Energy Content of Reduced-Fat Dried Distillers Grains and Solubles for Lactating Dairy Cows and Effects on Energy and Nitrogen Balance

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ENERGY CONTENT OF REDUCED-FAT DRIED DISTILLERS GRAINS AND
SOLUBLES FOR LACTATING DAIRY COWS AND EFFECTS ON ENERGY
AND NITROGEN BALANCE

by

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ENERGY CONTENT OF REDUCED-FAT DRIED DISTILLERS GRAINS AND SOLUBLES FOR LACTATING DAIRY COWS AND EFFECTS ON ENERGY AND NITROGEN BALANCE

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University of Nebraska, 2014

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Eight Holstein and 8 Jersey multiparous, lactating cows were used to complete 56 energy balances to determine the energy content of reduced-fat distillers grains and solubles (RFDDGS). A repeated switchback design was used to compare treatments with and without RFDDGS. Diets consisted of 24.2 % corn silage, 18.4 % alfalfa hay, 6.94 % brome hay with either 22.9 % rolled corn and 14.8 % soybean meal (Control), or 8.95 % rolled corn, 28.8 % RFDDGS, and 0 % soybean meal (Co-P; DM basis). The inclusion of RFDDGS did not affect ($P = 0.86$) DMI averaging 21.4 ± 0.53 kg DM for all cows but milk production tended ($P = 0.10$) to increase from 29.8 to 30.9 ± 1.46 kg/d for Control and Co-P treatments. There was no difference between treatments in milk fat percentage or ECM ($P = 0.81$ and 0.22, respectively), averaging 4.33 ± 0.14 % and 34.1 kg/d, respectively. Milk protein was decreased ($P < 0.01$) by the Co-P treatment (3.56 and 3.41 ± 0.08 % for Control and Co-P treatments), but protein yield was not affected ($P = 0.51$). Milk energies were 1.40 Mcal/d higher with Co-P ($P = 0.01$). Energy lost as methane was reduced ($P < 0.01$) by 0.31 Mcal/d with the addition of RFDDGS to the diet. Heat loss averaged 29.9 ± 0.55 Mcal/d and was not different between diets ($P = 0.49$). Average energy retained as tissue energy was -2.99 ± 0.93 Mcal/d ($P = 0.73$). Intake of digestible and metabolizable energy were not significantly different ($P = 0.16$).
and 0.14 for DE and ME, respectively) between the Control and Co-P treatments, averaging 2.68 and 2.31 Mcal/kg DM, respectively. Net energy of lactation values of Control and Co-P diets were calculated to be 1.43 and 1.47 Mcal/kg DM ($P = 0.10$), respectively.
“And whatever you do, whether in word or deed, do it all in the name of the Lord Jesus, giving thanks to God the Father through him.”

-Colossians 3:17 NIV

“I have fought the good fight, I have finished the race, I have kept the faith.”

-2 Timothy 4:7 NIV
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CHAPTER 1

INTRODUCTION

Ethanol production has increased over the past 10 years, resulting in greater quantities of ethanol byproducts known as distillers grains and solubles (Paz et al., 2013). These byproducts are utilized as animal feeds in beef and dairy diets, as well as other animal production systems (Berger and Singh, 2010). Distillers grains with solubles are a good source of rumen undegradable protein (RUP) and energy for ruminants and may be included up to approximately one third of the diet for lactating dairy cows (Schingoethe et al., 2009). More recently, a process has been developed to remove some of the oil from the byproducts and the remaining fraction is sold to cattle producers as reduced-fat distillers grains with solubles (RFDDGS). Reduced-fat distillers grains have been shown to reduce the risk of milk fat depression when fed to lactating dairy cows (Mjoun et al., 2010; Castillo-Lopez et al., 2014). The productive benefits of RFDDGS in dairy cow diets have been documented but energy values associated with reduced fat are still being researched to provide accurate values for diet formulation. The energy content of distillers grains may be determined through energy balance studies, as conducted by Birkelo et al. (2004).

Energy utilization is difficult to determine in lactating cows because of the multiple complex biological pathways used to produce milk. Energy must be digested, metabolized, and used to meet maintenance requirements before it can be partitioned to milk production. To account for energy losses, different methods have been used to calculate energy loss from heat produced by oxidation of carbohydrates, protein and fat (Nienaber et al., 2009).
In 1900, Kellner and Kohler developed the starch equivalent system where the energy value of feeds were determined relative to how starch was able to meet an animal’s need for growth (Johnson et al., 2003). Since then, new systems and methods have been developed to determine energy values of individual feed ingredients which more accurately explain the differences in nutritive value for the animal. The most common and perhaps most extensive method to determine energy values of feeds fed to lactating dairy cattle is open-circuit whole animal respiration chambers which indirectly measure heat production using gas exchange and methane production. Chambers have been constructed at the Beltsville, MD and Clay Center, NE, USDA research centers in the United States. Other chambers used in research are in The Netherlands, United Kingdom and other countries around the world (van Zijderveld et al., 2011; Reynolds et al., 2014; Williams et al., 2013; Yan et al., 1997). Chambers allow utilization of dietary energy to be studied in animal experiments and also to determine the energetic value of individual feeds. Other systems which are less expensive and less labor intensive have also been created and utilized to determine gas exchange and production, including indirect calorimeter headboxes, sulfur hexafluoride gas and comparative slaughter (Nienaber et al., 2009). The construction of headboxes can be more cost effective than whole animal chambers to run and maintain (Hellwing et al., 2012).

