calorimeters described by Foth et al. (2015) and Freetly et al. (2006). Prior to collections, 5 headboxes were used to test the rate of recovery of gas by burning 100% concentration of ethyl alcohol in the sealed headbox and comparing this measure to calculated gas concentrations. These calculations were based on weight of alcohol burned and a measured volume of gas sample. Five lamp runs were conducted. Recovery rates of oxygen (**O_2**) and carbon dioxide (**CO_2**) averaged 101.0 ± 0.04 and 100.8 ± 0.04 %, respectively. For each cow and within each period heat production was estimated 23 hours during which O2 consumption, and CO_2 and methane (CH_4) production was measured and adjusted for a 24 hour period. The design of the headboxes allowed for feed to be placed in the bottom of the box and ad libitum access to water was available for the cows from a water bowl located inside the headbox. Water meters (Model 11

010805, DLJ Meters, Hackensack, NJ) were attached to the water bowl to record water intake. Within the headbox, temperature and dew point were recorded every minute using a probe (Model TRH-100, Pace Scientific Inc., Moorseville, NC) that was connected to a data logger (Model XR440, Pace Scientific Inc., Moorseville, NC). Fifteen min prior to the start of the collection, the doors were closed and the motor was turned on, to allow for several air turnovers before gases were collected. Line pressure was measured using a manometer (Item # 1221-8, United Instruments, Westbury, NY). Barometric pressure of the room was also recorded using a barometer (Chaney Instruments Co., Lake Geneva, WI) and uncorrected for sea level. Total volume of gas that passed through the headbox during each run was measured using a dry gas meter (Model AL425, American Meter, Horsham, PA). From the headbox, continuous samples of outgoing and incoming air were diverted to 2 different collection bags (61 × 61 cm LAM-JAPCON-NSE, 44 L; PMC, Oak Park, IL) using glass tube rotameters (Model 1350E Sho-Rate "50", Brooks Instruments, Hatfield, PA). Collection bags with gas samples inside were analyzed at the University of Nebraska – Lincoln laboratory according to Nienaber and Maddy (1985). Heat production was estimated through calculation of O_2 consumption, and CO_2 and CH_4 production with correction for urinary N loss according to Brouwer (1965; Equation 1; Table 3.10). The gaseous products were reported in liters and the mass of urinary N in grams. Respiratory quotient was calculated using the ratio of CO_2 produced to the O_2 consumed and was not corrected for nitrogen. Volume of CH_4 produced was multiplied by a constant of 9.45 kcal/L to estimate the amount of energy formed from the gaseous

products. Energy balance was calculated for each cow and adjusted for excess N intake according to Freetly et al. (2006) according to the following equations:

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

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

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

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

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

Metabolizable energy for maintenance was calculated using the REG procedure of SAS (SAS Institute Inc., Cary, NC) by regression of RE on ME and is the ME at zero RE as illustrated in Figure 2.2. Tissue energy in protein describes the energy used for tissue protein synthesis (Equation 5). A total of 48 observations were collected and two animals were removed from all four periods of the data set as one cow suffered from a injured teat (ID 2177) and the second cow failed to consume feed while in the headbox (ID 1948). This resulted in a total of 40 energy balances.

Statistical Analysis

Data were analyzed using the mixed procedure of SAS (SAS Institute Inc., Cary, NC). Treatment, square, and period were modeled as fixed effects while cow within block was modeled as a random effect. The LSMEANS option was used to generate

least-squares means of treatments listed in this study. Significance was declared at $P \leq 0.05$ and trends at $0.05 < P \leq 0.10$.

RESULTS

Diet Composition

Chemical composition of the diets are listed in Table 2.4. The CP content of the control was slightly higher than the diets containing byproducts (18.2 ± 0.79, 16.8 ± 0.09, 17.3 ± 0.29, and 17.0 ± 0.40 % for CON, pDDGS, CanM, and aDDGS, respectively). Neutral detergent fiber content was relatively not different across all treatments, albeit slightly lower in the CON treatment (29.2 ± 1.1, 31.1 ± 1.8, 31.2 ± 1.6, and 31.2 ± 0.98 % for CON, pDDGS, CanM, and aDDGS, respectively). Starch content was also not different between diets (26.9 ± 1.9, 26.9 ± 1.4, 26.8 ± 1.2, and 26.5 ± 1.4 % for the CON, pDDGS, CanM, and aDDGS, respectively). Crude fat was greatest in the aDDGS treatment and least in CON (4.95 ± 0.21, 4.61 ±0.12, 4.43 ± 0.11, and 4.24 ± 0.17 % for aDDGS, pDDGS, CanM, and CON, respectively). Particle size of the TMR (as fed basis) were not different between treatments (Table 2.4). The top screen (> 19.0 mm) contained 3.46 ± 0.57, 3.67 ± 0.95, 4.04 ± 1.4, and 4.29 ± 1.4 % for CON, pDDGS, CanM, and aDDGS, respectively. The second screen (8.0-19.0 mm) contained 26.7 ± 3.0, 28.4 ± 1.5, 29.6 ± 1.6, and 28.3 ± 1.4 % for the CON, pDDGS, CanM, and aDDGS, respectively. The third screen (1.18- 8.0 mm) contained 51.2 ± 3.8, 44.7 ± 2.7, 46.7 ± 4.1, and 45.7 ± 2.0 % for CON, pDDGS, CanM, and aDDGS, respectively. Finally, the bottom pan (< 1.18 mm) contained 18.7 ± 2.5, 23.2 ± 2.7, 19.6 ± 2.8, and 21.7 ± 2.1 % for CON, pDDGS, CanM, and aDDGS, respectively.

Feed Intake, Milk Production and Composition

Results of milk production and composition are listed in Table 2.5. Dry matter intake was not different across treatments averaging 17.4 ± 0.56 kg/d (P = 0.437). Milk yield (P = 0.552) and ECM (P = 0.762) was also similar between treatments averaging 24.0 ± 0.80 kg/d and 33.3 ± 1.2 kg/d, respectively. The percent of milk fat (P = 0.937) and fat yield (P = 0.868) were not affected by treatment averaging 6.19 ± 0.17 %, and 1.5 ± 0.06 kg/d, respectively. The percent of milk protein (P = 0.423) and protein yield (P = 0.826) were not different across treatments averaging 3.64 ± 0.04 %, and 0.87 ± 0.03 kg/d, respectively. Lactose was not different (P = 0.878) between treatments and averaged 4.70 ± 0.03 %. Milk urea nitrogen was affected by treatment (P = 0.001; SEM = 0.62), specifically greatest in CON (20.6 mg/dl) and CanM (19.9 mg/dl) and lowest in the DDGS treatments (18.0 and 18.1 mg/dl for pDDGS and aDDGS, respectively). No differences in somatic cell count was detected across treatments (P = 0.346) and averaged 98.0 ± 66.1 cells/ml. Body weight (P = 0.169) and BCS (P = 0.316) was not different between all treatments and averaged 458 ± 11.5 kg, and 3.16 ± 0.09, respectively.

Oxygen Consumption, Carbon Dioxide, Methane, and Heat Production

Results of oxygen consumption, gas production and heat production are listed in Table 2.6. Oxygen consumption did not differ across treatments (P = 0.132) averaging 4058 ± 135 L/d. Carbon dioxide production had a tendency (P = 0.085; SEM = 132 L/d) to be affected by treatment, specifically the control produced the lowest CO_2 (3932 L/d), and this was similar to cows consuming two DDGS treatments (4041 and 4141 L/d, for pDDGS and aDDGS, respectively) but the greatest production was greatest in CanM

(4342 L/d). Methane production was not affected by treatment (P = 0.542) and averaged 340 ± 19.6 L/d across treatments. The respiration quotient averaged 1.01 ± 0.01 L/L and was not different across treatments (P = 0.748). Methane was also reported per unit of milk yield and DMI. Milk produced per L of methane did not differ across treatments (P = 0.628) and averaged 14.3 ± 1.0 kg/L. Methane produced per L of ECM was similar across treatments (P = 0.780) and averaged 10.3 ± 0.69 L/kg. Methane produced per kg of DMI was not affected by treatment (P = 0.787) and averaged 19.7 ± 1.3 L/kg. Heat production was not different across treatments (P = 0.118) and averaged 20.3 ± 0.67 Mcal/d. However, when expressed as a function of metabolic body weight, heat production tended to be affected by treatment (P = 0.058; SEM = 5.91 d/MBW), specifically this was lowest in CON (192 d/MBW), similarly higher in the DDGS treatments (200 and 204 d/MBW for pDDGS and aDDGS, respectively), and highest in CanM (215 d/MBW).

Energy Utilization

Intake, use, and output of energy results are listed in Table 2.7. Gross energy intake (GE intake) was not different between treatments (P = 0.697) and averaged 76.8 ± 2.6 Mcal/d. Digestible energy (DE) (P = 0.357) and metabolizable energy (ME) (P = 0.351) also did not differ between treatments and averaged 48.5 ± 2.2 and 41.7 ± 2.2 Mcal/d, respectively. As a percentage of GE intake, DE (P = 0.141), and ME (P = 0.166) were not affected by treatment and averaged 63.1 ± 1.2 %, and 54.2 ± 1.5 %, respectively. As a percentage of GE intake, fecal (P = 0.142) and urine (P = 0.700) loss were not affected by treatment, averaging 36.8 ± 1.24 % and 4.65 ± 0.34 %. Energy

balance was similar across treatments (P = 0.217) and averaged 21.6 ± 2.3 Mcal/d. No differences in milk energy were detected between treatments (P = 0.260) and when expressed as a percentage of GE intake (P = 0.267) averaged 23.2 ± 1.0 Mcal/d, and 30.6 ± 1.2 %, respectively. Tissue energy did not differ across treatments (P = 0.164) and averaged -1.62 ± 2.0 Mcal/d. Energy balance when expressed as a percentage of GE intake was not affected by treatment (P = 0.117) and averaged 27.9 ± 2.27%. When expressed as Mcal per kg of DM, GE intake was affected by treatment (P = 0.014; SEM = 0.07), specifically greatest in aDDGS (4.62 Mcal/ kg DM) not different from diets containing byproducts, in CON (4.40 Mcal/ kg DM), and similarly least in pDDGS and CanM (4.31 and 4.34 Mcal/kg DM, respectively). Digestible energy (DE) was affected by treatment (P = 0.018; SEM = 0.08) and followed the same pattern as GE specifically greatest in aDDGS (2.98 Mcal/ kg DM), not different from diets containing byproducts, in CON (2.98 Mcal/ kg DM), and least in pDDGS and CanM (2.83 and 2.67 Mcal/kg DM, respectively). Metabolizable energy was affected by treatment (P = 0.034; SEM = 0.09), and again displayed a similar pattern as GE and DE, specifically greatest in aDDGS (2.58 Mcal/ kg DM), not different from diets containing byproducts, in CON (2.46 Mcal/ kg DM), and least in pDDGS and CanM (2.29 and 2.27 Mcal/kg DM, respectively). Energy balance also tended to be affected by treatment (P = 0.062; SEM = 0.11), specifically greatest in CON and aDDGS (1.36 and 1.38 Mcal/ kg DM, respectively), not different form CON, aDDGS, and CanM, in pDDGS (1.14 Mcal/ kg DM) and lowest in CanM (1.06 Mcal/ kg DM).

Nitrogen Utilization

Nitrogen intake, use and excretion are listed in Table 2.8. Total N intake was affected by treatment (P = 0.045), specifically greatest in CON (539 g/d), not different from CON, pDDGS and aDDGS, in CanM (508 g/d), and similarly lowest in DDGS treatments (481 and 495 g/d for pDDGS and aDDGS, respectively). Fecal N was also affected by treatment (P = 0.044), specifically greatest in CanM (181 g/d), not different from diets containing byproducts, in CON (172 g/d) and lowest in DDGS diets (165 and 160 g/d for pDDGS and aDDGS, respectively). As a percentage of N intake, fecal N was affected by treatment (P = 0.038), specifically greatest in CanM (35.6 %), not different from CON, CanM and aDDGS, in pDDGS (34.3 %), and similarly lowest in CON and aDDGS (31.9 and 32.4 %, respectively). Total Urine N was not affected by treatment (P = 0.768) and averaged 230 ± 16.0 g/d. Total N excretion did not differ between treatments and averaged 399 ± 18.4 g/d (P = 0.449). Milk N was not affected by treatment (P = 0.477) and averaged 161 ± 8.34 g/d. Nitrogen balance was not affected by treatment (P = 0.651) and averaged -59.7 ± 26.1 g/d. Tissue energy in protein was not different between treatments (P = 0.632) and averaged -1.87 ± 0.89 g/d. As a percentage of N intake, urine N was not affected by treatment (P = 0.840) and averaged 45.6 ± 3.54 %. As a percentage of N intake, milk N was not affected by treatment (P = 0.188), averaging 32.1 ± 1.49 %. When expressed as a percentage of N intake, N balance was not affected by treatment (P = 0.514) and averaged -12.3 ± 5.19 % across treatments.

Nutrient Digestibility

Nutrient digestibility is listed in Table 2.9. Dry matter digestibility was affected by treatment (P = 0.050; SEM = 1.09), and was greatest in CON and aDDGS (66.7 and 66.0 %, respectively) not different from CON, CanM, and aDDGS, in pDDGS (64.2 %) and lowest in CanM (63.3 %). Organic matter digestibility tended to be affected by treatment (P = 0.062; SEM = 1.02) and followed the same pattern as DMD, specifically it was greatest in CON and aDDGS (68.8 and 68.2 %, respectively), not different from CON, CanM, and aDDGS, in pDDGS (66.3 %) and least in CanM (65.7 %). Ash digestibility was not affected by treatment (P = 0.786) and averaged 33.4 ± 0.79 %. Crude protein digestibility was affected by treatment (P = 0.038; SEM = 1.25), and was greatest in CON and aDDGS (68.1 and 67.6 %, respectively), not different from CON, CanM, and aDDGS, in pDDGS (65.6. %) and lowest in CanM (64.3 %). Starch digestibility was not affected by treatment (P = 0.292) and averaged 92.7 ± 0.29 %. Neutral detergent fiber digestibility was affected by treatment (P = 0.002; SEM = 1.70), and was greatest in CON and aDDGS (47.0 and 45.0 %, respectively), and similarly lower in pDDGS and CanM (41.4 and 39.5 %, respectively).

DISCUSSION

Byproducts have historically played an important role in dairy feeding practices by providing a cost effective source of protein and fiber for dairy farmers (Bradford and Mullins, 2012). The use of these byproducts has also been predicted to increase overall efficiency and productivity of the dairy industry as a whole by utilizing a product that would have be considered waste by the primary production process (VandeHaar and St-Pierre, 2006). The purpose of our research was to compare two of the more popular

byproducts today, canola meal and DDGS, and analyze their effect on digestibility, energy utilization, and milk production.

Diet Composition

Crude protein was formulated to be similar between the control and canola meal treatments, and similar between the DDGS treatments. Crude protein was lowest in pDDGS, not different between CanM and aDDGS, and greatest in the control. This is most likely a result of the increased CP found in the concentrate for the control diet, and the lower CP of the pDDGS which is supported by chemical analysis. Crude fat was formulated to be generally similar between treatments, albeit a bit higher in the aDDGS treatment, which was supported by chemical analysis. The aDDGS treatment contained DDGS that were not centrifuged and were higher in fat than the RFDDGS (10.0 vs 6.0 % crude fat). Both DDGS treatments were included at the same inclusion rate in the diet (10.1%), and considering the increased crude fat of the DDGS in the aDDGS treatment, this is most likely the reason for the increased fat content of the aDDGS diet. However, all diets contained less than the maximum recommended inclusion rate of 7% of DM (NRC, 2001). Fiber content was formulated to be lower in the control, and relatively similar between the diets containing byproducts, which is supported by nutrient composition analysis.

Heat damaged can be estimated by considering the ADICP content of the diet (Kajikawa et al., 2012). The DDGS used in this study were produced differently, specifically the pDDGS were cooked at lower temperatures than the aDDGS. Therefore, we would expect to see differences in the ADICP concentrations of these diets because of

possible heat damage to the aDDGS treatment. As expected lower concentrations of ADICP were observed in the pDDGS diet (1.12 % DM) compared to the aDDGS diet (1.21% DM). Additionally, the RFDDGS in the pDDGS diet contained lower concentrations of ADICP (2.01 % DM) compared with the DDGS used in the aDDGS diet (2.90 %). Greater ADICP may not necessarily lead to reduced N digestibility ((Nakamura et al., 1994), and this will be discussed in further detail in the current study.