To date, there have been no studies conducted to determine energy utilization when lactating dairy cattle are fed RFDDGS for lactating dairy cows. Therefore the objectives of this study were 1) to compare indirect calorimetry systems, 2) to construct and measure the accuracy of the headbox system, and 3) to determine the energy content
of RFDDGS in lactating dairy cow diets using headboxes as an indirect method to determine heat production and energy balance.
LITERATURE REVIEW

Corn-ethanol Production and Distillers Grains

Distillers Grains Production. To produce ethanol, corn is ground and mixed with water to create a slurry (Figure 1.1). The slurry is cooked at 104°C using pressurized steam. Alpha-amylase, glucoamylase and yeast are added to break down starch, allow saccharification and fermentation, respectively. Ethanol can then be removed and the remaining non-fermented corn fractions including germ, fiber, and protein remain as whole stillage which is then centrifuged to remove water and soluble solids as thin stillage. Thin stillage is evaporated and the soluble solids are added back, dried, and produce dried distillers grains with solubles (DDGS; Berger and Singh, 2010).

Other procedures have been developed to remove other products from DDGS or improve its nutritional value (Figure 1.1). In recent years, a common practice has been to partially remove corn oil from DDGS resulting in RFDDGS. Methods to remove oil include solvent extraction and centrifugation (Berger and Singh, 2010; Mjoun et al., 2010). One process used to recover oil from evaporated thin stillage is a disk stack centrifuge is able to recover approximately one third of the oil (Berger and Singh, 2010). The process of solvent extraction is able to recover a greater proportion of oil, resulting in approximately 2.7 % crude fat and 34.0 % crude protein (Saunders and Rosentrater, 2009; Mjoun et al., 2010). Extracted oil is commonly then used for biodiesel production.

Nutrient Value of RFDDGS. Dried distillers grains with solubles are utilized as a protein and energy source in lactating cow diets because they contain high amounts of RUP and energy. Rumen undegradable protein allows a supply of protein to escape
rumen microbial degradation, and pass through to the small intestine to be digested by the animal, and corn oil provides energy. Schingoethe et al. (2009) suggested that DDGS is approximately 47 to 64 % RUP as a percent of total CP, and Castillo-Lopez et al. (2014) calculated RUP of RFDDGS to be within the range of 46.5 to 50.9 %. Total dietary requirements for RUP in lactating dairy cows according to the Dairy NRC (NRC, 2001) is 35 to 40 % of total CP, which suggests that DDGS can be used to increase the consumption of RUP. The RUP in DDGS has also been found to be highly digestible by ruminants and of higher quality than expected for a byproduct, improving its value as a feed ingredient (Kononoff et al., 2007). In a meta-analysis, Paz et al. (2013) determined the average RUP digestibility to be 83.9 %.

A large portion of energy in DDGS originates from corn oil and digestible fiber which remain after the fermentation process (Schingoethe et al., 2009). A number of years ago, a small percentage of starch ranging from 5-10 % may have remained in the byproduct adding to its energy value, but with advances in technology, essentially all of the starch is removed (Schingoethe et al., 2009). Historically, DDGS consisted of approximately 10 to 14 % fat. There is a concern that fat from DDGS may result in milk fat depression with diets high in DDGS (Bauman and Griinari, 2003). By removing a portion of the oil to produce RFDDGS, it is possible to reduce the risk of milk fat depression and potentially increase feeding levels (Castillo-Lopez et al., 2014). Mjoun et al. (2010) tested the occurrence of milk fat depression with increasing levels of RFDDGS from 0 to 30 %. In doing so, ground corn, soybean meal, and soybean hulls were replaced to maintain isoenergetic rations and the investigators actually observed that milk fat percentage increased linearly with RFDDGS without affecting milk production.
resulting in a similar linear increase in FCM. However, they also found that animals receiving 30% RFDDGS were in a slight negative energy balance of -0.81 Mcal/d compared to positive values for all other treatments, most likely due to the increased energy partitioning to milk fat.

Another benefit of including DDGS with the removal of other feed ingredients in lactating cow diets is the resulting drop in methane ($\text{CH}_4$) production (Benchaar et al., 2013). Total-tract methane production including eructated and enteric sources followed a linear decrease with DDGS inclusion rates up to 30%, and this may have been caused by increased fat which inhibits growth of the protozoa population (Knapp et al., 2014). There is a symbiotic relationship between protozoa and methanogens with protozoa releasing free hydrogen ions which are then utilized by methanogens for the production of $\text{CH}_4$. By reducing protozoa populations, it has been suggested that $\text{CH}_4$ production may also be indirectly reduced. Benchaar et al. (2013) suggested $\text{CH}_4$ suppression might also be due to low dietary fiber from including concentrate at the expense of forage in the diet. This would lead to lower free hydrogen production from formation of propionate rather than acetate, again providing less hydrogen ions for methanogens. Reduced $\text{CH}_4$ production was also observed in beef feedlot steers fed DDGS with a 16% drop in eructated $\text{CH}_4$ (McGinn et al., 2009).

The chemical composition of DDGS varies due to differences between and within plants. NRC (2001) lists estimates for DDGS of 3.72, 3.03, and 1.97 Mcal/kg for digestible, metabolizable, and lactation energies, respectively, but due to changes in the production processes over time, newer more accurate values need to be determined. Wet distillers grains were found to contain 2.25 Mcal/kg of $\text{NE}_L$ which is 15% higher than
NRC estimates for DDGS (Birkelo et al., 2004). Positive production responses of DDGS have been reported but its nutritional value is still being studied. Thus the recommended diet inclusion rate is 20 % (DM basis) to maximize production but reduce potential nutrient over-supplementation or negative production affects (Schingoethe et al., 2009).

**Energy Utilization**

*Energy Balance.* The amount of energy an animal consumes is known as their gross energy intake (GEI). A large proportion of the GEI is digested and absorbed, but some energy sources may be indigestible and are therefore excreted without utilization. That energy which is not lost through fecal output is assumed to be digestible energy (DE; Equation 1). Energy that has been digested may also be lost without use to the animal. Urinary energy has been metabolized but is excreted, and eructed CH₄ which is produced by rumen microorganisms is potential energy that is also lost by the animal. Metabolizable energy (ME; Equation 2) is DE minus urinary and methane energy. Net energy of lactation (NE₇) is the energy required for maintenance, lactation, gestation and growth. Other biological functions such as digestion, absorption, fermentation and motility utilize energy and produce heat. Heat production (HP) is the difference between ME and NE₇ (Equation 3).

\[
\text{DE} = \text{GEI} - \text{fecal energy} \quad [1]
\]

\[
\text{ME} = \text{DE} - \text{urinary energy} - \text{methane energy} \quad [2]
\]

\[
\text{NE}_\text{L} = \text{ME} - \text{heat production} \quad [3]
\]

Another measure of energy utilization that is used to explain animal production is efficiency. Within the dairy industry, efficiency can be defined as the saleable product
per unit of feed input (Bauman et al., 1985). Brody (1945) defines gross efficiency as the percentage of energy of feed, including maintenance, recovered in the desired product such as milk, growth, and work. This concept accounts for the product formed, maintenance requirements, work of organizing precursors into product, an increase in metabolism due to greater organ activity from higher nutrient concentrations in blood, excreting wastes formed through transformation of precursors into products, and maintenance costs of forming product (Brody, 1945). Similarly, Bauman et al. (1985) defines productive efficiency of dairy cows as “…the yield of milk and milk components in ratio to the nutritional cost of maintenance, lactation and of returning the cow to the level of body condition that exists before the onset of lactation”. With this description of efficiency, an increase in milk yield will improve productive efficiency because of the “maintenance energy dilution effect”. This effect occurs because milk yield increases while maintenance requirements remain relatively unchanged regardless of production level. Freetly et al. (2006) observed a similar effect by comparing maintenance energy requirements of lactating beef cows to previous studies with dairy cows. Maintenance estimates were similar even though milk yields were much lower. Other commonly calculated efficiencies are percent of milk energy from ME and percent energy lost as methane compared to productive energy used to produce milk (Benchaar et al., 2013; van Zijderveld et al., 2011b; Reynolds et al., 2014; Xue et al., 2011; Tine et al., 2001; Wilkerson et al., 1997).

Milk production has been determined to be more energetically efficient than body fat deposition or growth because conversion of dietary nitrogen to amino acids requires less energy than synthesizing urea, and the shorter fatty acid chains found in milk require
less energy to form than body fat as long chain fatty acids (Blaxter, 1962; Brody, 1945; Bauman et al., 1985). However, excess dietary protein, will be utilized inefficiently in effort to metabolize the carbon skeleton of amino acids and excrete nitrogen as urea (Blaxter, 1962). By continuing to learn to understand digestive processes to precisely meet nutrient requirements for individual tissues, it is theoretically possible to improve productive efficiency (Bauman et al., 1985).

**Energy Metabolism.** Lactating animals have a high demand for energy to meet requirements for maintenance and milk production. If these demands are not met through dietary energy, body reserves will be catabolized, resulting in a negative body energy balance. To avoid or minimize negative energy balances, nutritionists balance diets to meet energy requirements. In order to accomplish this, the ability of individual feed ingredients and/or the diet as a whole to provide energy must be predicted. Total energy content and heat of combustion of a feed ingredient can be determined through bomb calorimetry by completely combusting a sample in an insulated chamber and measuring the change in temperature, but does not explain how a biological system will utilize the energy source (Blaxter, 1962). Utilization of energy can be extremely complex because energy partitioning depends on many factors including type of ration, stage of lactation, environmental conditions and animal size, and is variable (Saama et al., 1993). However, there is little variation reported among animals of similar physiological status in energy partitioning except for total energy balance (Saama et al., 1993). While measuring gross energy intake of an individual animal on a specific diet, as well as fecal, urinary and milk energy outputs can be labor intensive but relatively simple, the most difficult energy
expenditure of an animal to accurately measure is HP. In 1889, Richet and Rubner were
the first to create a gradient layer calorimeter to directly calculate an animal’s HP from a
known diet in order to determine the amount of energy utilized by the animal. Although
this method provided insight into energy utilization and HP, it was found to be difficult to
conduct accurately because of errors such as heat loss from absorption through the floor,
as well as the addition of heat from feces and urine which were excreted (Blaxter, 1962).

Understanding the metabolic pathways of nutrients may help determine energy
production and utilization through calculations based on chemical bonds and structure.
However, this is method can be extremely complex. For example, if the amounts of
organic compounds oxidized by the body are known, the total HP may be calculated by
summing the enthalpies of their oxidation (Blaxter, 1989). The Law of Hess states that
the change in heat of a reaction is independent of the path it took. This suggests that it is
possible to simplify predictions by indirectly determining HP without accounting for the
many different possible pathways for energy to follow (Saama et al., 1993). If this is
true, heat of combustion can also be predicted based on the amount of carbon, hydrogen
and oxygen present in polysaccharides consumed by the animal (Blaxter, 1962).

However, error in this method may be due to the difference in oxidation of carbohydrates
compared to other nutrient sources such as amino acids, resulting in an over- or under-
estimation of total HP. Measuring the carbon and nitrogen balance of animals can also
indirectly measure energy retained (RE) in kcal by the animal using Equation 4.

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

Brouwer (1965) published an equation to indirectly calculate HP in ruminants to
be used with energy balance experiments. Brouwer’s equation takes into account oxygen
(O_2) consumption, carbon dioxide (CO_2) and CH_4 production, and urinary nitrogen as in Equation 5 with HP in kcal, O_2, CO_2 and CH_4 in liters, and N (urine N) in g. The theory behind Brouwer’s equation is that heat from the oxidation of carbohydrates, fat and protein, along with heat produced from the production of urea is equal to total heat given off by the animal. This method of determining energy balance is widely used but complete accuracy cannot be reached because of the assumption that all dietary components are completely oxidized (Blaxter, 1962). The main factor in accurately determining gas exchange is the precision of gas analysis which indicates the importance of using and maintaining a high quality analyzer (Young et al., 1975). Also based on gas exchange, the respiratory quotient (RQ) can be used to indicate metabolic processes or the metabolism of different substrates. The RQ can be defined as the ratio of CO_2 production to O_2 consumption which changes based on the product oxidized (Nienaber et al., 2009). When carbohydrates are being oxidized the RQ is equal to 1.000 because the volume of O_2 consumed is proportional to the volume of CO_2 produced. For example, the oxidation of glucose consumes 6 molecules of O_2 and produces 6 molecules of CO_2, as seen in Equation 6. The oxidation of lipid and protein lead to RQ values of 0.711 and 0.809, respectively. RQ values greater than 1 indicate synthesis of lipid.