General recommendations of particle size are 2 to 8 % for the >19.0-mm screen, 30 to 50 % for the 8.0 and 1.18-mm screen, and ≤ 20 % the bottom pan (Heinrichs and Kononoff, 2002). All treatments were slightly lower than 30 % for the 8.0 to 19.0-mm screen. The CON treatment had a slightly greater amount of feed than recommended for the 1.18 to 8.0 screen (51.2 %). And finally, the pDDGS treatment had a greater amount of particles than recommended on the bottom pan (23.2 %). One consequence of feeding a diet of fine particle size may be less rumination and as a consequence less salvia may be produced to buffer the rumen (NRC, 2001; Zebeli et al., 2010, White et al., 2017). Although ruminal pH and rumination were not recorded for the current study, milk fat percentage was maintained across all treatments suggesting that effective fiber was adequate.

Nutrient Digestibility

Previous research conducted using in vitro or in situ methods have reported intestinal RUP digestibility values for DDGS to be greater than canola meal (NRC, 2001; Paz et al., 2014). However, more recently Lawrence and Anderson (2018) reported the intestinal CP digestibility in vitro to be greatest in soybean meal (81 %) and in contrast

with Paz et al (2014), reported digestibility to be similar between canola meal (71 %) and DDGS (63 %). In the current study, the RUP and dRUP were greatest for RFDDGS (94 % and 84 %, respectively), lower in DDGS (74 % and 77 %, respectively) and lowest in canola meal (58 % and 72 %, respectively). Additionally, total tract digestibility of CP was observed to be lowest in cattle consuming canola meal (64.3 %) compared with cattle consuming pDDGS (65.6 %) or aDDGS (67.6 %). These results are similar with values generated using in vitro or in situ procedures to estimate of the digestibility of CP of canola (Paz et al., 2014; NRC, 2001). Canola meal has been predicted to have lower fiber digestibility than soybean meal, due to its greater content of indigestible NDF (Shingfield et al., 2003; Huhtanen et al., 2011). In the current study, compared to the control the NDF digestibility of cattle consuming diets containing canola meal was lowest. This is likely a result of a greater lignin content of the CanM treatment which was greater than the CON treatment in the current study (3.99 % and 3.18 % of DM, respectively), and is known to reduce digestibility of NDF. This increased lignin content of canola meal may be due to hardness of the seed coat of canola seeds and this property may resist degradation by rumen microbes (Bell, 1993). About 16 % of the seed weight represents the hull, which is primarily fiber, and is carried through processing to canola meal, representing about 30 % of meal weight (Bell and Shires, 1982).

As mentioned previously, the DDGS used in the current study differed in way of processing; specifically the RFDDGS used in the pDDGS treatment were developed through heating at lower temperatures compared to the DDGS in the aDDGS treatment. Previous research has suggested that DDGS cooked at a lower temperature have resulted

in a modest improvement in in vitro NDF digestibility when compared to DDGS cooked at higher temperatures (Dufour et al., 2017). Therefore, we hypothesized that the pDDGS would have a higher NDF digestibility than aDDGS and canola meal. However, this was not observed, as NDF digestibility was greater for the aDDGS (45.0 %) compared with pDDGS (41.4 %).

It was also predicted that the pDDGS may have a higher CP digestibility than the aDDGS, also due to processing differences. Cooking the product at higher temperatures may increase the chance for heat damage, which may damage a considerable portion of the protein, making it unavailable for the animal (Kleinschmit et al., 2007). Previous research has shown ADICP to be a predictor of heat damage (Kajikawa et al., 2012). Considering pDDGS contained a lower content of ADICP compared to aDDGS, it could be predicted that digestibility of this treatment would be greater than aDDGS. However, somewhat unexpectedly in the current study aDDGS, which were produced in a corn ethanol process that uses more heat, resulted in a greater digestibility of CP. These results support the suggestion that unless the feeds have experienced extensive heat damage that ADICP is not an accurate predictor of the digestibility of protein (Nakamura et al., 1994).

Previous comparison of wheat DDGS and canola meal treatments have investigated digestibility and reported no differences in DM, NDF or OM digestibility (Chibisa et al., 2012; Mutsvangwa et al., 2016). In the current study, the control and aDDGS contained the greatest CP and NDF digestibility, which contributed to the greatest DMD. The canola meal treatment contained the lowest NDF and CP digestibility, which contributed to the lowest DMD.

Energy Utilization

Nutrients contain different heats of combustion, or provide different amounts of gross energy to the animal, specifically 4.2 Mcal/kg for carbohydrates, 5.6 Mcal/kg for proteins, and 9.4 Mcal/kg for long chain fatty acids (Maynard et al., 1979). Therefore, it was expected that aDDGS would contain a greater concentration of GE due to its greater concentration of crude fat. Crude fat content was greatest in the aDDGS (4.95 %) compared to the pDDGS or CanM diets (4.61 and 4.43 %, respectively), which likely explains the increased amount of energy reported in our results. Gross energy (Mcal/kg of DM) was greatest in aDDGS, lower in CON, and similarly lowest in pDDGS and CanM. The same pattern is reported in DE and ME, with the greatest concentrations in aDDGS. The energy balance was greatest in the CON and aDDGS diets.

One item of interest when comparing the two sources of DDGS for the current study, was if the pDDGS, which were cooked at a lower temperature, would have an increased amount of energy available to the animal compared to the aDDGS. This was of interest due to the increased total tract NDF digestibility observed by Dufour et al. (2017) and the possibility of greater CP digestion due to less heat damage. However, results of the current study demonstrate pDDGS contained less gross, digestible, and metabolizable energy than the aDDGS. This is primarily a consequence of the greater crude fat concentrations (Maynard et al., 1979) observed in the aDDGS treatment.

The pattern of energy utilization when expressed as Mcal/d or percent of GE did not differ between treatments and is generally comparable to other energy balance studies. As a percentage of GE, energy lost as feces averaged 37 % which is compared to values corresponding with control diets fed to lactating dairy cattle of 31 % (Tine et al., 2001), 33 % (Birkelo et al., 2004; Foth et al., 2015). Urine as a percentage of GE averaged 4.6 % which is slightly greater than reported by Tine et al. (2001), Birkelo et al. (2004) and Foth et al. (2015) who reported urine losses to be 3.9, 3.7, and 3.6 %, respectively. The increased urine energy in the current study may be a result of catabolized protein from body stores are that were used for energy and excreted as urea in urine (Maltz and Silanikove, 1996), which is discussed in further detail in the nitrogen utilization section of the current study. Methane loss as a percentage of GE averaged 4.2 % which is lower than Tine et al. (2001) and Foth et al. (2015) who reported methane loss to be 5.7 % of GE, but comparable to Birkelo et al. (2004) at 4.4 % of GE.

Oxygen Consumption and Carbon Dioxide, Methane, and Heat Production

Carbon dioxide and methane are natural byproducts of microbial fermentation of carbohydrates and to a smaller degree amino acids in the rumen and hindgut (Hristov et al., 2013). When substrates are fermented in the rumen, volatile fatty acids and metabolic hydrogens are produced. These metabolic hydrogens and CO_2 combine to form methane (Knapp et al., 2014). Methane production is an efficient way to remove these hydrogens, which is necessary for fermentation to continue (McAllister and Newbold, 2008). Propionate production in the rumen, which is associated with concentrate diets, is a consumer of hydrogens. Therefore, an increase in propionate production can result in a

decrease in methane production per unit of feed fermented, although the opposite is true for acetate and butyrate, which are primarily produced on forage diets (Van Nevel and Demeyer, 1996). Methane production accounts for a 2 to 12 % loss of feed energy intake of dairy cows (Johnson and Johnson, 1995). Decreasing methane emissions may increase the ME available and improve energy utilization of the animal (Hynes et al., 2016). In the current study, carbon dioxide production was increased with the addition of byproduct in the diet compared to the control. Methane production was unaffected by treatment. This is in contrast to Foth et al. (2015) who reported a decrease in carbon dioxide and methane production with the inclusion of reduced fat DDGS (RFDDGS) in the diet. In agreement with the current study, Castillo-Lopez et al. (2017) reported total methane production was not reduced when diets containing RFDDGS or DDGS were fed. Previous studies have evaluated the effect canola meal has on methane production. Gidlund et al. (2015) reported methane emissions per unit of ECM decreased when canola meal was used as the protein source in place of soybean meal for lactating dairy cows. The response in this study was due partly to slightly reduced emissions per kg of DMI, and greater ECM yield. Additionally, Paula et al. (2017) found that methane production (g/kg of DM) tended to be decreased by canola meal versus soybean meal in vitro.

The respiratory quotient (RQ) is the ratio of the volume of carbon dioxide produced by the volume of oxygen consumed (Kim et al., 2015). The RQ may be used as a rough proxy for the type of substrate being utilized or what metabolic process is occurring in the animal. For the oxidation of carbohydrates, the RQ is expected to be

close to 1.00, as 6 mols of carbon dioxide are produced and 6 mols of oxygen are consumed. In comparison, when fat is oxidized the RQ is closer to 0.7 (Brody, 1945). The RQ of the current study was similar between treatments and averaged 1.01 ± 0.01, which is to be expected because the primary energy source was carbohydrates. This result is also in similar with previous energy studies with lactating dairy cattle, where the RQ ranged from 0.99 to 1.01 (Derno et al., 2009; Aubry and Yan, 2015). When expressed as a function of MBW, heat production was the greatest for animals consuming CanM, followed by aDDGS, pDDGS, and the CON. Heat production is important because it contributes to the amount of NE_L that can progress to milk production (i.e. higher heat production leads to less energy available for milk production). It is also important to note that energy lost as milk was not affected by treatment, which would imply that cattle consuming canola meal, although losing more energy as heat, were still able to divert generally the same energy towards milk production as cattle consuming the CON and DDGS treatments.

Milk Production and Composition

Previous research comparing DDGS and canola meal treatments have reported similar DMI (Mulrooney et al., 2009; Mutsvangwa et al., 2016). However, this is in contrast with Chibisa et al. (2012) who reported increasing DMI when wheat DDGS replaced canola meal at increasing levels in the diet. In the current study, the addition of byproducts to the diet did not affect DMI and percent refusals (orts/DMI intake) averaged 3.4 ± 0.03 % for all cows for the entire study. Dry matter intake increases with decreased particle size (Kononoff et al., 2003). The similar DMI could be explained by the similar

particle size of the byproducts and the products they were replacing (ground corn and soybean meal). Another explanation may be that the low inclusion rate of byproducts (10 %) in the current study was lower than previous research. Chibisa et al., (2012) reported differences in DMI with wheat DDGS inclusion rates of up to 20 %. In addition, Janicek et al. (2008) reported that dairy diets containing as much as 30 % of DDGS may result in an increase in DMI. Substituting byproducts in diets with high starch concentrations has been reported to have positive associate effects such as increased milk fat (Weiss, 2012), NDF digestibility (Allen and Grant, 2000), and increased feed efficiency (Boddugari et al., 2001). In the current study, the starch content was similar across all diets, ranging from 26.5 to 26.9 % of DM. Perhaps the low inclusion rate of byproducts in the current study did not displace enough starch to display the positive associate effects that are described by previous research.

In the current study, differences in energy content and digestibility of treatments did not translate into differences in milk production or composition. Milk yield and ECM were similar between the canola meal and DDGS treatments, which is similar with several previous studies (Maxin et al., 2013; Acharya et al., 2015; Mutsvangwa et al., 2016) and expected, as diets were formulated to for equal milk production between diets. However, in contrast to the current study, Chibisa et al. (2012) observed a 1.2 to 1.8 kg increase in milk yield when replacing canola meal with wheat DDGS, which was attributed to an increase in DMI. Dried distillers grains have been reported to contain rumen-available unsaturated fatty acids, which can contribute to milk fat depression (Hippen et al., 2010). However, Weiss (2012) reported that addition of 25 % of a corn

milling product increased milk fat when replacing a concentrate, but decreased milk fat when replacing forage and concentration. Additionally, Weiss et al. (2015) reported milk fat to linearly increase from 3.28 % to 3.34 % fat when the inclusion of canola meal in the diet was increased from 3.9 % to 13.9 % and fed to Holstein dairy cattle. The results of our study suggest that milk fat can be maintained when DDGS containing varying crude fat levels and canola meal are replacing corn and soybean meal at 10 % of the diet. The inclusion of DDGS in diets may decrease milk protein due to an unbalanced supply of amino acids, particularly lysine (Carvalho et al., 2006). However, Nichols et al. (1998) reported improved milk protein production when ruminally protected Lys and Met were fed in diets containing 20 % DDGS. In the current study ruminally protected lysine and methionine were supplemented to ensure amino acid requirements were met. Milk protein was maintained and milk urea nitrogen (MUN) was reduced when byproducts were added to the diets compared to the control. This is surprising because CP digestibility and N balance were greatest in the control, possibly predicting that less N would be translated into milk. This small difference may be due to the greater protein content in the CON treatment (18.2 %) versus the byproducts treatments and therefore the cows excreted more N.

Nitrogen Utilization

Several studies have examined the N balance or N efficiencies between DDGS and canola meal. Contrary to the current study, similar N intakes have been reported between DDGS and canola meal (Chibisa et al., 2012; Maxin et al. 2013; Mutsvangwa et al., 2016). In the current study, nitrogen intake was affected by treatment and was

specifically greatest for CON, lower in CanM, and similarly lowest in pDDGS and aDDGS. Total fecal N output was also affected by treatment with the greatest output from CanM, lower in CON, and similarly lowest in pDDGS and aDDGS. As a percentage of N intake, fecal output was also greatest in CanM, lower in pDDGS, and similarly lowest in aDDGS and CON. The greater fecal output in canola meal is likely a result of the lower CP digestibility. Greater N outputs may result in environmental consequences such as nitrate leaching into water, which leads to eutrophication (Arriaga et al., 2009). Nitrogen balance was not affected by treatment but was numerically negative for all treatments, specifically lowest in the pDDGS treatment. Nitrogen balance is the N remaining after subtracting N lost in milk, feces, and urine. Negative nitrogen balances have been associated with negative energy balances, where catabolized protein from body stores are used for energy and excreted as urea in urine (Maltz and Silanikove, 1996). This may have been the case for the current study, as pDDGS contained a low energy content and for all treatments N loss was greatest through the urine.

CONCLUSIONS

Results of this current study support previous research that the addition of byproducts such as DDGS and canola meal maintain milk production and composition in lactating dairy cattle. Metabolizable and digestible energy were increased in the in DDGS containing highest concentrations of crude fat treatment. As hypothesized, digestibility of CP was lowest in the cattle consuming canola meal. Future research should be conducted

to gather a better understanding of how the digestibility of CP in canola meal could be improved.

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TABLES AND FIGURES

Table 2.1. Diet composition of control (CON), reduced-fat distillers grains and solubles (pDDGS), canola meal (CanM), and an alternative source of dried distillers grains and solubles (aDDGS)

	Treatment			
	CON	**pDDGS**	**CanM**	**aDDGS**
	% of DM			
Corn silage	40.5	40.5	40.5	40.5
Alfalfa hay	13.3	13.3	13.3	13.3
Brome hay	1.15	1.15	1.15	1.15
Ground corn	16.8	15.0	15.8	15.0
Soybean meal	13.7	5.50	4.70	5.50
DDGS	--	10.1	--	10.1
Canola meal	--	--	10.1	--
Soybean hulls	2.52	2.52	2.52	2.52
Expellers soybean meal[1]	4.58	4.58	4.58	4.58
Calcium carbonate	1.37	1.37	1.37	1.37
Tallow	1.95	1.95	1.95	1.95
Bloodmeal	1.53	1.53	1.53	1.53
Ca salts of LCFA[2]	0.80	0.80	0.80	0.80
Magnesium oxide	0.32	0.32	0.32	0.32
Sodium bicarbonate	0.57	0.57	0.57	0.57
Dicalcium phosphate	0.34	0.34	0.34	0.34
Salt	0.25	0.25	0.25	0.25
Vitamin mineral premix[3]	0.05	0.05	0.05	0.05
Trace mineral premix[4]	0.04	0.04	0.04	0.04
Rumen protected lysine[5]	0.05	0.05	0.05	0.05
Rumen protected methionine[6]	0.05	0.05	0.05	0.05
Formulated chemical composition[7]				
CP, % DM	18.6	17.1	18.1	16.9
Crude fat, % DM	5.5	5.8	5.7	6.0
NDF, % DM	28.7	31.0	30.5	31.0
Lignin, % DM	2.90	3.00	3.60	3.0
Ash, % DM	8.40	8.40	8.60	8.40
Starch, % DM	28.3	27.2	27.7	27.2
NFC[8], % DM	41.7	40.6	40.8	40.6
Gross energy, cal/g				
ME, Mcal/kg	2.71	2.73	2.71	2.73
NE_L, Mcal/kg	1.74	1.76	1.74	1.76

[1]Soypass, LignoTech, Overland Park, KS. [1]SoyPass, LignoTech, Overland Park, KS.
[2] Calcium salts of long-chain fatty acids marketed as Megalac by Church & Dwight Co. Inc. Princeton, NJ.
[3] 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.
[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] AjiPro-L, Ajinomoto Heartland Inc., Chicago, IL.
[6]SmartamineM, Adisseo Inc., Antony, France.
[7]Values formulated from CPM dairy model.
[8]NFC = Nonfiber carbohydrate calculated by difference 100 – (% NDF + % CP + % Fat + % Ash).