\[
\text{HP} = 3.866 \times \text{O}_2 + 1.200 \times \text{CO}_2 - 0.518 \times \text{CH}_4 - 1.431 \times \text{N} \quad [5]
\]

\[
\text{C}_6\text{H}_{12}\text{O}_6 + 6 \text{O}_2 \rightarrow 6 \text{CO}_2 + 6 \text{H}_2\text{O} + \text{heat} \quad [6]
\]

**Methane Production.** The energy used for production of CH_4 by rumen microorganisms is considered non-metabolizable because the animal is not able to use the energy for production or growth. It is therefore desirable to reduce CH_4 losses as one
means to improve energetic efficiency, as well as reducing CH$_4$ released as a greenhouse gas (Johnson and Johnson, 1995). Methane is predicted to contribute to 15 to 17% of global warming and slightly less than 2% of CH$_4$ contributions are expected to come from cattle (Johnson and Johnson, 1995). Cattle begin producing CH$_4$ around 4 weeks of age and production increases as the animal grows and consumes more feed, reaching levels of 109 to 126 kg per year for mature dairy cows (Johnson and Johnson, 1995).

Average CH$_4$ production of cattle is approximately 6% of total GEI but can vary based on a number of factors (Johnson and Johnson, 1995). First, carbohydrates supply hydrogen ions when fermented by influencing the volatile fatty acid ratio between acetate and propionate. This ratio will affect the amount of free hydrogen ions available for methanogens to utilize in synthesis of CH$_4$ and can be affected by rumen pH level. The pH of the rumen will change the environment of the microbes, altering microbial populations. Reynolds et al. (2014) observed at lower ruminal pH, acetate concentrations decreased and propionate concentrations increased in lactating dairy cows, and CH$_4$ production decreased. The investigators suggested the reason for this was a shift in hydrogen utilization from methane to propionate synthesis and this may have been a result of the drop in pH from less dietary fiber, thereby inhibiting methanogenesis.

Secondly, the fermentability of ingested carbohydrates may also influence CH$_4$ production. A greater extent of fermentation may lead to lower CH$_4$ production because higher fermentable feeds are typically processed which usually leads to smaller particle size, and therefore higher passage rates. This will reduce rumen retention time and as a result, the time microorganisms have access to feed particles (Johnson and Johnson, 1995). However, Wilkerson et al. (1997) observed no difference in CH$_4$ energy when
comparing rolled and ground corn processing techniques, and determined 5.2 % of GE intake was utilized for methane production. Thirdly, concentration of fat in diets may influence CH$_4$ production, as discussed previously. This is believed to occur because fat inhibits protozoa which reduces the concentration of free hydrogen for methanogens to use (Benchaar et al., 2013; Johnson and Johnson, 1995). In a study by Whitelaw et al. (1984), defaunation of rumen protozoa reduced CH$_4$ production by 50 %. Additionally, biohydrogenation of unsaturated fatty acids utilizes hydrogen ions that would otherwise be used to synthesize CH$_4$. However, biohydrogenation only accounts for approximately 1 % of the total hydrogen utilized in the rumen. Reduction of CO$_2$ to CH$_4$, VFA synthesis and bacterial cell synthesis account for 48, 33 and 12 % of ruminal hydrogen utilization (Czerkawski, 1986). Therefore fat typically reduces fermentability of substrates rather than directly influencing methanogenesis (Johnson and Johnson, 1995). Similarly, Andrew et al. (1991) observed CH$_4$ energy was reduced when a calcium salt of long-chain fatty acids was added to the diet of lactating dairy cows at 2.95 % (DM basis). The high concentration of fat in the diet was most likely responsible for the reduction in methane. Finally, directly inhibiting pathways in the production of CH$_4$ is possible but there is limited knowledge of these pathways. Alternative hydrogen sinks in the rumen are potential inhibitors of CH$_4$ production, once again utilizing free hydrogen ions instead of allowing methanogens to use them (Johnson and Johnson, 1995). Acetogenesis is a hydrogen disposal mechanism and 3 species that carry out this process have been found present in rumen contents (Greening and Leedle, 1989). However, there is little evidence of acetogenesis activity in the rumen, but more likely occurs in the hind gut. If added to the diet, other compounds such as 3-nitrooxypropanol (3NP) may cause changes in CH$_4$
production pathways. This compound is a potential inhibitor of methyl-coenzyme M reductase which is involved in the reduction of CO\textsubscript{2} to CH\textsubscript{4} in the rumen by methanogens. Reynolds et al. (2014) added 3NP to lactating cow diets to observe the effects and found a 7 to 10\% decrease in CH\textsubscript{4} production, but volume of CH\textsubscript{4} per kg milk produced was not affected. They also observed residual effects of 3NP with CH\textsubscript{4} production remaining low throughout the day and not only immediately after dosing. This suggests that it is possible to identify pathways in the synthesis of CH\textsubscript{4} which can then be targeted in order to reduce CH\textsubscript{4} emissions.