Table 2.2. Chemical composition for individual ingredients of corn silage, alfalfa hay, brome hay, reduced-fat dried distillers' grains with solubles (RFDDGS) concentrate, canola meal concentrate, and dried distillers' grains with solubles (DDGS) concentrate (DM basis)[1]

	Corn Silage		Alfalfa Hay		Brome Hay		Control Concentrate		pDDGS Concentrate		CanM Concentrate		aDDGS Concentrate	
Chemical	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
DM, %	95.8	0.507	95.2	0.616	93.7	0.804	96.2	0.287	96.3	0.311	95.9	0.330	96.3	0.173
CP, % DM	8.15	0.436	14.6	1.086	7.83	0.419	28.5	1.374	25.6	0.450	26.6	0.263	25.8	0.638
Soluble protein, % DM	4.68	0.877	6.35	0.889	2.40	0.594	5.48	1.159	6.90	0.523	7.13	1.063	6.10	1.116
ADICP[2], % DM	0.87	0.388	1.92	0.297	1.18	0.319	0.62	0.327	1.11	0.361	1.41	0.877	1.31	0.194
NDICP[3], % DM	0.94	0.361	3.50	0.326	2.23	1.291	2.12	0.318	2.20	0.079	2.98	0.141	2.62	0.129
ADF, % DM	23.0	2.651	45.8	2.048	41.9	0.520	5.63	0.759	7.28	0.929	9.50	1.169	8.35	0.473
NDF, % DM	37.3	3.612	55.2	2.865	60.9	8.592	13.5	1.079	17.68	0.991	17.9	0.768	18.0	0.968
Lignin, % DM	3.36	0.400	10.1	0.553	5.92	0.915	0.92	0.336	1.47	0.159	2.73	0.145	1.98	0.453
NFC[4], % DM	47.1	4.685	20.0	1.726	20.8	8.802	40.5	3.289	39.23	1.350	38.59	0.872	39.1	1.602
Sugar, % DM	0.78	0.206	3.35	0.545	7.33	0.822	6.30	0.356	4.53	0.340	5.18	0.287	5.25	0.695
Starch, % DM	35.6	3.559	0.85	0.580	0.78	0.918	27.5	2.640	27.38	0.568	27.1	0.497	26.4	0.594
Crude fat, % DM	2.79	0.198	0.75	0.185	1.22	0.212	6.65	0.225	7.49	0.386	7.08	0.095	8.23	0.367
Ash, % DM	4.61	0.744	9.41	0.502	9.19	0.261	10.9	1.555	10.04	0.798	9.78	0.655	8.91	0.369
Ca, % DM	0.21	0.021	0.89	0.087	0.36	0.019	2.19	0.484	1.99	0.040	2.14	0.209	1.81	0.127
P, % DM	0.23	0.029	0.35	0.008	0.27	0.010	0.64	0.047	0.72	0.096	0.70	0.048	0.69	0.022
Mg, % DM	0.15	0.025	0.20	0.014	0.12	0.006	0.59	0.066	0.62	0.033	0.65	0.019	0.61	0.053
K, % DM	1.08	0.082	3.96	0.099	2.33	0.085	1.36	0.074	1.21	0.048	1.14	0.045	1.24	0.048
S, % DM	0.13	0.006	0.16	0.016	0.15	0.031	0.34	0.005	0.47	0.034	0.42	0.008	0.44	0.005
Na, % DM	0.02	0.000	0.03	0.000	0.02	0.000	0.67	0.135	0.86	0.282	0.62	0.058	0.69	0.038
Cl, % DM	0.14	0.040	0.11	0.006	0.28	0.010	0.45	0.130	0.64	0.405	0.35	0.038	0.40	0.021
Fe, mg/kg	157	45.29	187	42.51	182	26.61	383	126.9	468	41.26	425	35.98	437	65.88
Zn, mg/kg	27.5	3.697	20.5	1.291	19.5	1.915	194	34.61	252	142.3	206	40.72	181	16.42
Cu, mg/kg	9.50	5.686	8.00	0.000	7.00	0.816	34.0	5.354	45.3	14.84	31.5	3.109	36.8	2.986
Mn, mg/kg	31.5	3.873	24.5	2.646	49.8	1.258	116	23.26	157	44.44	120	13.19	112	25.79

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

[2]ADICP = Acid detergent insoluble crude protein.

[3]NDICP = Neutral detergent insoluble crude protein.

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

Table 2.3. Chemical composition of byproducts[1,2]

	RFDDGS		CM		HFDDGS	
Chemical	Mean	SD	Mean	SD	Mean	SD
DM, %	89.8	0.698	89.7	0.071	89.7	0.071
CP, % DM	30.8	0.538	41.1	0.354	32.2	0.495
Soluble protein, % DM	8.53	0.695	13.3	0.636	7.65	0.217
RUP[3], % CP	94.3	2.376	58.3	0.000	74.2	0.000
dRUP[3], % RUP	83.7	1.658	71.8	0.000	77.3	0.000
ADICP[3], % DM	2.01	0.155	2.39	0.106	2.90	0.127
NDICP[4], % DM	2.69	0.549	4.28	0.078	3.83	0.078
ADF, % DM	11.7	1.748	19.3	0.283	11.9	0.141
NDF, % DM	32.2	2.171	27.5	0.495	31.8	0.990
Lignin, % DM	3.40	0.132	9.02	0.170	3.59	0.764
NFC[5], % DM	24.5	3.23	17.9	0.120	20.1	1.499
Sugar, % DM	5.15	0.404	10.9	0.354	5.20	0.283
Starch, % DM	6.68	1.684	0.30	0.141	2.60	0.141
Ether extract, % DM	6.05	0.379	3.46	0.085	10.0	0.134
Ash, % DM	6.40	1.008	10.3	0.177	5.85	0.120
Ca, % DM	0.08	0.062	2.01	0.092	0.05	0.000
P, % DM	0.91	0.077	1.17	0.000	0.97	0.014
Mg, % DM	0.35	0.010	0.70	0.000	0.38	0.000
K, % DM	1.44	0.123	1.41	0.120	1.48	0.028
S, % DM	1.14	0.055	0.81	0.007	1.09	0.035
Na, % DM	0.35	0.089	0.12	0.007	0.22	0.000
Cl, % DM	0.46	0.552	0.06	0.000	0.20	0.007
Fe, mg/kg	104	22.17	207	14.14	142	2.121
Zn, mg/kg	59.0	5.944	92.5	7.778	64.5	3.536
Cu, mg/kg	2.75	0.500	26.5	0.707	5.00	0.000
Mn, mg/kg	17.3	2.630	92.5	3.536	19.5	0.707

[1]Values determined by Cumberland Valley Analytical Services, Hagerstown, MD.
[2] RFDDGS= Reduced-fat dried distillers' grains with solubles, CM = Canola Meal, HFDDGS = High fat dried distillers' grains with solubles
[3]Values determined at Cumberland Valley Analytical Services Inc. (Waynesboro, PA) according to Ross et al. (2013).
[4]ADICP = Acid detergent insoluble crude protein.
[5]NDICP = Neutral detergent insoluble crude protein.
[6]NFC = Nonfiber carbohydrate calculated by difference 100 – (% NDF + % CP + % Fat + % Ash).

Table 2.4. Chemical composition and particle distribution of treatment diets differing in byproducts based on the feed ingredients

	Treatment[1,2]							
	CON		**pDDGS**		**CanM**		**aDDGS**	
Chemical	Mean	SD	Mean	SD	Mean	SD	Mean	SD
DM, %	70.6	1.219	70.7	1.072	70.8	1.237	70.5	1.137
CP, % DM	18.2	0.789	16.8	0.095	17.3	0.292	17.0	0.405
Soluble protein, % DM	5.23	0.270	5.87	0.496	5.97	0.643	5.51	0.766
ADICP[3], % DM	0.90	0.248	1.12	0.352	1.26	0.440	1.21	0.130
NDICP[4], % DM	1.82	0.154	1.86	0.133	2.21	0.094	2.05	0.116
ADF, % DM	18.4	1.298	19.1	0.962	20.1	1.422	19.6	1.114
NDF, % DM	29.2	1.083	31.1	1.779	31.2	1.620	31.2	0.985
Lignin, % DM	3.18	0.206	3.43	0.240	3.99	0.260	3.66	0.258
NFC[5], % DM	40.2	1.867	39.7	2.082	39.4	2.089	39.6	1.110
Sugar, % DM	3.68	0.222	2.88	0.163	3.17	0.201	3.21	0.357
Starch, % DM	26.9	1.886	26.9	1.367	26.8	1.246	26.5	1.365
Crude fat, % DM	4.24	0.175	4.61	0.116	4.43	0.112	4.95	0.213
Ash, % DM	8.12	0.776	7.74	0.615	7.63	0.334	7.24	0.122
Ca, % DM	1.19	0.230	1.10	0.005	1.17	0.083	1.02	0.061
P, % DM	0.43	0.030	0.46	0.042	0.46	0.013	0.45	0.011
Mg, % DM	0.35	0.040	0.37	0.012	0.38	0.013	0.36	0.029
K, % DM	1.60	0.033	1.54	0.025	1.50	0.043	1.55	0.048
S, % DM	0.23	0.006	0.28	0.013	0.26	0.002	0.27	0.006
Na, % DM	0.32	0.061	0.40	0.127	0.29	0.026	0.32	0.017
Cl, % DM	0.27	0.068	0.36	0.168	0.23	0.021	0.25	0.025
Fe, mg/kg	263.2	56.71	301.4	32.88	282.2	20.90	287.3	17.69
Zn, mg/kg	101.5	14.31	127.5	65.49	106.7	17.50	95.4	6.738
Cu, mg/kg	20.3	1.006	25.4	8.857	19.2	3.139	21.5	2.980
Mn, mg/kg	68.7	11.22	87.4	20.51	70.6	7.127	66.9	10.36
Particle size[6]								
> 19.0 mm	3.46	0.574	3.67	0.951	4.04	1.411	4.29	1.406
8.0 – 19.0 mm	26.7	3.052	28.4	1.544	29.6	1.624	28.3	1.379
1.18 – 8.0 mm	51.2	3.850	44.7	2.708	46.7	4.136	45.7	2.004
< 1.18 mm	18.7	2.540	23.2	2.743	19.6	2.775	21.7	2.128

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

[2] CON = Control, pDDGS= Reduced-fat dried distillers grains and solubles, CanM = Canola meal, aDDGS = High fat dried distillers grains and solubles.

[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 as fed basis (Heinrichs and Kononoff, 2002).

Table 2.5. DMI, milk production and components, body weight, and BCS of treatments differing in byproducts

	Treatment[1]					
	CON	**pDDGS**	**CanM**	**aDDGS**	**SEM[2]**	***P*-value**
DMI, kg/d	17.5	17.4	17.6	17.0	0.558	0.437
Milk yield, kg/d	23.4	24.2	24.2	24.4	0.804	0.552
ECM[3], kg/d	32.7	33.3	33.4	33.8	1.234	0.762
Fat, %	6.23	6.11	6.20	6.17	0.167	0.937
Fat yield, kg/d	1.46	1.48	1.49	1.50	0.063	0.868
Protein, %	3.67	3.63	3.60	3.64	0.045	0.423
Protein yield, kg/d	0.86	0.87	0.87	0.89	0.030	0.826
Lactose, %	4.71	4.69	4.70	4.71	0.033	0.878
MUN[4], mg/dl	20.6[a]	18.0[b]	19.9[a]	18.1[b]	0.620	0.001
SCC[5], cells/ml	43.5	190	91.8	66.6	66.14	0.346
Body weight, kg	461	460	454	459	11.50	0.169
BCS[6]	3.23	3.21	3.03	3.19	0.088	0.316

[1]CON = Control, pDDGS = Reduced-fat dried distillers grains and solubles, CanM = Canola meal, aDDGS = High fat dried distillers grains and solubles.

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

[3]Energy correct 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).

[4]MUN = Milk urea nitrogen.

[5]SCC = Somatic cell count.

[6]BCS = Body condition score, 1-5 according to Wildman et al. (1982).

Table 2.6. Daily consumption of oxygen and production of carbon dioxide and methane for treatments differing in byproducts

	Treatment[1]					
	Control	**pDDGS**	**CanM**	**aDDGS**	**SEM[2]**	***P*- value**
O_2 consumption, L/d	3873	4007	4262	4092	135	0.132
CO_2 production, L/d	3932[b]	4041[ab]	4342[a]	4141[ab]	132	0.085
CH_4 production, L/d	335	329	360	337	19.6	0.542
RQ[3], L/L	1.01	1.01	1.02	1.01	0.007	0.748
Milk produced/CH_4, kg/L	14.4	13.9	15.1	13.9	1.00	0.628
CH_4/ECM[4], L/kg	10.3	10.2	10.8	10.0	0.695	0.780
CH_4 /DMI, L/kg	19.3	19.0	20.5	19.9	1.27	0.787
Heat production[4], Mcal/d	19.2	19.9	21.2	20.3	0.668	0.118
Heat production[5], d/MBW	192.4[b]	200.0[ab]	214.9[a]	203.8[ab]	5.91	0.058

[1]CON = Control, pDDGS = Reduced-fat dried distillers grains and solubles, CanM = Canola meal, aDDGS = High fat dried distillers grains and solubles.

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

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

[4]Energy correct 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]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 2.7. Energy partitioning of treatments differing in byproducts

	Treatment[1]					
Item[2]	**Control**	**pDDGS**	**CanM**	**aDDGS**	**SEM[3]**	***P*- value**
			Mcal/d			
GE intake	76.8	75.1	76.6	78.5	2.58	0.697
DE	49.4	46.8	47.1	50.7	2.20	0.357
ME	42.9	40.1	40.0	43.9	2.23	0.351
Component						
Feces	27.4	28.3	29.6	27.8	1.09	0.371
Urine	3.30	3.59	3.68	3.67	0.238	0.639
Methane	3.16	3.11	3.40	3.18	0.185	0.542
Heat	19.2	19.9	21.2	20.3	0.668	0.118
Milk	22.0	23.8	22.7	24.5	1.05	0.260
Tissue	1.76	-3.52	-3.81	-0.90	1.99	0.164
Balance[4]	23.7	20.2	18.9	23.6	2.27	0.217
			% of GE			
Feces	35.7	37.7	38.4	35.6	1.24	0.142
Urine	4.30	4.81	4.80	4.70	0.34	0.700
Methane	4.17	4.17	4.46	4.06	0.27	0.621
Heat	25.1	26.6	27.8	25.9	1.04	0.292
Milk	29.1	32.0	29.8	31.5	1.19	0.267
Tissue	0.16	-0.05	-0.05	-0.02	0.03	0.168
Balance[4]	30.7	26.6	24.6	29.7	2.27	0.117
DE	64.3	62.2	61.6	64.4	1.24	0.141
ME	55.8	53.3	52.3	55.6	1.48	0.166
			Mcal/kg of DM			
GE	4.40^{ab}	4.31^{b}	4.34^{b}	4.62^{a}	0.068	0.014
DE	2.83^{ab}	2.68^{b}	2.67^{b}	2.98^{a}	0.082	0.018
ME	2.46^{ab}	2.29^{b}	2.27^{b}	2.58^{a}	0.089	0.034
Balance[4]	1.36^{a}	1.14^{ab}	1.06^{b}	1.38^{a}	0.111	0.062

[1]CON = Control, pDDGS = Reduced-fat dried distillers grains and solubles, CanM = Canola meal, aDDGS = High fat dried distillers grains and solubles.

[2]GE = gross energy; DE = digestible energy, ME = metabolizable energy.

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

[4]Balance = Milk Energy + Tissue Energy.

Table 2.8. Nitrogen partitioning of treatments differing in byproducts

	Treatment[1]					
Item	**CON**	**pDDGS**	**CanM**	**aDDGS**	**SEM[2]**	***P*- value**
Mass	g/d					
N intake	539[a]	481[b]	508[ab]	495[b]	17.97	0.045
Fecal N	172[ab]	165[b]	181[a]	160[b]	7.142	0.044
Urine N	234	216	236	233	15.70	0.768
Total N excretion[3]	406	381	417	393	18.42	0.449
Milk N	168	163	150	163	8.34	0.477
N balance[4]	-34.0	-81.1	-61.2	-62.7	26.15	0.651
TE in protein[5]	-0.97	-2.62	-1.82	-2.08	0.8943	0.632
N intake	% of N intake					
Fecal N	31.9[b]	34.3[ab]	35.6[a]	32.4[b]	1.246	0.038
Urine N	43.1	45.3	46.6	47.3	3.539	0.840
Milk N	31.5	34.0	29.6	33.2	1.493	0.188
N balance[4]	-6.30	-17.4	-12.2	-13.3	5.188	0.514

[1]CON = Control, RFDDGS= Reduced-fat dried distillers grains and solubles, CanM = Canola meal, aDDGS = High fat dried distillers grains and solubles.

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

[3]Fecal N + Urine N.

[4]Nitrogen balance = intake N – fecal N – urine N – milk N.

[5]TE = Tissue Energy.

Table 2.9. Apparent digestibility of treatments differing in byproducts

	Treatment[1]					
Component	**CON**	**pDDGS**	**CanM**	**aDDGS**	**SEM[2]**	***P*-value**
DM, %	66.7[a]	64.2[ab]	63.3[b]	66.0[a]	1.094	0.050
OM, %	68.8[a]	66.3[ab]	65.7[b]	68.2[ab]	1.025	0.062
Ash, %	34.5	34.9	31.3	32.9	3.425	0.786
CP, %	68.1[a]	65.6[ab]	64.3[b]	67.6[a]	1.246	0.038
Starch, %	92.5	92.8	92.3	93.2	0.430	0.292
NDF[3], %	47.0[a]	41.4[b]	39.5[b]	45.0[a]	1.701	0.002

[1]CON = Control, pDDGS = Reduced-fat dried distillers grains and solubles, CanM = Canola meal, aDDGS = High fat dried distillers grains and solubles.
[2]Lowest standard error of treatment means is shown.
[3]Ash corrected NDF.

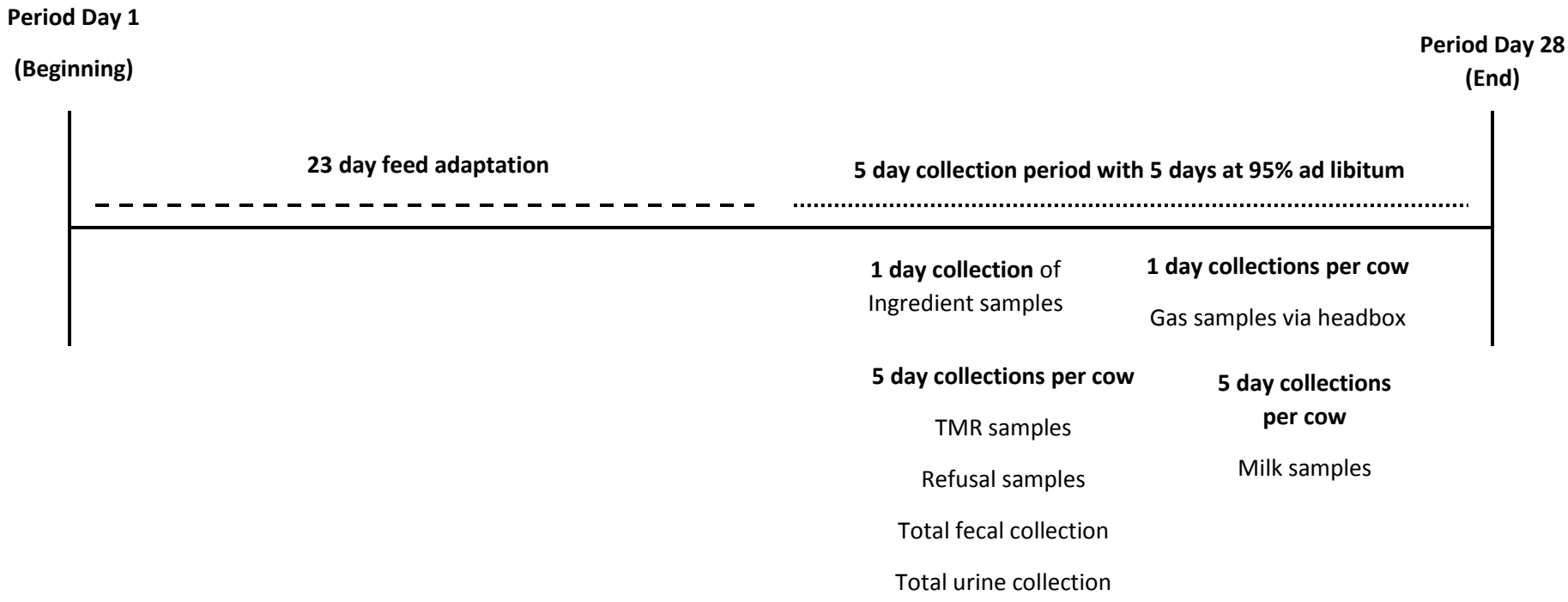

Figure 2.1. Timeline for each period, which includes a 23 day feed adaptation period and 5 days of collection and sampling.

Figure 2.2. Cattle lying down while inside a headbox at the University of Nebraska Lincoln, Dairy Metabolism Unit (Lincoln, NE).

Figure 2.3. Large garbage container and plastic bag used in total fecal collections.

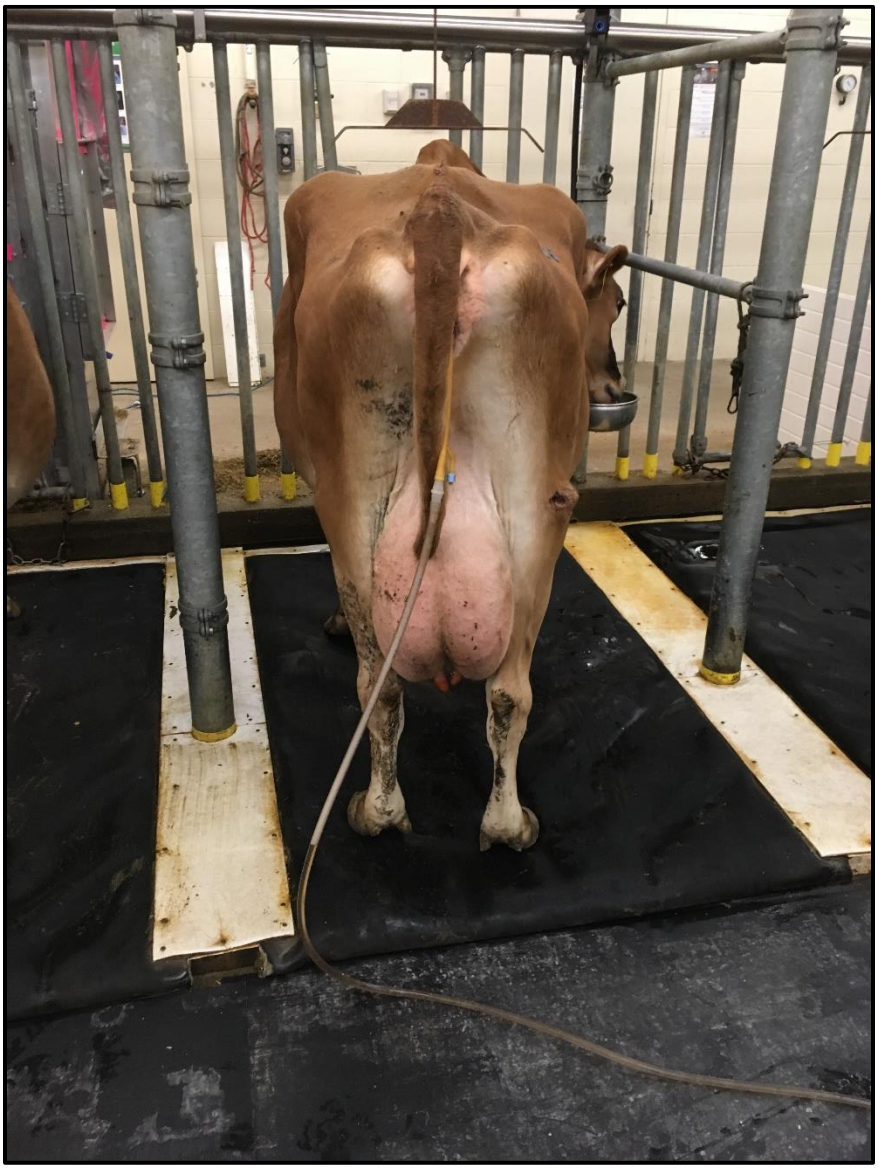

Figure 2.4. French foley catheter and tygon tubing used in total urine collection.

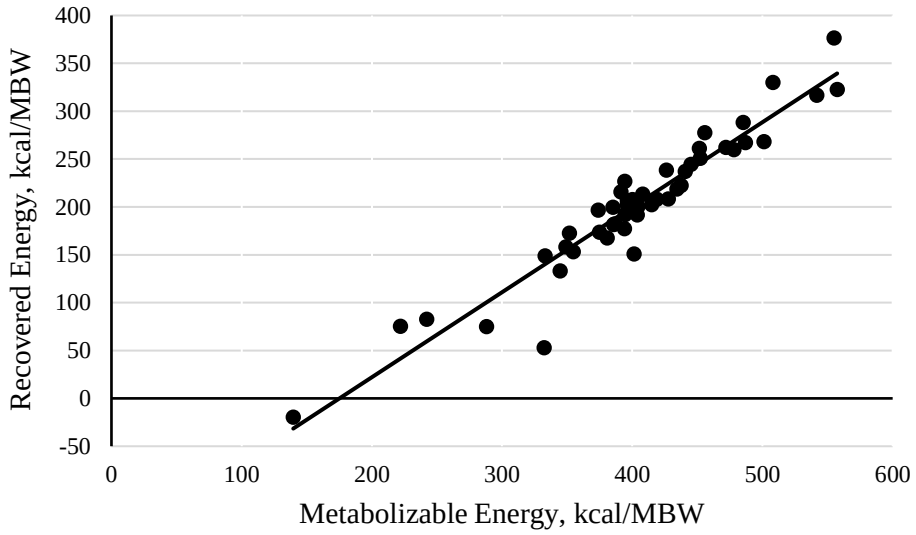

Figure 2.5. Regression of recovered energy on metabolizable energy intake in kilocalories per metabolic body weight (kcal/MBW; y = 0.8879x – 155.5; R2 = 0.91). Recovered energy = 0 at 175 kcal/MBW and efficiency of converting ME to lactation energy is 88 %, MSE 483.

CHAPTER 3

INTERPRETIVE SUMMARRY: Myers *et al.* (2018). "Technical Note: Comparison of sample preparation methods of urine to determine gross energy" describes a comparison of sample preparation methods of urine to be analyzed for energy content by bomb calorimetry. The two methods to be tested included a lyophilization and oven drying method. Results of this study suggest that there was a significant difference in total urine energy depending on which sample preparation method was used. The lyophilization methods resulted in a higher total urine energy compared to oven drying method, creating a negative method difference.

Running Head: TECHNICAL NOTE: BOMB CALORIMETRY OF URINE

Technical Note: Comparison of sample preparation methods of urine to determine gross energy.

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INTRODUCTION

In animal energetic studies, the gross energy (GE) of the feed inputs, and outputs of fecal, urine, and milk energy are determined through the use of bomb calorimetry. In this method, the concentration of energy in a sample is calculated by combusting the sample and measuring the increase in temperature of the water inside the bomb calorimeter multiplied by the heat capacity of the water, thus providing a number for heat or energy produced in the reaction (Blaxter, 1962). Considerations in sample preparation for the bomb calorimetry must strive to conserve all the nutrients in the original sample (Owens et al., 1969) but also to increase efficiency of the procedure.

Recently, multiple energy utilization studies using dairy cattle have used the same method for preparing urine for bomb calorimetry (Drehmel et. al, 2017; Judy et al., 2017). This method involves boiling the urine to remove a large proportion of the moisture, followed by lyophilizing the sample, before placing it in the bomb calorimeter. Boiling is preformed to reduce the extent of lyophilization needed for further analysis of the sample. However, a number of concerns exist with this boiling step including the fact that after the lyophilization, the samples may retract moisture, and this moisture has made samples especially hard to handle and prepare for analysis (Drehmel et al., 2017). Although accurate, the procedure is also laborious, thus we sought to determine how the boiling step could be replaced by placing a known amount of a wet sample on a known mass of cotton, and analyzing this mixture through the bomb to obtain a GE content.

There is very limited information on the accuracy of drying urine samples from lactating dairy cattle in preparation for bomb calorimetry. However, this has been explored in other species recently. Jacobs et al. (2011) tested the impact of different drying methods, lyophilizing, oven drying at 55° C, or oven drying at 100° C, on GE concentrations of swine urine. These researchers concluded no differences in GE concentrations of the urine between drying methods. The objective of this research was to compare two sample preparation methods of urine for determination of GE through bomb calorimetry. The two methods to be tested included a lyophilizing and oven drying method. Our hypothesis was that the two methods would produce similar concentrations of GE, and thus the oven drying method could be used for future energy experiments.

MATERIALS AND METHODS

Raw urine samples were collected from a study using lactating Jersey cows housed in the University of Nebraska-Lincoln Dairy Metabolism Facility. The experimental design was three times replicated 4 × 4 Latin square and used 12 lactating Jersey dairy cows (88.4 ± 27.7 DIM at the first collection; 445.3 ± 45.2 kg BW) and 35 d periods thus a total of 48 samples were collected and used in this analysis. Cows were milked three times daily at 0700, 1400, and 2100 hr representing an AM, mid-day and PM milking. A total of 4 treatments were used, namely 1) control diet; no feather meal, 2) low feather meal (LFM): 3.29 % diet DM 3) medium feather meal (MFM): 6.59 % diet DM, 4) high feather meal (HFM): 10 % diet DM (Table 1).

Total urine was collected by inserting a 30 cc French foley catheter into each cow's bladder with a stylus (Tamura et al., 2014). The balloon was inflated to 55 mL

with physiological saline and tygon tubing drained into a plastic carboy (15 quart; Midwest Can Company, Franklin Park, IL) behind the cow. Using the funnel spout of the plastic carboy, urine was deposited into a 55-L plastic container 4 times a day and was acidified with HCl targeting a pH below 5.0 prior to subsampling (500 mL). Two 250 mL bottles and one 100 mL composite samples were collected for each cow and period.

Laboratory Analysis

The procedure for the lyophilization method is listed in Appendix C. Briefly, urine was thawed and boiled to reduce the moisture content. To boil the urine, 2 thawed samples of approximately 250 mL urine were placed into a 600 mL beaker. Urine filled beakers were placed into a boiling water bath (Ankom Technology, Macedon, NY) underneath a hood (Figure 3.2.). The water bath was on for approximately 6 hours each day for a total of 2 to 3 days, until moisture removal was complete. After the majority of moisture was boiled away, the remaining dark brown paste was then composited by cow and period (Figure 3.3.). The brown paste was then lyophilized (VirTis Freezemobile 25ES, SP Scientific, Gardiner, NY) (Figure 3.4.). Urine samples were analyzed at the University of Nebraska for GE (Parr 6400 Calorimeter, Moline, IL). A standard of 0.4 g of mineral oil was run through the bomb prior to running the urine samples to calibrate the machine. Next, 0.2 g of urine sample and 0.4 g of mineral oil were combined in a capsule and ran through the bomb calorimeter to produce GE of the urine. The GE of the urine was then multiplied by the total kg (DM) of urine the cow produced to obtain total urine energy.

The procedure for the drying method is listed in Appendix D, with the only modification being the cotton round (~0.7 g, Swisspers, U.S. Cotton, Gastonia, NC) which was cut into four pieces, and one of the four pieces was weighed, and placed in a capsule (Figure 3.5.). This modification was made to fit the entire piece of cotton and urine sample into the capsule. After the urine samples were fully thawed, 4 mL of wet urine sample was placed into one capsule on top of the cotton round using a pipette (MU09577, Fisher Scientific, Hampton, NH). Samples were then placed into an oven at 105° C for 24 hours (Figure 3.6. and Figure 3.7.). Before analyzing the dried urine samples for GE, two standards were run. These standards were composed of one fourth of a cotton round and 0.4 g mineral oil. Urine samples were then analyzed for GE (Parr 6400 Calorimeter, Moline, IL). Gross energy calculations for the urine are listed in Equation 1 through 4. The energy for cotton is calculated in Equation 1. The proportional GE calculates the total energy in the sample for that specific cotton weight (Equation 2). The difference between the proportional GE and the cotton GE is the amount of energy due to the addition of urine (4 mL), which is divided by 4 to obtain the energy value per 1 mL of urine (Equation 3). The GE of the urine was then multiplied by the total urine the cow produced (kg) to obtain a value for total urine energy (Equation 4).

$$\text{Cotton GE (cal)} = \text{cotton weight} \times \text{average GE of standard} \quad [1]$$

$$\text{Proportional GE (cal)} = \text{cotton weight} \times \text{GE of sample} \quad [2]$$

$$\text{Urine Energy (cal)} = (\text{Proportional GE} - \text{Cotton GE}) / 4 \text{ mL} \quad [3]$$

$$\text{Total Urine Energy (Mcal)} = \text{Urine Energy} \times \text{Total Urine Output (kg)} / 1{,}000 \quad [4]$$

Statistical Analysis

Correlation of sample preparation methods was calculated using the REG procedure of SAS (SAS Institute Inc., Cary, NC) as illustrated in Figure 3.1. A paired t test was used to determine whether differences between methods were significant. Statistical calculations for the paired t test were carried out through the PROC UNIVARIATE procedure of SAS version 9.4.