**Heat Production.** As stated previously, heat is produced from the physiological digestion and oxidation of compounds (Blaxter, 1989). The efficiency with which a cow can convert those compounds into milk is important because it affects the amount of energy which is remaining for milk production. Thus, by reducing the amount of heat lost, it is possible to improve productive efficiency. However, HP does not vary greatly from animal to animal and is difficult to reduce with high milk production because of the increased metabolic activity that must accompany greater nutrient digestion and conversion to milk compounds (Belyea and Adams, 1990). Differing response of HP to level of intake have been observed. Wilkerson et al. (1997) observed an increase in HP with diets that increased milk production, whereas Andrew et al. (1991) observed HP was not affected with a 2.3 kg/d increase in milk production. Higher intakes were also found to increase HP when animals had ad libitum access to feed compared to restricted diets but were lower when expressed as a percent of GE intake (Tine et al., 2001). Belyea and Adams (1990) compared 6 high and 6 low genetic merit lactating Holstein cows for
producing milk and found no difference in HP but when expressed per kg metabolic BW ($BW^{0.75}$), the low producing animals had higher HP resulting in 61.7 % of ME lost as heat, compared to 52.9 % in high producing animals. This suggests that cows with higher genetic merit are able to convert ME into milk more efficiently, indicating the possibility of lowering HP through genetic selection.

Energy Requirements for Maintenance. Maintenance requirements are held relatively constant among animals and between cattle breeds but will vary slightly depending on diet and physiological state (Bauman et al., 1985). If more nutrients are required to grow, produce milk or support a fetus, maintenance energy will increase to support digestion of dietary nutrients and synthesis of precursors into needed compounds such as muscle, fat or milk. It can be difficult to establish accurate maintenance energy requirements because of complications in adaptation to levels of alimentation, changes in diet digestibility, fermentation, microbial growth, protein supply, production level, variable nutrient flux, metabolism, hormonal control and product composition (Johnson et al., 2003). However, over the past 100 years, energy requirements for maintenance are believed to have not changed dramatically but Evans et al. (2000) found a slight increase over the past 20 years. Many studies involving energetics estimate maintenance values to be 73 kcal of $NE_L/kg\,BW^{0.75}$ as averaged by Tyrrell and Moe (1972) in a meta-analysis on earlier studies. Maintenance estimates for lactating dairy cows since that time vary from 90 to 170 kcal of $ME/kg\,BW^{0.75}$ as seen in Table 1.1 (Flatt et al., 1967a; Flatt et al., 1967b; Van Es and van der Honing, 1976; Vermorel et al., 1982; Moe and Tyrrell, 1971; Yan et al., 1997; Reynolds and Tyrrell, 2000; Birkelo et al., 2004; Freetly et al., 2006;
Xue et al., 2011). This suggests that maintenance requirements have increased or previous values were under-estimated.

Yan et al. (1997) proposed an increase in maintenance energy estimates with increased milk production may be due to greater energy requirements partitioned to milk production, as well as increased mass of hepatic, gastro-intestinal, and renal organs to support higher GEI. Differences between breeds has been studied by Xue et al. (2011) using Holstein and Jersey-Holstein cross primiparous lactating animals. They determined maintenance of Holsteins to be 170 kcal of ME/kg BW\(^{0.75}\) and Jersey-Holstein crosses were 160 kcal of ME/kg BW\(^{0.75}\), which was significantly lower than the purebreds suggesting efficiency of converting ME to milk of crossbreds is greater than Holsteins. However, Reynolds and Tyrrell (2000) found no difference in maintenance between lactating Holstein and Hereford-Angus crossbred animals with maintenance averages at 120 kcal of ME/kg BW\(^{0.75}\). The difference between studies could stem from Xue using first lactation animals while animals in the Reynolds and Tyrrell study were multiparous. In general, maintenance requirements vary among animals and breeds, diets, physiological states, and with analytical differences, but total variation is limited to a range of 73 – 170 kcal of ME/kg BW\(^{0.75}\).

**Energy Requirements for Lactation.** The most important value in energy partitioning for dairy producers is the NE\(_L\) because it directly influences the economic value of the animals. In the study by Belyea and Adams (1990) which compared high and low producing dairy cows, they found high producing cows had higher NE\(_L\) values of 7.8 Mcal/d because of reduced maintenance energy requirements. This allowed the
animals to partition more energy towards milk production while still filling maintenance requirements. Interestingly, high producing cows also mobilized less body fat, suggesting they were more efficient at converting ME to NE\textsubscript{L}.

A number of studies have looked at how stage of lactation affects partitioning of energy to NE\textsubscript{L}. Tine et al. (2001) and Xue et al. (2011) found less ME partitioned towards milk as lactation progressed resulting in greater fat accretion, but Williams et al. (2013) did not see a difference in the efficiency of animals to convert dietary energy to NE\textsubscript{L} throughout lactation. There is most likely a metabolic change that takes place as lactation progresses which influences energy partitioning and allows the animal to regain the lost body energy reserves from early lactation.

Diet is believed to have a major effect on NE\textsubscript{L} with increased dietary energy supplying greater amounts of ME to be partitioned to milk and milk fat (Andrew et al., 1991). However, different methods of processing feeds can also influence NE\textsubscript{L} as described by Wilkerson et al. (1997). Corn stored dry was found to have approximately 80\% of the value of high moisture corn for conversion of ME to NE\textsubscript{L}. Van Knegsel et al. (2007) compared a glucogenic diet to a lipogenic diet with the same concentration of energy in both diets and found the lipogenic treatment had a greater proportion of ME which was converted to NE\textsubscript{L} and increased milk fat with 51.7 and 55.2\% of ME converted to milk energy for glucogenic and lipogenic diets, respectively. They also found animals receiving the glucogenic diet had lower priority in converting energy to milk and therefore partitioned excess energy to body reserves resulting in higher total energy balances. The lipogenic animals were found to have increased body fat.
mobilization compared to glucogenic animals in order to fill the requirements for higher milk fat.

Energy partitioning is complex but there may be ways to manipulate genetics, maintenance requirements, and the animal’s ability to utilize feeds to improve efficiency of cows to produce milk (Belyea and Adams, 1990). Improving our understanding of physiological state, diet, and animal effects influence energy partitioning, the more accurately we can estimate nutrient requirements and optimize milk production. More research needs to be conducted on how maintenance energy requirements have changed in lactating dairy cows and how to reduce energy loss through methane production.