RESULTS AND DISCUSSION

Results of this study are listed in Table 3.1 and illustrated in Figure 3.1. Results of the paired t test were significantly different ($P < 0.01$) between the oven drying method and the lyophilizing method. The samples from the lyophilizing method contained a significantly greater amount of gross energy ($2.1 \times 10^{-3} \pm 2.4 \times 10^{-3}$ Mcal/g) compared with the oven dried samples ($6.9\times 10^{-5} \pm 2.1\times 10^{-5}$), which resulted in a negative method difference ($-2.0 \times 10^{-3} \pm 2.6 \times 10^{-4}$). Total urine energy was also greater in the lyophilization method (2.10 ± 0.398 Mcal) compared with the oven dried samples (1.32 ± 0.439 Mcal). This resulted in a method difference of -0.78 ± 0.545 Mcal. This was surprising, as the hypothesis for this study was the two methods would result in similar energy values. This is the first evaluation of dairy cattle urine sample preparation for bomb calorimetry, however comparisons do exist for other species. For example, Jacobs et al. (2011) compared the gross energies of urine samples that were lyophilized, oven dried at 55° C or 100° C. The researchers reported no differences in GE concentrations based on drying method. However, the researchers reported that urinary DM was greatest

in the lyophilized samples compared to samples oven dried at 100° C (4.50 % and 3.60 % DM, respectively). This could be a partial explanation for the results of the current study. If lyophilizing is more efficient at dehydrating the sample, the energy would be more concentrated in the sample, thus total energy would be greater. One difference between Jacobs et al (2011) and the current study is the researchers used a cellulose pellet to absorb the urine and subsequently dry the urine for the oven dried methods, whereas in the current study a cotton round was used to absorb the urine before drying. The weight and GE of the cotton round standard contained great variability between samples (17 % CV; 0.164 ± 0.019 g and 3693 ± 631 cal/g, respectively), which could be another potential reason for why the results are so different. Jacob et al. (2011) also used a cotton ball and plastic bags to absorb urine in a crucible for lyophilizing, whereas the current method involved boiling the urine, and after most of the moisture was removed, the brown paste was transferred to a plastic bag (532 mL, Whirlpack, NASCO, Fort Atkinson, WI) and lyophilized.

Energy utilization experiments typically acidify the urine prior to analysis to prevent loss of N due to microbial growth (Pedersen et al., 2007). Some researchers have suggested neutralizing the urine prior to analysis (US ISO, 2009). Urine analyzed in Jacobs et al. (2011) was not acidified or acidified after thawing with the addition of 1.5mL of 6 M HCl, targeting a pH of 2. The researchers reported no differences in GE concentration of urine that was acidified prior to analysis or not at all. This would imply that in the current experiment, where urine was acidified by addition of HCl to target a

pH of 5, the acidification process was not responsible for the lower GE content of the oven-dried samples.

CONCLUSION

The results of this study suggest there are significant differences in the GE concentrations of urine, which was boiled and lyophilized, or oven dried, prior to analysis. This is most likely due to greater dehydration of the sample when lyophilizing. Future research should measure the N content of the samples prior to and after drying is performed to account for possible N loss from the sample.

ACKNOWLEDGEMENTS

The authors would like to thank the researchers at the U.S. Meat and Animal Research Center in Clay Center, Nebraska for their cooperation in the study. We would also like to thank the lab staff at the University of Nebraska-Lincoln for their technical services relating to the bomb calorimeter.

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Table 3.1. Total energy (Mcal) and method difference (Mcal) of urine samples prepared by lyophilizing or oven drying

	Lyophilized	SD	Oven Dry	SD	Method Difference	SD	*P* - Value
Total Urine Energy, Mcal/d	2.10	0.398	1.32	0.439	-0.78	0.547	< 0.01
Gross Energy, Mcal/g	2.1×10^{-3}	2.4×10^{-3}	6.9×10^{-5}	2.1×10^{-5}	-2.0×10^{-3}	2.6×10^{-4}	< 0.01

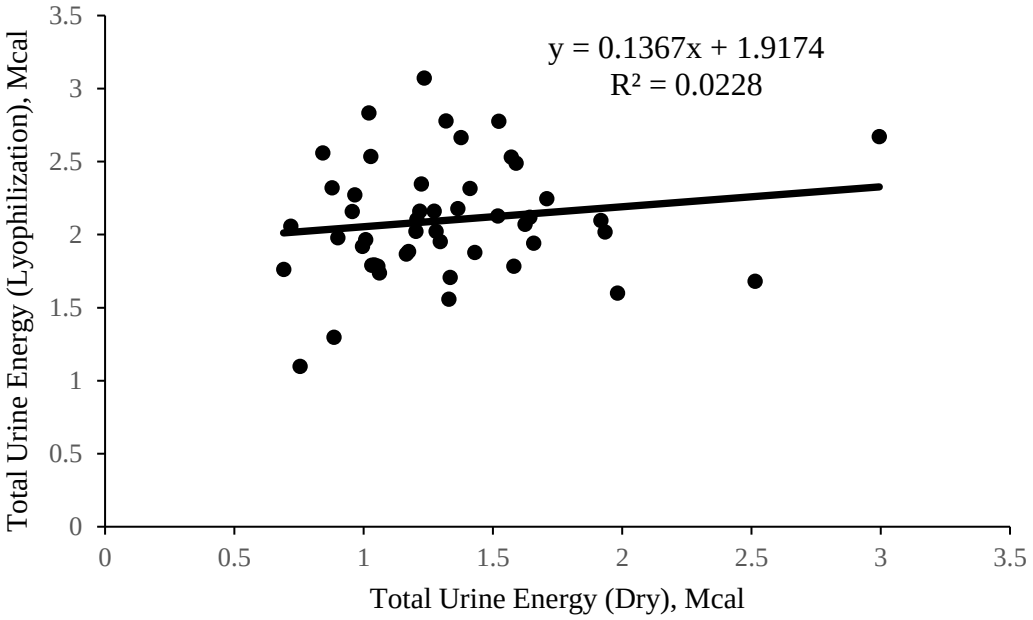

Figure 3.1. Correlation of sample preparation methods for determining gross energy of urine

Figure 3.2. Urine in beakers prior to boiling.

Figure 3.3. Urine samples post boiling, prior to lyophilization.

Figure 3.4. Urine post lyophilization.

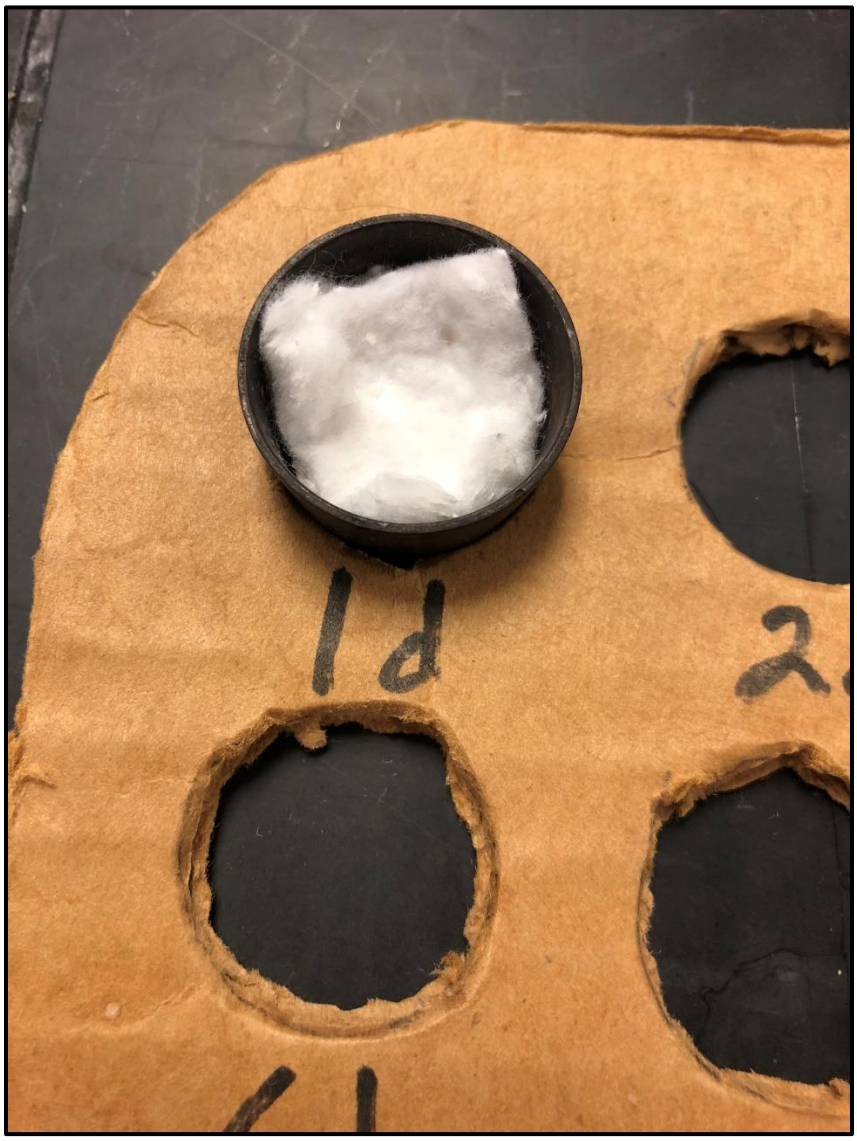

Figure 3.5. Cotton round in capsule prior to addition of 4 mL of urine.

Figure 3.6. Cotton round in capsule with 4 mL of urine prior to drying**.**

Figure 3.7. Urine (4mL) and cotton round post drying for 24 h at 105°C.

GENERAL SUMMARY AND CONCLUSIONS

The first study in Chapter 2 used indirect calorimetry and lactating jersey dairy cattle. In this study, the feeding value of dried distillers grains and solubles (DDGS) and canola meal, two common byproducts, was tested. The second study in Chapter 3 used bomb calorimetry and urine samples from lactating dairy cattle. In this study, effects of sample preparation methods on the gross energy content of urine was tested.

In the first study, we chose to evaluate the feeding value of canola meal and DDGS in a single study because of their popularity among dairy producers and because comparisons of these products using indirect calorimetry did not exist. Specifically, our study aimed to test the effects of feeding canola meal and DDGS on feed intake, milk production and composition, total tract digestibility and energy utilization in lactating Jersey dairy cows. In this study we also tested the feeding value of reduced fat DDGS (pDDGS) and an alternative source of DDGS (aDDGS), which were cooked at different temperatures, specifically the pDDGS were cooked at a lower temperature compared with aDDGS. Our results demonstrated that DMI, milk production, and milk composition were maintained when lactating dairy cattle were fed pDDGS, aDDGS, or canola meal at a 10 % inclusion rate. However, inclusion of these byproducts did result in differences in energy utilization. Specifically, the alternative DDGS, which were cooked at a greater temperature, supplied the greatest digestible and metabolizable energy, which is likely due to the higher concentrations of fat in this feed. Previous in vitro research has suggested DDGS cooked at a lower temperature to have modest improvements in fiber digestibility. This was not observed in vivo as total tract NDF digestibility was higher in

animals consuming the aDDGS compared to pDDGS or canola meal which had the lowest NDF digestibility. Previous in vitro research has also suggested that the digestibility of CP in canola meal is lower than either DDGS or soybean meal. This is supported by our study as total tract digestibility was lowest in cows consuming canola meal.

Future research of these byproducts should focus in on why differences in digestibility exist. In the case of canola meal, research should be conducted on the development of feed processing methods that could be used to increase total tract digestibility of both NDF and CP. In the case of DDGS research should be conducted to further explore the effects of fat removal on nutrient digestibility. In doing so researchers may want to consider testing feeds originating from the same source so that tests of fat content are not confounded by grain source.

There is very little research on sample preparation methods for bomb calorimetry of urine. In our lab, urine sample have been previously prepared multiple ways including boiling prior to lyophilization. The goal of the second study was to test the effects of simply drying down the urine on a cotton round prior to estimation of gross energy through a bomb calorimeter compared to boiling and lyophilizing. This procedure had the potential of being more time efficient in analyzing urine samples, because it did not include the boiling or lyophilization steps. However, results of this study showed that tremendous differences were observed in total urine energy depending on which method was used. The drying method resulted in lower total urine energy compared to boiling with lyophilization. While the exact reason for this is still unknown, we suspect that

boiling prior to lyophilization, and lyophilization itself, resulted in greater dehydration of the sample. Future research should measure the N content of the following samples to determine which step is resulting in the greatest loss of N from the sample. In the drying method the N content of the wet urine sample could be compared with the N content of the urine sample post drying. The N content of the cotton ball should also be determined pre and post drying to account for any N loss from the cotton ball. In the lyophilization method, the N content could be measured post boiling and compared with the N content post lyophilization. Other possible methods to be tested should include decreasing the oven temperature to dry urine, and also determining the GE of the urine paste post boiling.

APPENDIX A: CON, pDDGS, CanM, AND aDDGS ACCORDING TO THE CPM DAIRY RATION ANALYZER (2000)

Control (CON) diet:

CPM-Dairy — CNCPS Evaluation — 9/6/2016 3:36:39 PM

File: F:\Studies\1607DA\CPM\1607DA Control Ration-AA Final
Farm: UNL Dairy Research Unit — BW: 850 lb — DIM: 120
Ration: 1607DA Control — BCS: 3.25 — Milk: 70.00 lb
Ration By: Kononoff & Judy — Growth: 0.10 lb/d — Fat: 5.30 %
Organization: University of Nebraska-Lincoln — Lact#: 3 — TP: 3.70 %

						Ingredient	DM (lb/d)
Cost ($)	4.92	IOF ($)	-4.92				
DMI (lb/d)	43.7	Model	41.0	% Model	106.6	Alfalfa hay	5.800
ME Bal (mCal)	1.4	CP (%)	18.6	NDF (%)	28.7	BrmdHy10Cp70Ndf9LNdf	0.500
MP Bal (g)	114.5	RUP (% CP)	46.2	ForageNDF (% NDF)	77.5	Corn silage	17.700
NP / MP (%)	61.1	LCFA (%)	4.6	ForageNDF (% DM)	22.2	Ground Corn	7.350
BactMP (% MP)	44.4	EE (%)	5.5	peNDF (%)	21.7	SoybeanML47.5Solv	6.000
Rumen N Balance				Lignin (%)	2.9	RFDDGS	0.000
Pept (g)	45	Pept & NH3 (g)	70	NFC (%)	41.6	CanolaMealSolv	0.000
% rqd	126	% rqd	123	Sil Acids (%)	3.2	SoybeanHullsGrnd	1.100
Amino Acid Balance				Sugar (%)	3.3	Soy Pass	2.000
Met (g)	11.8	Lys (g)	37.4	Starch (%)	28.3	FatTallowBeef	0.850
Met (% rqd)	126	Lys (% rqd)	126	Sol Fiber (%)	6.8	CalciumCarbonate	0.600
Met (% mp)	2.18	Lys (% mp)	7.06	Lys:Met	3.24:1	BloodMeal	0.670
Possible production due to ME and MP						Megalac	0.350

	Milk(lb)	Fat (%)	TP (%)	Milk(lb)	Fat (%)	TP (%)	
Trg:	70.0	5.30	3.70	70.0	5.30	3.70	SodiumBicarbonate 0.250
		Yield Constant			Composition Constant		CalciumPhosDi 0.150
ME:	70.0	n/a	n/a	72.2	5.30	n/a	MagOx 0.140
MP:	70.0	n/a	3.93	74.4	5.30	3.70	SaltNaCl 0.110
Adjustments based on Rulquin AA Ratios:							Vitamin Premix 0.022
	70.0	n/a	0.02	0.4	5.30	3.70	Trace Premix 0.017
							Agipro-L 0.022
n/a - Equations not available							SmartamineM 0.030
Ration DM (%)	55.52		Forage (% DM)		54.97		Total 43.661

Control (CON) diet:

CPM-Dairy — Amino Acids — 9/6/2016 3:36:39 PM

File: F:\Studies\1607DA\CPM\1607DA Control Ration-AA Final
Farm: UNL Dairy Research Unit — BW: 850 lb — DIM: 120
Ration: 1607DA Control — BCS: 3.25 — Milk: 70.00 lb
Ration By: Kononoff & Judy — Growth: 0.10 lb/d — Fat: 5.30 %
Organization: University of Nebraska-Lincoln — Lact#: 3 — TP: 3.70 %