**Calorimetry Methods**

Calorimetry has been used for many decades as a way to determine nutritional energetics or HP by humans or animals. Nienaber et al. (2009) defines animal calorimetry as the science of measuring heat transfer between an animal and its environment. Throughout history, nutritional energetics have been used to pursue three main objectives (Johnson et al., 2003). The first is to determine the relationship between gas exchange and HP. The second objective is to find a method in which to evaluate foods or feed ingredients and determine energy requirements and expenditures of the animal. The final purpose is to determine dietary energy partitioning, where the energy is used in the body and how much is usable energy. Overall, calorimetry is used to define the amount of energy an animal requires for metabolism of nutrients by determining heat production or loss. There are two general methods used to determine HP which are direct and indirect calorimetry (Blaxter, 1962; Nienaber et al., 2009). Both methods are
accepted as valid and accurate methods to study energetics, but are not directly comparable due to different underlying analytical principles.

**Direct Calorimetry.** Direct calorimetry measures heat loss in the form of sensible and evaporative heat losses from the animal (Nienaber et al., 2009; Blaxter, 1989) and have been used mostly in human and small animal studies, and is less commonly for large animals (Johnson et al., 2003). Animals have little control over sensible heat loss due to environmental effects, but evaporative heat losses can be changed by the animal through O₂/CO₂ exchange or perspiration. There is also a small amount of heat lost through heating of ingested food and water (Blaxter, 1962).

There are a number of different techniques used to determine heat loss directly. Respiration calorimeters are whole animals chambers which prevent heat loss or gain from the chamber to measure sensible heat loss from the animal (Nienaber et al., 2009). A common design has an air space between the chamber and the outside environment that is maintained at the same temperature as inside the chamber so there will be no transfer of heat. Temperature is constantly monitored and the amount of heat produced is considered sensible heat loss from the animal.

Gradient layer calorimetry is another technique used to directly measure heat loss. An advantage of this chamber is that it can partition sensible heat loss into radiation and convection by placing heat flow meters on each inside wall, floor and ceiling of the chamber to measure heat loss through the walls. Another advantage is that it has the ability to respond quickly to heat losses from the animal when it moves or changes.
position. A gradient layer calorimeter is currently used with mice by Dr. Nielsen at the University of Nebraska-Lincoln (Nienaber et al., 2009).

The general calculation for metabolizable energy based on direct calorimetry is Equation 7 where RE is retained energy and HP is heat produced (Johnson et al., 2003). Retained energy is due to thermal insulation of the tissues, specifically the skin and hair. The use of direct calorimetry does not allow for measurement of retained energy, but over the long run an equilibrium between RE and HP will be met.

\[
\text{ME} = \text{RE} + \text{HP} \tag{7}
\]

**Indirect Calorimetry.** Direct and indirect calorimetry are equal unless work such as growth, milk production or egg laying is conducted. However, over the long term, they will remain similar (Blaxter, 1962). While direct calorimetry measures heat loss, indirect calorimetry is based on heat production. Nienaber et al. (2009) defines indirect calorimetry as the measurement of energy exchange taking place within the animal’s living tissues. This includes both metabolism of food and catabolism of body tissue. Indirect calorimetry operates on the basis of gas exchange being correlated to HP. The first indirect calorimeter was designed by Lavoisier and Laplace by observing the relationship between ice melting and carbon dioxide production of a guinea pig based on oxidation of carbohydrates as in Equation 6 (Brody, 1945), and numerous indirect calorimeter methods have been developed.

The main advantage of indirect compared to direct calorimetry is that different environmental conditions can be tested with indirect methods. There is also greater flexibility in calorimeter design with indirect techniques. Blaxter (1962) suggests it is
difficult to attain absolute precision of HP when using indirect calorimetry because of the many assumptions made when dealing with elemental comparisons. However the errors incurred are relatively small (Blaxter, 1962).

**Indirect Calorimetry Methods**

There are two main subtypes of indirect calorimeters, closed- and open-circuit. The first closed-circuit indirect calorimeter was designed by Regnault and Reiset in 1849 (Blaxter, 1962). Closed-circuit calorimeters absorb CO\textsubscript{2} and water vapor as it is produced and replaces O\textsubscript{2} as it is consumed. Oxygen replenishment is measured, which is equal to the volume of O\textsubscript{2} consumed, providing a direct estimate of gas exchange (Blaxter, 1989). One difficulty with closed-circuit chambers is the change in O\textsubscript{2} admission into the system due to changes in temperature and pressure. Any small change may cause significant errors in determining oxygen consumption. Most closed-circuit chambers have been used for human and small animal experiments, but some large animals have also been tested. Research with ruminants also poses a challenge in the form of methane gas which must also be removed from the system through the use of absorbents.

Open-circuit chambers use airflow rate and a difference in O\textsubscript{2} and CO\textsubscript{2} concentrations to determine HP (Nienaber et al., 2009). The first open-circuit calorimeter was designed by Pettenkofer and Voit (Blaxter, 1962). Precise measurements of air volume passing through the chamber must be made and true samples of incoming and outgoing air must be collected in order to accurately determine gas concentrations. Any small error in gas estimates can over- or under-estimate gas exchange, significantly
influencing HP calculations using Brouwer’s equation (Equation 5). Chamber ventilation rates may also affect gas exchange analysis. Decreased ventilation rates will allow for a greater difference in gas concentrations between incoming and outgoing air, resulting in a more accurate calculation of HP. However, this may cause accumulation of CO₂ in the chamber, simulating animal respiration and increasing water vapor. The result is unnatural gas exchange and inaccurate estimation of HP (Blaxter, 1962). Typically, a difference in gas concentrations of 0.7 to 1.0 % from incoming to outgoing air is targeted and the air flow rate is adjusted to meet this setting. This will lead to a reduction in error in gas analysis but maintain adequate levels of oxygen for the animal (Young et al., 1975).

**Carbon Dioxide Entry Rate Technique (CERT) Method.** The CO₂ entry rate technique (CERT) uses a ¹⁴C isotope to measure CO₂ production in ruminants. The ¹⁴C can be lost through CO₂ from the lungs, CO₂ or CH₄ from fermentation in the rumen, feces and urine, although fecal and urinary losses are relatively insignificant. The isotope is infused as ¹⁴C-bicarbonate into the animal and allowed to reach equilibrium with the body CO₂ pool. Once equilibrium has been reached, saliva from the parotid gland is collected into a backpack through tubing running through the animal’s cheek. The saliva is then tested for presence and concentration of the ¹⁴C marker and CO₂ is calculated based on its dilution. Sahlu et al. (1988) tested this method on wethers and compared it to whole animal chamber measurements of CO₂. They observed no difference in estimating CO₂ production between methods, suggesting the CERT method may accurately provide a value for CO₂ production. They used Brouwer’s equation (1965) to
calculate heat production but urinary nitrogen was ignored because less than 1% of total HP is from synthesis of urea. However, estimation of HP based on CERT depend on RQ which is in itself an estimation and can result in large errors. Another potential issue with CERT is radioactive contamination from $^{14}$C being infused into the animal which may harm the animal (Sahlu et al., 1988).

**Sulfur Hexafluoride ($\text{SF}_6$) Method.** In the last 20 years, sulfur hexafluoride ($\text{SF}_6$) has been frequently utilized as a marker to determine total rumen CH$_4$ production from animals in a more natural setting. For this method, the $\text{SF}_6$ marker is released into the rumen from a permeation tube placed directly into the rumen. The $\text{SF}_6$ is allowed to equilibrate before the release rate is determined. Once the release rate is known, total CH$_4$ production can be determined. A sample of air from around the nostrils is directed into a canister usually placed around the animal’s neck and concentrations of CH$_4$ and $\text{SF}_6$ are determined using gas analyzers (Figure 1.2). Total daily CH$_4$ production is calculated based on the concentration and release rate of $\text{SF}_6$ (Grainger et al., 2007). This method has been shown to accurately determine CH$_4$ production of ruminants but there are limitations (Grainger et al., 2007; Boadi and Wittenberg, 2002). For example, hind gut fermentation is also responsible for 2 to 12% of total methane production (Johnson and Johnson, 1995) but Boadi and Wittenberg (2002) suggest some hind gut CH$_4$ is absorbed into the blood and expired through the lungs where it adds to the measured concentration. This loss of CH$_4$ from hind gut fermentation may be minute but it has resulted in underestimation of CH$_4$ production using $\text{SF}_6$ at a rate of 93 to 95% when compared to whole animal chambers which can account for all gas excretions (McGinn et
Grainger et al. (2007) compared CH₄ production of high production lactating dairy cows using the SF₆ method and whole animal chambers and found coefficients of variation within individual cows to be 6.1 and 4.3 %, respectively. Within treatments, the coefficients of variation were 19.6 and 17.8 %, respectively, suggesting more replications are necessary for the SF₆ method to reach the same level of accuracy as chambers. Methane production averages were determined to be 331 ± 74.6 and 322 ± 57.5 g/d for SF₆ and chamber methods, respectively, but no significant difference was observed. It was concluded that using the SF₆ tracer provided accurate estimates of CH₄ production in animals with high intakes, but DMI greater than 20 kg/d may result in overestimation of CH₄. Boadi and Wittenberg (2002) suggested among animal variation was due to differences in intake, eating behavior, animal selectivity, rumen capacity and rate of passage. They also suggested variation within animals was generally caused by differences in intake level which can account for 64 % of the variation because of the correlation between CH₄ production and DMI, but SF₆ may be more greatly influenced by digestive tract characteristics. The greatest benefit of this method is that it allows animals to remain untethered and behave normally during collections to reduce error due to changes in daily routine.

**Comparative Slaughter Technique.** Another method for indirectly determining HP is by partitioning RE and ME as in Equation 7 in the hind limb, gastrointestinal tract, liver, gravid uterus, or fetus after slaughter based on body composition (Nienaber et al., 2009). This method requires the animal to be slaughtered and is therefore not as useful as
a live animal technique. Also, this method is not applicable to lactating dairy animals. The slaughter method will not be discussed further in this review.

**Whole Animal Chambers.** Whole animal chambers are historically the most common type of indirect calorimeter used in energetic studies with lactating dairy cows. These chambers are designed to be large enough to house the entire animal, as well as equipped to allow for feed, water, and feces and urine collection equipment (Figure 1.3, Figure 1.4). Chambers are typically under slight negative pressure because these systems are not air tight and a negative pressure will ensure no gas expired by the animal will leave through any location other than to be measured or analyzed (Young et al., 1975). Gas flow is held constant for the duration of gas collection, and air volume is corrected for standard temperature and pressure (STP) including air temperature within the chamber, atmospheric pressure, negative pressure imposed on the system, and dew point. Sources of error may include gas analysis, gas temperature, moisture and pressure, and the calculation used to determine HP. However, whole animal chambers are considered to be the most accurate method of gas exchange because expired air from the lungs, eructated gas from the rumen, and gas lost as flatulence are all accounted for. As seen previously, other gas collection methods are compared to chambers to determine their accuracy (Sahlu et al., 1988; Boadi and Wittenberg, 2002; Grainger et al., 2007).

The coefficients of variation of multiple energy values determined through the use of whole animal chambers were studied by Bauman et al. (1985) in a review on energetic efficiency of dairy cows. They found variation in GE and milk energy to be 18 to 23 % but variation in DE or ME was only 1.9 to 2.5 %. This suggests that efficiency of
nutrient absorption and genetics do not affect milk yield, but intake and production levels have the greatest influence on energy balance. They did however, find a difference among animals in nutrient partitioning. Animals with a higher genetic potential for milk production were able to yield more milk due to increased intakes and mobilization of body reserves than lower genetic potential cows. Overall, there was found to be variation between cows using whole animal chambers, but the variation was due to differences in animal efficiencies rather than gas collection or analysis.