			Metabolizable Amino Acids						Duodnl Amino Acids		
	RR*	% Rqd	Diff	Rqd	Total	Tissue	Bact	Feed	Total	Bact	Feed
			g/d	g/d	g/d	g/d	g/d	g/d	g/d	g/d	g/d
Met	2.18	126	11.8	44.6	56.4	0.0	30.8	25.6	71.3	42.3	28.9
Lys	7.06	126	37.4	145.3	182.7	0.0	94.3	88.4	221.8	121.2	100.6
Arg	6.42	120	27.2	138.8	166.1	0.0	80.1	86.0	196.6	98.4	98.2
Thr	4.85	156	44.9	80.5	125.5	0.0	64.3	61.2	150.6	80.1	70.5
Leu	8.52	112	23.9	196.6	220.5	0.0	86.4	134.1	270.1	114.7	155.5
Ile	4.67	99	-1.3	122.0	120.7	0.0	67.6	53.1	148.9	86.8	62.1
Val	5.73	107	9.7	138.5	148.2	0.0	70.8	77.3	183.3	93.4	89.9
His	2.91	150	25.0	50.2	75.2	0.0	30.9	44.3	90.0	39.3	50.7
Phe	5.32	172	57.8	79.8	137.6	0.0	59.3	78.3	169.9	79.5	90.5
Trp	1.44	155	13.3	24.0	37.3	0.0	18.7	18.6	47.9	26.6	21.3

*** RR = Rulquin Ratio (Amino acids as a percentage of metabolizable protein).**

Control (CON) diet:

CPM-Dairy — Diet Summary - Both — 9/6/2016 3:36:39 PM

File: F:\Studies\1607DA\CPM\1607DA Control Ration-AA Final
Farm: UNL Dairy Research Unit — BW: 850 lb — DIM: 120
Ration: 1607DA Control — BCS: 3.25 — Milk: 70.00 lb
Ration By: Kononoff & Judy — Growth: 0.10 lb/d — Fat: 5.30 %
Organization: University of Nebraska-Lincoln — Lact#: 3 — TP: 3.70 %

Ingredient	Cost $ / T	DM %	AF lb/d	DM lb/d	% AF	% DM
Alfalfa hay	110.00	89.90	6.45	5.80	8.20	13.28
BrmdHy10Cp70Ndf9LNdf	60.00	88.00	0.57	0.50	0.72	1.15
Corn silage	33.00	35.50	49.86	17.70	63.40	40.54
Ground Corn	130.00	88.00	8.35	7.35	10.62	16.83
SoybeanML47.5Solv	435.80	90.00	6.67	6.00	8.48	13.74
RFDDGS	120.00	89.00	0.00	0.00	0.00	0.00
CanolaMealSolv	310.00	90.17	0.00	0.00	0.00	0.00
SoybeanHullsGrnd	123.60	91.00	1.21	1.10	1.54	2.52
Soy Pass	404.80	90.14	2.22	2.00	2.82	4.58
FatTallowBeef	752.60	99.00	0.86	0.85	1.09	1.95
CalciumCarbonate	26.80	99.50	0.60	0.60	0.77	1.37
BloodMeal	1247.00	90.00	0.74	0.67	0.95	1.53
Megalac	1500.60	97.00	0.36	0.35	0.46	0.80
SodiumBicarbonate	543.80	99.50	0.25	0.25	0.32	0.57
CalciumPhosDi	26.80	99.50	0.15	0.15	0.19	0.34
MagOx	799.60	99.50	0.14	0.14	0.18	0.32
SaltNaCl	243.80	99.50	0.11	0.11	0.14	0.25
Vitamin Premix	26.80	95.75	0.02	0.02	0.03	0.05
Trace Premix	26.80	95.97	0.02	0.02	0.02	0.04
Agipro-L	0.00	97.00	0.02	0.02	0.03	0.05
SmartamineM	0.00	98.00	0.03	0.03	0.04	0.07
Total			78.64	43.66		

Macro Nutrients		
Nutrient	DM	AF
Dry Matter (%)	100.00	55.52
Forage (%)	54.97	32.80
Crude Prot (%)	18.63	10.34
RUP (%CP)	46.17	46.17
RDP (%CP)	53.83	53.83
RDP (%)	10.03	5.57
Sol Prot (%CP)	23.16	23.16
ME (mCal/lb)	1.26	0.70
NEl (mCal/lb)	0.81	0.45
Nem (mCal/lb)	0.81	0.45
NEg (mCal/lb)	0.55	0.30
ADF (%)	19.31	10.72
NDF (%)	28.69	15.93
For NDF (%NDF)	77.50	43.03
Forage NDF (%)	22.23	12.34
peNDF (%)	21.73	12.06
Lignin (%)	2.91	1.62
NFC (%)	41.65	23.12
Sil Acids (%)	3.22	1.79
Sugar (%)	3.34	1.85
Starch (%)	28.34	15.73
Sol Fiber (%)	6.76	3.75
EE Total (%)	5.47	3.04
EE 1 (%)	2.79	1.55
EE 2 (%)	2.00	1.11
EE 3 (%)	0.68	0.38
LCFA Total (%)	4.63	2.57
Ash (%)	8.44	4.69
Cost ($/d)	4.92	4.92
Cost ($T)	225.40	125.14

Minerals and Vitamins		
Nutrient	DM	AF
Dry Matter (%)	100.00	55.52
Calcium (%)	0.99	0.55
Phosphorus (%)	0.39	0.22
Magnesium (%)	0.35	0.19
Potassium (%)	1.34	0.74
Sulfur (%)	0.24	0.13
Sodium (%)	0.28	0.15
Chlorine (%)	0.23	0.13
Iron (ppm)	210.31	116.76
Zinc (ppm)	48.79	27.09
Copper (ppm)	13.70	7.61
Manganese (ppm)	43.88	24.36
Selenium (ppm)	0.14	0.08
Cobalt (ppm)	0.25	0.14
Iodine (ppm)	0.34	0.19
Vitamin A (KIU/lb)	1.32	0.73
Vitamin D (KIU/lb)	0.33	0.19
Vitamin E (IU/lb)	20.66	11.47
DCAD1 (meq/100g)	24.96	13.86
DCAD2 (meq/100g)	31.13	17.28
Cost ($/d)	4.92	4.92
Cost ($T)	225.40	125.14

Control (CON) diet:

CPM-Dairy | Fatty Acids - Summary | 9/6/2016 3:36:39 PM

File: F:\Studies\1607DA\CPM\1607DA Control Ration-AA Final
Farm: UNL Dairy Research Unit | BW: 850 lb | DIM: 120
Ration: 1607DA Control | BCS: 3.25 | Milk: 70.00 lb
Ration By: Kononoff & Judy | Growth: 0.10 lb/d | Fat: 5.30 %
Organization: University of Nebraska-Lincoln | Lact#: 3 | TP: 3.70 %

	Intake g/d	Lipolysed g/d	Duodenal g/d	Absorbed g/d	Fecal g/d	Digested % Duodenal
C12:0	1.46	1.25	1.46	1.39	0.07	95
C14:0	17.30	15.50	17.30	12.88	4.41	74
C16:0	225.18	179.26	239.27	177.89	61.38	74
C16:1	14.20	13.72	4.38	2.80	1.58	64
C18:0	79.81	73.93	461.47	335.37	126.10	73
C18:1T	14.25	13.74	65.12	51.12	14.01	78
C18:1C	272.79	237.07	92.18	80.76	11.42	88
C18:2	234.26	223.36	30.92	26.35	4.56	85
C18:3	34.84	33.54	2.25	1.75	0.51	78
Other	23.61	22.53	50.67	29.38	21.29	58
Ration	917.71	813.91	965.01	719.68	245.33	75

Reduced fat dried distillers grains and solubles (pDDGS) diet:

CPM-Dairy — CNCPS Evaluation — 9/6/2016 3:37:27 PM

File: F:\Studies\1607DA\CPM\1607DA RFDDGS-AA Final
Farm: UNL Dairy Research Unit — BW: 850 lb — DIM: 120
Ration: 1607DA RFDDGS Ration — BCS: 3.25 — Milk: 70.00 lb
Ration By: Kononoff & Judy — Growth: 0.10 lb/d — Fat: 5.30 %
Organization: University of Nebraska-Lincoln — Lact#: 3 — TP: 3.70 %

						Ingredient	DM (lb/d)
Cost ($)	4.29	IOF ($)	-4.29				
DMI (lb/d)	43.7	Model	41.0	% Model	106.6	Alfalfa hay	5.800
ME Bal (mCal)	0.7	CP (%)	17.1	NDF (%)	31.0	BrmdHy10Cp70Ndf9LNdf	0.500
MP Bal (g)	16.6	RUP (% CP)	48.9	ForageNDF (% NDF)	71.8	Corn silage	17.700
NP / MP (%)	64.4	LCFA (%)	4.8	ForageNDF (% DM)	22.2	Ground Corn	6.550
BactMP (% MP)	45.0	EE (%)	5.8	peNDF (%)	22.0	SoybeanML47.5Solv	2.400
Rumen N Balance				Lignin (%)	3.0	RFDDGS	4.400
Pept (g)	14	Pept & NH3 (g)	33	NFC (%)	40.6	CanolaMealSolv	0.000
% rqd	109	% rqd	111	Sil Acids (%)	3.2	SoybeanHullsGrnd	1.100
Amino Acid Balance				Sugar (%)	3.3	Soy Pass	2.000
Met (g)	9.9	Lys (g)	19.8	Starch (%)	27.2	FatTallowBeef	0.850
Met (% rqd)	122	Lys (% rqd)	113	Sol Fiber (%)	7.0	CalciumCarbonate	0.600
Met (% mp)	2.19	Lys (% mp)	6.63	Lys:Met	3.03:1	BloodMeal	0.670
Possible production due to ME and MP						Megalac	0.350

	Milk(lb)	Fat (%)	TP (%)	Milk(lb)	Fat (%)	TP (%)	
Trg:	70.0	5.30	3.70	70.0	5.30	3.70	SodiumBicarbonate 0.250
		Yield Constant			Composition Constant		CalciumPhosDi 0.150
							MagOx 0.140
ME:	70.0	n/a	n/a	71.1	5.30	n/a	SaltNaCl 0.110
MP:	70.0	n/a	3.73	70.6	5.30	3.70	Vitamin Premix 0.022
Adjustments based on Rulquin AA Ratios:							Trace Premix 0.017
	70.0	n/a	-0.02	-0.4	5.30	3.70	Agipro-L 0.022
n/a - Equations not available							SmartamineM 0.030
Ration DM (%)	55.50		Forage (% DM)		54.97		Total 43.661

Reduced fat dried distillers grains and solubles (pDDGS) diet:

CPM-Dairy — Amino Acids — 9/6/2016 3:37:27 PM

File: F:\Studies\1607DA\CPM\1607DA RFDDGS-AA Final

Farm: UNL Dairy Research Unit	BW: 850 lb	DIM: 120
Ration: 1607DA RFDDGS Ration	BCS: 3.25	Milk: 70.00 lb
Ration By: Kononoff & Judy	Growth: 0.10 lb/d	Fat: 5.30 %
Organization: University of Nebraska-Lincoln	Lact#: 3	TP: 3.70 %

			Metabolizable Amino Acids						Duodnl Amino Acids		
	RR*	% Rqd	Diff	Rqd	Total	Tissue	Bact	Feed	Total	Bact	Feed
			g/d	g/d	g/d	g/d	g/d	g/d	g/d	g/d	g/d
Met	2.19	122	9.9	45.0	54.9	0.0	30.3	24.6	69.8	41.6	28.1
Lys	6.63	113	19.8	146.7	166.5	0.0	92.7	73.7	204.2	119.1	85.1
Arg	6.04	109	12.1	139.5	151.7	0.0	78.7	73.0	181.7	96.7	85.0
Thr	4.67	144	36.0	81.4	117.4	0.0	63.2	54.2	142.3	78.8	63.6
Leu	8.56	109	16.9	198.1	215.0	0.0	84.9	130.0	265.6	112.7	152.8
Ile	4.55	93	-8.3	122.6	114.3	0.0	66.5	47.8	142.3	85.3	56.9
Val	5.85	105	7.5	139.4	146.8	0.0	69.7	77.2	182.6	91.8	90.8
His	2.82	140	20.2	50.8	70.9	0.0	30.4	40.5	85.6	38.6	47.0
Phe	5.22	163	50.6	80.6	131.2	0.0	58.3	72.8	163.6	78.1	85.4
Trp	1.47	153	12.8	24.1	37.0	0.0	18.4	18.6	47.7	26.1	21.6

*** RR = Rulquin Ratio (Amino acids as a percentage of metabolizable protein).**

Reduced fat dried distillers grains and solubles (pDDGS) diet:

CPM-Dairy — Diet Summary - Both — 9/6/2016 3:37:27 PM

File: F:\Studies\1607DA\CPM\1607DA RFDDGS-AA Final
Farm: UNL Dairy Research Unit — BW: 850 lb — DIM: 120
Ration: 1607DA RFDDGS Ration — BCS: 3.25 — Milk: 70.00 lb
Ration By: Kononoff & Judy — Growth: 0.10 lb/d — Fat: 5.30 %
Organization: University of Nebraska-Lincoln — Lact#: 3 — TP: 3.70 %

	Cost		AF	DM		
Ingredient	$ / T	DM %	lb/d	lb/d	% AF	% DM
Alfalfa hay	110.00	89.90	6.45	5.80	8.20	13.28
BrmdHy10Cp70Ndf9LNdf	60.00	88.00	0.57	0.50	0.72	1.15
Corn silage	33.00	35.50	49.86	17.70	63.37	40.54
Ground Corn	130.00	88.00	7.44	6.55	9.46	15.00
SoybeanML47.5Soiv	435.80	90.00	2.67	2.40	3.39	5.50
RFDDGS	120.00	89.00	4.94	4.40	6.28	10.08
CanolaMealSolv	518.00	90.17	0.00	0.00	0.00	0.00
SoybeanHullsGrnd	123.60	91.00	1.21	1.10	1.54	2.52
Soy Pass	404.80	90.14	2.22	2.00	2.82	4.58
FatTallowBeef	752.60	99.00	0.86	0.85	1.09	1.95
CalciumCarbonate	26.80	99.50	0.60	0.60	0.77	1.37
BloodMeal	1247.0(	90.00	0.74	0.67	0.95	1.53
Megalac	1500.60	97.00	0.36	0.35	0.46	0.80
SodiumBicarbonate	543.80	99.50	0.25	0.25	0.32	0.57
CalciumPhosDi	26.80	99.50	0.15	0.15	0.19	0.34
MagOx	799.60	99.50	0.14	0.14	0.18	0.32
SaltNaCl	243.80	99.50	0.11	0.11	0.14	0.25
Vitamin Premix	26.80	95.75	0.02	0.02	0.03	0.05
Trace Premix	26.80	95.97	0.02	0.02	0.02	0.04
Agipro-L	0.00	97.00	0.02	0.02	0.03	0.05
SmartamineM	0.00	98.00	0.03	0.03	0.04	0.07
Total			78.67	43.66		

Macro Nutrients		
Nutrient	DM	AF
Dry Matter (%)	100.00	55.50
Forage (%)	54.97	32.79
Crude Prot (%)	17.11	9.50
RUP (%CP)	48.89	48.89
RDP (%CP)	51.11	51.11
RDP (%)	8.75	4.85
Sol Prot (%CP)	22.66	22.66
ME (mCal/lb)	1.24	0.69
NEl (mCal/lb)	0.80	0.44
Nem (mCal/lb)	0.80	0.44
NEg (mCal/lb)	0.53	0.30
ADF (%)	19.71	10.94
NDF (%)	30.96	17.18
For NDF (%NDF)	71.80	39.85
Forage NDF (%)	22.23	12.34
peNDF (%)	22.01	12.21
Lignin (%)	3.01	1.67
NFC (%)	40.62	22.54
Sil Acids (%)	3.22	1.79
Sugar (%)	3.26	1.81
Starch (%)	27.17	15.08
Sol Fiber (%)	6.98	3.87
EE Total (%)	5.79	3.21
EE 1 (%)	3.14	1.74
EE 2 (%)	1.98	1.10
EE 3 (%)	0.68	0.38
LCFA Total (%)	4.83	2.68
Ash (%)	8.43	4.68
Cost ($/d)	4.29	4.29
Cost ($T)	196.35	108.97

Minerals and Vitamins		
Nutrient	DM	AF
Dry Matter (%)	100.00	55.50
Calcium (%)	0.98	0.54
Phosphorus (%)	0.43	0.24
Magnesium (%)	0.35	0.20
Potassium (%)	1.27	0.71
Sulfur (%)	0.29	0.16
Sodium (%)	0.30	0.17
Chlorine (%)	0.24	0.13
Iron (ppm)	208.69	115.81
Zinc (ppm)	49.81	27.64
Copper (ppm)	12.29	6.82
Manganese (ppm)	42.21	23.43
Selenium (ppm)	0.17	0.09
Cobalt (ppm)	0.25	0.14
Iodine (ppm)	0.34	0.19
Vitamin A (KIU/lb)	1.32	0.73
Vitamin D (KIU/lb)	0.33	0.19
Vitamin E (IU/lb)	20.66	11.46
DCAD1 (meq/100g)	20.44	11.34
DCAD2 (meq/100g)	26.82	14.89
Cost ($/d)	4.29	4.29
Cost ($T)	196.35	108.97

Reduced fat dried distillers grains and solubles (pDDGS) diet:

CPM-Dairy — Fatty Acids - Summary — 9/6/2016 3:37:28 PM

File: F:\Studies\1607DA\CPM\1607DA RFDDGS-AA Final
Farm: UNL Dairy Research Unit — BW: 850 lb — DIM: 120
Ration: 1607DA RFDDGS Ration — BCS: 3.25 — Milk: 70.00 lb
Ration By: Kononoff & Judy — Growth: 0.10 lb/d — Fat: 5.30 %
Organization: University of Nebraska-Lincoln — Lact#: 3 — TP: 3.70 %

	Intake g/d	Lipolysed g/d	Duodenal g/d	Absorbed g/d	Fecal g/d	Digested % Duodenal
C12:0	1.57	1.37	1.57	1.50	0.07	95
C14:0	16.72	14.94	16.72	12.45	4.27	74
C16:0	229.34	183.38	243.32	180.82	62.50	74
C16:1	14.31	13.83	4.40	2.82	1.59	64
C18:0	79.88	74.00	481.70	350.10	131.60	73
C18:1T	14.07	13.56	72.49	56.91	15.59	78
C18:1C	287.53	251.61	95.79	83.94	11.85	88
C18:2	257.08	245.91	32.91	27.99	4.92	85
C18:3	32.48	31.22	2.15	1.67	0.48	78
Other	23.74	22.66	50.71	29.40	21.31	58
Ration	956.73	852.48	1001.77	747.60	254.17	75

Canola meal (CanM) diet:

CPM-Dairy | CNCPS Evaluation | 9/6/2016 3:38:16 PM

File: F:\Studies\1607DA\CPM\1607DA Canola Meal-AA Final

Farm: UNL Dairy Research Unit	BW: 850 lb	DIM: 120
Ration: 1607DA Canola Meal Ration	BCS: 3.25	Milk: 70.00 lb
Ration By: Kononoff & Judy	Growth: 0.10 lb/d	Fat: 5.30 %
Organization: University of Nebraska-Lincoln	Lact#: 3	TP: 3.70 %

Cost ($)	4.69	IOF ($)	-4.69			Ingredient	DM (lb/d)
DMI (lb/d)	43.7	Model	41.0	% Model	106.6	Alfalfa hay	5.800
ME Bal (mCal)	0.1	CP (%)	18.1	NDF (%)	30.5	BrmdHy10Cp70Ndf9LNdf	0.500
MP Bal (g)	21.6	RUP (% CP)	47.6	ForageNDF (% NDF)	72.9	Corn silage	17.700
NP / MP (%)	64.2	LCFA (%)	4.7	ForageNDF (% DM)	22.2	Ground Corn	6.900
BactMP (% MP)	44.7	EE (%)	5.7	peNDF (%)	22.5	SoybeanML47.5Solv	2.050
Rumen N Balance				Lignin (%)	3.6	RFDDGS	0.000
Pept (g)	32	Pept & NH3 (g)	56	NFC (%)	40.8	CanolaMealSolv	4.400
% rqd	119	% rqd	118	Sil Acids (%)	3.2	SoybeanHullsGrnd	1.100
Amino Acid Balance				Sugar (%)	3.4	Soy Pass	2.000
Met (g)	10.4	Lys (g)	32.0	Starch (%)	27.7	FatTallowBeef	0.850
Met (% rqd)	123	Lys (% rqd)	122	Sol Fiber (%)	6.5	CalciumCarbonate	0.600
Met (% mp)	2.20	Lys (% mp)	7.10	Lys:Met	3.22:1	BloodMeal	0.670
Possible production due to ME and MP						Megalac	0.350

	Milk(lb)	Fat (%)	TP (%)	Milk(lb)	Fat (%)	TP (%)	
Trg:	70.0	5.30	3.70	70.0	5.30	3.70	SodiumBicarbonate 0.250
		Yield Constant			Composition Constant		CalciumPhosDi 0.150
ME:	70.0	n/a	n/a	70.1	5.30	n/a	MagOx 0.140
MP:	70.0	n/a	3.74	70.8	5.30	3.70	SaltNaCl 0.110
Adjustments based on Rulquin AA Ratios:							Vitamin Premix 0.022
	70.0	n/a	0.03	0.5	5.30	3.70	Trace Premix 0.017
n/a - Equations not available							Agipro-L 0.022
							SmartamineM 0.030
Ration DM (%)	55.53		Forage (% DM)	54.97			Total 43.661

Canola meal (CanM) diet:

CPM-Dairy — Amino Acids — 9/6/2016 3:38:16 PM

File: F:\Studies\1607DA\CPM\1607DA Canola Meal-AA Final
Farm: UNL Dairy Research Unit — BW: 850 lb — DIM: 120
Ration: 1607DA Canola Meal Ration — BCS: 3.25 — Milk: 70.00 lb
Ration By: Kononoff & Judy — Growth: 0.10 lb/d — Fat: 5.30 %
Organization: University of Nebraska-Lincoln — Lact#: 3 — TP: 3.70 %

			Metabolizable Amino Acids						Duodnl Amino Acids		
	RR*	% Rqd	Diff	Rqd	Total	Tissue	Bact	Feed	Total	Bact	Feed
			g/d	g/d	g/d	g/d	g/d	g/d	g/d	g/d	g/d
Met	2.20	123	10.4	45.2	55.6	0.0	30.3	25.3	70.8	41.6	29.3
Lys	7.10	122	32.0	147.2	179.2	0.0	92.6	86.6	220.4	119.0	101.4
Arg	6.30	114	19.2	139.8	159.0	0.0	78.6	80.3	191.4	96.6	94.8
Thr	4.85	150	40.8	81.7	122.5	0.0	63.2	59.4	149.2	78.7	70.5
Leu	8.44	107	14.5	198.6	213.1	0.0	84.8	128.3	264.9	112.6	152.3
Ile	4.79	98	-2.0	122.9	120.8	0.0	66.4	54.4	150.7	85.3	65.5
Val	5.99	108	11.6	139.7	151.3	0.0	69.6	81.7	189.0	91.7	97.2
His	3.08	152	26.7	51.0	77.7	0.0	30.4	47.3	94.1	38.6	55.5
Phe	5.26	164	52.0	80.8	132.8	0.0	58.3	74.5	166.4	78.1	88.3
Trp	1.42	149	11.7	24.2	35.9	0.0	18.4	17.5	46.7	26.1	20.6

*** RR = Rulquin Ratio (Amino acids as a percentage of metabolizable protein).**

Canola meal (CanM) diet:

CPM-Dairy — Diet Summary - Both — 9/6/2016 3:38:16 PM

File: F:\Studies\1607DA\CPM\1607DA Canola Meal-AA Final
Farm: UNL Dairy Research Unit — BW: 850 lb — DIM: 120
Ration: 1607DA Canola Meal Ration — BCS: 3.25 — Milk: 70.00 lb
Ration By: Kononoff & Judy — Growth: 0.10 lb/d — Fat: 5.30 %
Organization: University of Nebraska-Lincoln — Lact#: 3 — TP: 3.70 %

Ingredient	Cost $ / T	DM %	AF lb/d	DM lb/d	% AF	% DM
Alfalfa hay	110.00	89.90	6.45	5.80	8.21	13.28
BrmdHy10Cp70Ndf9LNdf	60.00	88.00	0.57	0.50	0.72	1.15
Corn silage	33.00	35.50	49.86	17.70	63.42	40.54
Ground Corn	130.00	88.00	7.84	6.90	9.97	15.80
SoybeanML47.5Solv	435.80	90.00	2.28	2.05	2.90	4.70
RFDDGS	120.00	89.00	0.00	0.00	0.00	0.00
CanolaMealSolv	310.00	90.17	4.88	4.40	6.21	10.08
SoybeanHullsGrnd	123.60	91.00	1.21	1.10	1.54	2.52
Soy Pass	404.80	90.14	2.22	2.00	2.82	4.58
FatTallowBeef	752.60	99.00	0.86	0.85	1.09	1.95
CalciumCarbonate	26.80	99.50	0.60	0.60	0.77	1.37
BloodMeal	1247.0(	90.00	0.74	0.67	0.95	1.53
Megalac	1500.60	97.00	0.36	0.35	0.46	0.80
SodiumBicarbonate	543.80	99.50	0.25	0.25	0.32	0.57
CalciumPhosDi	26.80	99.50	0.15	0.15	0.19	0.34
MagOx	799.60	99.50	0.14	0.14	0.18	0.32
SaltNaCl	243.80	99.50	0.11	0.11	0.14	0.25
Vitamin Premix	26.80	95.75	0.02	0.02	0.03	0.05
Trace Premix	26.80	95.97	0.02	0.02	0.02	0.04
Agipro-L	0.00	97.00	0.02	0.02	0.03	0.05
SmartamineM	0.00	98.00	0.03	0.03	0.04	0.07
Total			78.62	43.66		

Macro Nutrients		
Nutrient	DM	AF
Dry Matter (%)	100.00	55.53
Forage (%)	54.97	32.81
Crude Prot (%)	18.06	10.03
RUP (%CP)	47.65	47.65
RDP (%CP)	52.35	52.35
RDP (%)	9.46	5.25
Sol Prot (%CP)	25.58	25.58
ME (mCal/lb)	1.23	0.68
NEl (mCal/lb)	0.79	0.44
Nem (mCal/lb)	0.79	0.44
NEg (mCal/lb)	0.52	0.29
ADF (%)	20.71	11.50
NDF (%)	30.48	16.92
For NDF (%NDF)	72.95	40.51
Forage NDF (%)	22.23	12.35
peNDF (%)	22.46	12.47
Lignin (%)	3.58	1.99
NFC (%)	40.80	22.66
Sil Acids (%)	3.22	1.79
Sugar (%)	3.38	1.88
Starch (%)	27.65	15.36
Sol Fiber (%)	6.55	3.64
EE Total (%)	5.66	3.14
EE 1 (%)	2.98	1.65
EE 2 (%)	2.00	1.11
EE 3 (%)	0.68	0.38
LCFA Total (%)	4.73	2.63
Ash (%)	8.62	4.79
Cost ($/d)	4.69	4.69
Cost ($T)	214.72	119.24

Minerals and Vitamins		
Nutrient	DM	AF
Dry Matter (%)	100.00	55.53
Calcium (%)	1.18	0.66
Phosphorus (%)	0.44	0.25
Magnesium (%)	0.38	0.21
Potassium (%)	1.25	0.70
Sulfur (%)	0.27	0.15
Sodium (%)	0.29	0.16
Chlorine (%)	0.23	0.13
Iron (ppm)	216.97	120.50
Zinc (ppm)	51.33	28.51
Copper (ppm)	13.32	7.40
Manganese (ppm)	48.94	27.18
Selenium (ppm)	0.13	0.07
Cobalt (ppm)	0.25	0.14
Iodine (ppm)	0.34	0.19
Vitamin A (KIU/lb)	1.32	0.73
Vitamin D (KIU/lb)	0.33	0.19
Vitamin E (IU/lb)	20.66	11.47
DCAD1 (meq/100g)	21.12	11.73
DCAD2 (meq/100g)	28.56	15.86
Cost ($/d)	4.69	4.69
Cost ($T)	214.72	119.24

Canola meal (CanM) diet:

CPM-Dairy | Fatty Acids - Summary | 9/6/2016 3:38:16 PM

File: F:\Studies\1607DA\CPM\1607DA Canola Meal-AA Final
Farm: UNL Dairy Research Unit | BW: 850 lb | DIM: 120
Ration: 1607DA Canola Meal Ration | BCS: 3.25 | Milk: 70.00 lb
Ration By: Kononoff & Judy | Growth: 0.10 lb/d | Fat: 5.30 %
Organization: University of Nebraska-Lincoln | Lact#: 3 | TP: 3.70 %

	Intake g/d	Lipolysed g/d	Duodenal g/d	Absorbed g/d	Fecal g/d	Digested % Duodenal
C12:0	1.46	1.25	1.46	1.39	0.07	95
C14:0	16.84	15.05	16.84	12.54	4.30	74
C16:0	223.03	177.16	237.71	176.75	60.95	74
C16:1	14.88	14.38	4.37	2.80	1.57	64
C18:0	79.16	73.30	475.06	345.26	129.79	73
C18:1T	14.51	13.99	64.35	50.51	13.85	78
C18:1C	298.68	262.58	98.32	86.15	12.16	88
C18:2	226.96	216.27	30.02	25.62	4.40	85
C18:3	36.43	35.11	2.33	1.81	0.52	78
Other	24.41	23.32	50.61	29.35	21.27	58
Ration	936.35	832.42	981.06	732.18	248.89	75

High fat dried distillers grains and solubles (aDDGS) diet:

CPM-Dairy — CNCPS Evaluation — 9/7/2016 2:41:28 PM

File: F:\Studies\1607DA\CPM\1607DA Consumer Supply RFDDGS-AA Final

Farm: UNL Dairy Research Unit	BW: 850 lb	DIM: 120
Ration: 1607DA Consumer Supply RFDDGS Ration	BCS: 3.25	Milk: 70.00 lb
Ration By: Kononoff & Judy	Growth: 0.10 lb/d	Fat: 5.30 %
Organization: University of Nebraska-Lincoln	Lact#: 3	TP: 3.70 %

Cost ($)	4.29	IOF ($)	-4.29			Ingredient	DM (lb/d)
DMI (lb/d)	43.7	Model	41.0	% Model	106.6	Alfalfa hay	5.800
ME Bal (mCal)	0.8	CP (%)	16.9	NDF (%)	31.0	BrmdHy10Cp70Ndf9LNdf	0.500
MP Bal (g)	-0.4	RUP (% CP)	48.8	ForageNDF (% NDF)	71.8	Corn silage	17.700
NP / MP (%)	65.0	LCFA (%)	5.0	ForageNDF (% DM)	22.2	Ground Corn	6.550
BactMP (% MP)	45.3	EE (%)	6.0	peNDF (%)	22.0	SoybeanML47.5Solv	2.400
Rumen N Balance				Lignin (%)	3.0	RFDDGS	4.400
Pept (g)	13	Pept & NH3 (g)	31	NFC (%)	40.6	CanolaMealSolv	0.000
% rqd	108	% rqd	110	Sil Acids (%)	3.2	SoybeanHullsGrnd	1.100
Amino Acid Balance				Sugar (%)	3.2	Soy Pass	2.000
Met (g)	9.7	Lys (g)	19.3	Starch (%)	27.2	FatTallowBeef	0.850
Met (% rqd)	121	Lys (% rqd)	113	Sol Fiber (%)	7.0	CalciumCarbonate	0.600
Met (% mp)	2.19	Lys (% mp)	6.66	Lys:Met	3.04:1	BloodMeal	0.670
Possible production due to ME and MP						Megalac	0.350

	Milk(lb)	Fat (%)	TP (%)	Milk(lb)	Fat (%)	TP (%)	
Trg:	70.0	5.30	3.70	70.0	5.30	3.70	SodiumBicarbonate 0.250
		Yield Constant			Composition Constant		CalciumPhosDi 0.150
							MagOx 0.140
ME:	70.0	n/a	n/a	71.3	5.30	n/a	SaltNaCl 0.110
MP:	70.0	n/a	3.70	70.0	5.30	3.70	Vitamin Premix 0.022
Adjustments based on Rulquin AA Ratios:							Trace Premix 0.017
	70.0	n/a	-0.01	-0.3	5.30	3.70	Agipro-L 0.022
n/a - Equations not available							SmartamineM 0.030
Ration DM (%)	55.50		Forage (% DM)		54.97		Total 43.661

High fat dried distillers grains and solubles (aDDGS) diet:

CPM-Dairy — Amino Acids — 9/7/2016 2:41:28 PM

File: F:\Studies\1607DA\CPM\1607DA Consumer Supply RFDDGS-AA Final
Farm: UNL Dairy Research Unit — BW: 850 lb — DIM: 120
Ration: 1607DA Consumer Supply RFDDGS Ration — BCS: 3.25 — Milk: 70.00 lb
Ration By: Kononoff & Judy — Growth: 0.10 lb/d — Fat: 5.30 %
Organization: University of Nebraska-Lincoln — Lact#: 3 — TP: 3.70 %

			Metabolizable Amino Acids						Duodnl Amino Acids		
	RR*	% Rqd	Diff	Rqd	Total	Tissue	Bact	Feed	Total	Bact	Feed
			g/d	g/d	g/d	g/d	g/d	g/d	g/d	g/d	g/d
Met	2.19	121	9.7	45.0	54.7	0.0	30.3	24.4	69.5	41.6	27.9
Lys	6.66	113	19.3	146.7	166.0	0.0	92.6	73.4	203.8	119.0	84.8
Arg	6.05	108	11.4	139.5	150.9	0.0	78.6	72.3	180.9	96.6	84.2
Thr	4.68	144	35.4	81.4	116.8	0.0	63.2	53.7	141.7	78.7	63.0
Leu	8.55	108	15.4	198.1	213.4	0.0	84.9	128.6	263.8	112.6	151.2
Ile	4.56	93	-8.9	122.6	113.8	0.0	66.4	47.3	141.7	85.3	56.4
Val	5.85	105	6.6	139.4	145.9	0.0	69.6	76.3	181.6	91.7	89.9
His	2.83	139	19.9	50.8	70.6	0.0	30.4	40.2	85.2	38.6	46.7
Phe	5.23	162	49.9	80.6	130.4	0.0	58.3	72.1	162.7	78.1	84.7
Trp	1.47	152	12.6	24.1	36.7	0.0	18.4	18.3	47.4	26.1	21.3

*** RR = Rulquin Ratio (Amino acids as a percentage of metabolizable protein).**

High fat dried distillers grains and solubles (aDDGS) diet:

CPM-Dairy — Diet Summary - Both — 9/7/2016 2:41:28 PM

File: F:\Studies\1607DA\CPM\1607DA Consumer Supply RFDDGS-AA Final
Farm: UNL Dairy Research Unit — BW: 850 lb — DIM: 120
Ration: 1607DA Consumer Supply RFDDGS Ration — BCS: 3.25 — Milk: 70.00 lb
Ration By: Kononoff & Judy — Growth: 0.10 lb/d — Fat: 5.30 %
Organization: University of Nebraska-Lincoln — Lact#: 3 — TP: 3.70 %

Ingredient	Cost $ / T	DM %	AF lb/d	DM lb/d	% AF	% DM
Alfalfa hay	110.00	89.90	6.45	5.80	8.20	13.28
BrmdHy10Cp70Ndf9LNdf	60.00	88.00	0.57	0.50	0.72	1.15
Corn silage	33.00	35.50	49.86	17.70	63.37	40.54
Ground Corn	130.00	88.00	7.44	6.55	9.46	15.00
SoybeanML47.5Solv	435.80	90.00	2.67	2.40	3.39	5.50
RFDDGS	120.00	89.00	4.94	4.40	6.28	10.08
CanolaMealSolv	518.00	90.17	0.00	0.00	0.00	0.00
SoybeanHullsGrnd	123.60	91.00	1.21	1.10	1.54	2.52
Soy Pass	404.80	90.14	2.22	2.00	2.82	4.58
FatTallowBeef	752.60	99.00	0.86	0.85	1.09	1.95
CalciumCarbonate	26.80	99.50	0.60	0.60	0.77	1.37
BloodMeal	1247.0(	90.00	0.74	0.67	0.95	1.53
Megalac	1500.60	97.00	0.36	0.35	0.46	0.80
SodiumBicarbonate	543.80	99.50	0.25	0.25	0.32	0.57
CalciumPhosDi	26.80	99.50	0.15	0.15	0.19	0.34
MagOx	799.60	99.50	0.14	0.14	0.18	0.32
SaltNaCl	243.80	99.50	0.11	0.11	0.14	0.25
Vitamin Premix	26.80	95.75	0.02	0.02	0.03	0.05
Trace Premix	26.80	95.97	0.02	0.02	0.02	0.04
Agipro-L	0.00	97.00	0.02	0.02	0.03	0.05
SmartamineM	0.00	98.00	0.03	0.03	0.04	0.07
Total			78.67	43.66		

Macro Nutrients		
Nutrient	DM	AF
Dry Matter (%)	100.00	55.50
Forage (%)	54.97	32.79
Crude Prot (%)	16.94	9.40
RUP (%CP)	48.83	48.83
RDP (%CP)	51.17	51.17
RDP (%)	8.67	4.81
Sol Prot (%CP)	22.74	22.74
ME (mCal/lb)	1.24	0.69
NEl (mCal/lb)	0.80	0.44
Nem (mCal/lb)	0.80	0.44
NEg (mCal/lb)	0.54	0.30
ADF (%)	19.71	10.94
NDF (%)	30.96	17.18
For NDF (%NDF)	71.80	39.85
Forage NDF (%)	22.23	12.34
peNDF (%)	22.01	12.21
Lignin (%)	3.01	1.67
NFC (%)	40.60	22.53
Sil Acids (%)	3.22	1.79
Sugar (%)	3.25	1.80
Starch (%)	27.16	15.08
Sol Fiber (%)	6.97	3.87
EE Total (%)	5.97	3.31
EE 1 (%)	3.32	1.84
EE 2 (%)	1.98	1.10
EE 3 (%)	0.68	0.38
LCFA Total (%)	4.98	2.76
Ash (%)	8.43	4.68
Cost ($/d)	4.29	4.29
Cost ($T)	196.35	108.97

Minerals and Vitamins		
Nutrient	DM	AF
Dry Matter (%)	100.00	55.50
Calcium (%)	0.98	0.54
Phosphorus (%)	0.43	0.24
Magnesium (%)	0.35	0.20
Potassium (%)	1.27	0.71
Sulfur (%)	0.29	0.16
Sodium (%)	0.30	0.17
Chlorine (%)	0.24	0.13
Iron (ppm)	208.69	115.81
Zinc (ppm)	49.81	27.64
Copper (ppm)	12.29	6.82
Manganese (ppm)	42.21	23.43
Selenium (ppm)	0.17	0.09
Cobalt (ppm)	0.25	0.14
Iodine (ppm)	0.34	0.19
Vitamin A (KIU/lb)	1.32	0.73
Vitamin D (KIU/lb)	0.33	0.19
Vitamin E (IU/lb)	20.66	11.46
DCAD1 (meq/100g)	20.44	11.34
DCAD2 (meq/100g)	26.82	14.89
Cost ($/d)	4.29	4.29
Cost ($T)	196.35	108.97

High fat dried distillers grains and solubles (aDDGS) diet:

CPM-Dairy — Fatty Acids - Summary — 9/7/2016 2:41:28 PM

File: F:\Studies\1607DA\CPM\1607DA Consumer Supply RFDDGS-AA Final
Farm: UNL Dairy Research Unit — BW: 850 lb — DIM: 120
Ration: 1607DA Consumer Supply RFDDGS Ration — BCS: 3.25 — Milk: 70.00 lb
Ration By: Kononoff & Judy — Growth: 0.10 lb/d — Fat: 5.30 %
Organization: University of Nebraska-Lincoln — Lact#: 3 — TP: 3.70 %

	Intake g/d	Lipolysed g/d	Duodenal g/d	Absorbed g/d	Fecal g/d	Digested % Duodenal
C12:0	1.60	1.40	1.60	1.53	0.07	95
C14:0	16.76	14.98	16.76	12.48	4.28	74
C16:0	233.33	187.30	247.17	183.61	63.56	74
C16:1	14.35	13.86	4.43	2.83	1.59	64
C18:0	80.56	74.67	494.97	359.76	135.21	73
C18:1T	14.08	13.57	78.62	61.72	16.90	79
C18:1C	294.51	258.46	97.54	85.48	12.07	88
C18:2	273.00	261.56	34.61	29.39	5.22	85
C18:3	32.96	31.69	2.20	1.71	0.50	78
Other	23.96	22.88	50.83	29.47	21.36	58
Ration	985.11	880.37	1028.74	767.99	260.75	75

APPENDIX B: 2017 ADSA ANNUAL MEETING POSTER

Abstract #M295: Milk and methane production in lactating dairy cattle consuming distillers dried grains or canola meal

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INTRODUCTION

- The use of co-products as an alternative feed source is a common practice when formulating dairy rations.
- These co-products are a good source of protein and energy for ruminants.
- Two of the more popular co-products in the United States are canola meal and dried distillers grains with solubles (DDGS).
- Previous research has found canola meal to be a suitable replacement for DDGS in dairy cattle diets (Christen et. al, 2010; Mulrooney et al, 2009)
- Methane (CH_4) production has been observed to be reduced with increasing concentrations of conventional DDGS (ConDG) (Benchaar et al., 2013) as well as the addition of reduced-fat DDGS (RFDDGS) (Foth et al. 2015); this may potentially be due to difference in fat or the concentration or composition of fiber.

OBJECTIVE AND HYPOTHESIS

- To test the effects of feeding corn milling co-products, specifically reduced-fat dried distillers grains with solubles (RFDDGS) and canola meal (CM) on dry matter intake (DMI), milk production, composition, and methane production in lactating dairy cows.
- Our hypothesis was rations containing the coproducts will reduce methane without affecting milk production and composition.

MATERIALS AND METHODS

- 12 multiparous (79 ± 16 DIM) (mean ± SD) lactating Jersey cows housed in a tie stall barn
- A replicated 4 X 4 Latin square design was used to compare four different dietary treatments.
- Treatments (Table 2) were composed of a control (CON) containing no co-products, a treatment diet containing 10% (DM basis) DDGS supplied by POET LLC (Sioux Falls, SD) (pDDGS), 10% DDGS treatment with an alternative distillers grains source (aDDGS), and a 10% canola meal (CanM) treatment as described in Table 2.
- Co-products were included in partial replacement for corn and soybean meal and all diets were formulated using the CPM model.
- Indirect calorimeters were used to sample methane.
- 28-d periods, last 5d of each period for data collection
  - Daily feed intake (fed once a day, allowing ≈ 10% orts)
  - Daily milk production (2X milking)
  - Milk composition
  - Methane production collected via the indirect calorimetry method
- Data were analyzed using the MIXED procedure of SAS
  - Fixed effects: Period and Treatment
  - Random effect: Cow

Table 1. Feed chemical analysis for reduced-fat dried distillers grains with solubles (RFDDGS), and alternative source of distillers grains with solubles (DDGS), and canola meal (CM)

Item[1]	RFDDGS[2]	CM	DDGS
		% of DM	
DM	89.8	89.7	89.6
CP	30.8	41.1	32.2
Soluble Protein	8.53	13.2	7.65
Acid Detergent Insoluble Crude Protein	2.01	2.39	2.90
Neutral Detergent Insoluble Crude Protein	2.69	4.28	3.83
ADF	11.7	19.3	11.9
NDF	32.2	27.1	31.8
Lignin	3.40	9.02	3.59
Starch	6.68	0.30	2.60
Ether Extract	6.05	3.46	10.0
Ash	6.40	10.4	5.85
Phosphorus	0.91	1.17	0.97
Sulfur	1.14	0.82	1.09

[1] Values determined by Cumberland Valley Analytical Services, Hagerstown, MD
[2] RFDDGS supplied by POET nutrition LLC Sioux Falls, SD

Table 2. Diet composition of control, reduced-fat dried distillers grains with solubles (pDDGS), an alternative source of dried distillers grains with solubles (aDDGS) , and canola meal (CanM)

Item	Control	pDDGS	CanM	aDDGS
		% of DM		
Corn Silage	40.5	40.5	40.5	40.5
Alfalfa Hay	13.3	13.3	13.3	13.3
Brome Hay	1.15	1.15	1.15	1.15
Ground Corn	16.8	15.0	15.8	15.0
Soybean Meal	13.7	5.50	4.70	5.50
DDGS	–	10.1	–	10.1
Canola Meal	–	–	10.1	–
Soybean Hulls	2.52	2.52	2.52	2.52
Bypass Soy[3]	4.58	4.58	4.58	4.58
Calcium Carbonate	1.37	1.37	1.37	1.37
Tallow	1.95	1.95	1.95	1.95
Bloodmeal	1.53	1.53	1.53	1.53
Ca salts of LCFA[2]	0.80	0.80	0.80	0.80
Vitamin Mineral Premix[4]				
Rumen Protected Lysine[4]	0.05	0.05	0.05	0.05
Rumen Protected Methionine[4]	0.07	0.07	0.07	0.07
CP	18.6	17.1	18.1	16.9
Ether Extract	5.50	5.80	5.70	6.00
NDF	28.7	31.0	30.5	31.0
Starch	28.3	27.2	27.7	27.2

[3] SoyPass, UgroTech, Overland Park, KS.
[2] Calcium salts of long-chain fatty acids marketed as Megalac by Church & Dwight Co. Inc. Princeton, NJ.
[4] Formulated to supply approximately 130,000 IU/d vitamin A, 38,000 IU/d vitamin D and 902 IU/d vitamin E in total rations. Formulated to supply approximately 2,900 mg/kg Co, 29,000 mg/kg Cu, 2,600 mg/kg I, 1,000 mg/kg Fe, 190,000 mg/kg Mn, 820 mg/kg Se and 180,000 mg/kg Zn in total rations.
[4] AgTrace, AJ Livestock Heartland Inc., Chicago, IL
[4] Smartamine M, Adisseo Inc., Antony, France

RESULTS

Table 3. DMI, milk production and composition, BW and BCS[1] of Control (CON), POET reduced-fat dried distiller's grains with solubles (pDDGS), canola meal (CanM), and an alternative reduced-fat dried distillers grains with solubles treatments (aDDGS)

Item	CON	pDDGS	CanM	aDDGS	SEM[2]	P-value
DMI, kg/d	17.7	17.5	17.7	17.0	0.58	0.78
Milk Yield, kg/d	23.4	24.2	24.2	24.4	0.80	0.55
ECM[3], kg/d	32.7	33.3	33.4	33.8	1.23	0.76
Fat, %	6.23	6.11	6.21	6.17	0.17	0.94
Fat Yield, kg/d	1.46	1.48	1.49	1.50	0.06	0.87
Protein, %	3.67	3.63	3.60	3.64	0.04	0.43
Protein Yield, kg/d	0.86	0.87	0.87	0.89	0.03	0.83
SCC, cells/mL	52.7	72.9	52.7	75.8	17.4	0.36
MUN[4], mg/dl	20.7[a]	18.1[b]	19.9[a]	18.1[b]	0.62	<0.01
CH_4 Production, L/d	335	329	360	337	19.6	0.54
CH_4/ECM, L/kg/d	10.3	10.2	10.8	10.0	0.70	0.78
CH_4/DMI, L/kg/d	19.0	18.9	20.4	19.8	1.26	0.75
Heat production, Mcal/d	19.5	20.2	21.5	20.6	0.67	0.11
Heat production/d/MBW[5]	203[b]	211[ab]	228[a]	215[ab]	6.27	0.06
Body Weight, kg	462	461	454	459	11.5	0.17
BCS[1]	3.23	3.21	3.06	3.18	0.09	0.39

[1] BCS = Body Condition Score 1-5 scale according to Wildman et al. (1982)
[2] Lowest standard error of treatment means is shown
[3] Energy corrected milk = 0.327 × milk yield [kg] + 7.2 × protein [kg] adjusted for 3.5% fat and 3.2% total protein (CNE Glossary, 2014)
[4] MUN = Milk urea nitrogen
[a,b] Means within rows lacking common superscript differ (P < 0.05)
[5] Heat production calculated with Brouwer's (1965) equation from oxygen consumption (L), carbon dioxide production (L), methane production (L) (HP = 3.866 × O2 + 1.200 × CO2 – 0.518 × CH4)

DISCUSSION AND CONCLUSIONS

- Results of this study indicate that milk production and the composition of milk can be maintained when the feed co-products DDGS and canola meal replace corn and soybean meal (Table 3).
- Total methane production per cow per day or per unit of milk or DMI was not affected when feeding coproducts (Table 3).
- The inclusion of canola meal tended to increase heat production in lactating dairy cattle (Table 3).

ACKNOWLEDGMENTS

The authors would like to thank POET Nutrition LLC for partial support of the study. We would also like to thank the dairy team at University of Nebraska-Lincoln for their part in data collection.

APPENDIX C: PROCEDURE FOR BOILING URINE

Urine Boiling Procedure:

1. Thaw frozen urine

2. Pour two 250 ml bottles or approximately 500 ml of urine into a 600 ml beaker. Place 14 beakers in a boiling water bath underneath a hood. This will remove water out of the urine and turn it into a dark brown paste. Try to keep the water level in the bath the same as the urine level in the beaker. Add distilled water to the bath as needed

3. Turn the water bath on each morning and off each afternoon or the beaker will burn and the sample will be ruined. This procedure may take 2 to 5 days

APPENDIX D: PROCEDURE FOR DRYING URINE FOR DETERMINATION OF GROSS ENERGY

1. Thaw urine
2. Weigh and record weight of cotton round
3. Fold cotton round and place into crucible
4. Pour 4 ml of urine onto cotton round in the crucible
5. Place crucibles in the drying oven at 105 degrees for 24 h
6. Once samples are dried, run on bomb calorimeter, entering the weight of the cotton round when prompted for sample weight
7. Record gross heat (cal/g)