*Headboxes.* Headboxes are relatively new developments as indirect calorimeters. They function similarly to whole animal chambers but enclose only the animal’s head, and therefore do not account for gases lost from hind gut fermentation (Figure 1.5). However, they are significantly less expensive and less complicated to run. They also allow lactating animals to be milked without disrupting gas collections.

**SUMMARY**

After ethanol is produced, distillers grains remain as a byproduct and can be utilized in ruminant feeds as a energy and protein source. It is beneficial for dairy producers to purchase distillers grains because of its high nutrient value and relatively low cost compared to corn grain. With recent developments, a portion of oil can be removed from distillers grains, producing RFDDGS with 3 to 8% fat which has been shown to reduce the risk of milk fat depression. This progress in technology allows producers to include RFDDGS at a higher proportion of the diet than previously and reduce feed costs.
Energy metabolism is a complex biological system. Energy can be lost if it remains undigested and is lost through feces, or after digestion is lost as CH₄ and urine, lost as heat if metabolized, or used for work such as growth or lactation. With each source of energy loss, there is potential to improve energetic efficiency by minimizing losses. Methane can be reduced by diet which affects the rumen environment and therefore the organisms present. If conditions for CH₄ producing bacteria are not ideal, ruminant CH₄ production can be reduced. Heat production can be correlated to intake level, but is more greatly influenced by genetic potential. Cows that have higher genetic potential for milk production have lower HP which allows more energy to be partitioned towards synthesis of milk. Maintenance energy requirements are greater for high producing cows because of increased need for nutrients, but values for lactating cows range from 73-170 kcal of ME/kg BW⁰.⁷⁵ regardless of production level, physiological state or breed. The ability of an animal to convert ME to NE₅₀ is mostly due to diet, but can also be influenced by stage of lactation, with diets high in energy and early lactation improving efficiency of energy conversion to milk. These potential targets for reducing energy loss from lactating cows allows for manipulation of energy efficiency, and therefore milk production.

There are numerous methods that have been used in the past and are currently in use to determine energy balance and gas exchange in lactating dairy cows. The most common system used is open-circuit indirect calorimetry which allows indirect calculation of HP or gas exchange based on a sample of gas from the animal. The standard used to compare other designs to are whole animal chambers which account for respiration gases, eructated gas and gas from hind gut fermentation. Other systems
include CERT, SF\textsubscript{6}, and comparative slaughter techniques, as well as headboxes. These systems do not account for gas produced during hind gut fermentation, but the loss of energy through flatulence is minimal compared to eructated gas. Therefore, these systems have been shown to be accurate methods to indirectly determine HP or gas production.
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faunated and ciliate-free cattle and its relationship with rumen volatile fatty acid

lactating cows fed diets containing dry or high moisture corn in either rolled or

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efficiency of Holstein and Jersey-Holstein crossbred dairy cows offered diets

### Table 1.1. List of energy balance studies and determined maintenance energy values (Mcal ME/kg BW\(^{0.75}\)) of lactating dairy cows

<table>
<thead>
<tr>
<th>Author</th>
<th>Maintenance Energy Value (kcal ME/kg BW(^{0.75}))</th>
<th>Cow Breed</th>
</tr>
</thead>
<tbody>
<tr>
<td>Flatt et al. (1967a)</td>
<td>110</td>
<td>Holstein</td>
</tr>
<tr>
<td>Flatt et al. (1967b)</td>
<td>141.5</td>
<td>Holstein, dry and lactating</td>
</tr>
<tr>
<td>Van Es and van der Honing (1976)</td>
<td>117</td>
<td>Holstein</td>
</tr>
<tr>
<td>Vermorel et al. (1982)</td>
<td>121</td>
<td>Holstein-Friesian</td>
</tr>
<tr>
<td>Moe and Tyrrell (1971)</td>
<td>110</td>
<td>Holstein and Jersey</td>
</tr>
<tr>
<td>Yan et al. (1997)</td>
<td>160</td>
<td>Holstein-Friesian</td>
</tr>
<tr>
<td>Reynolds and Tyrrell (2000)</td>
<td>120</td>
<td>Hereford-Angus heifers</td>
</tr>
<tr>
<td>Birkelo et al. (2004)</td>
<td>136.2</td>
<td>Holstein</td>
</tr>
<tr>
<td>Freetly et al. (2006)</td>
<td>146</td>
<td>MARC III heifers</td>
</tr>
<tr>
<td>Xue et al. (2011)</td>
<td>169</td>
<td>Holstein and Jersey-Holstein</td>
</tr>
</tbody>
</table>
Figure 1.1. Dry grind ethanol process producing distillers grains byproduct (DDGS) with modified processes in dashed boxes (Berger and Singh, 2010)
Figure 1.2. Sulfur hexafluoride (SF$_6$) method for indirect calculation of methane production
Figure 1.3. Schematic of an indirect open-circuit whole animal chamber (Nienaber and Maddy, 1985)
Figure 1.4. Photo of (a) Armsby indirect open-circuit whole animal chambers and (b) gas analysis system (University Park, PA; photo credit Dr. Paul Kononoff)
Figure 1.5. Photo of headbox collecting gas from a Holstein cow (Place et al., 2011)
APPENDIX A: EQUATIONS

\[ DE = GEI – \text{fecal energy} \]  \[ 1 \]

\[ ME = DE – \text{urinary energy – methane energy} \]  \[ 2 \]

\[ NE_L = ME – \text{heat production} \]  \[ 3 \]

\[ \text{kcal RE} = (12.55 \times \text{g C retained}) – (6.90 \times \text{g N retained}) \]  \[ 4 \]

\[ HP = 3.866 \times \text{O}_2 + 1.200 \times \text{CO}_2 – 0.518 \times \text{CH}_4 – 1.431 \times \text{N} \]  \[ 5 \]

\[ \text{C}_6\text{H}_{12}\text{O}_6 + 6 \text{O}_2 \rightarrow 6 \text{CO}_2 + 6 \text{H}_2\text{O} + \text{heat} \]  \[ 6 \]

\[ ME = RE + HE \]  \[ 7 \]
CHAPTER 2

Technical Note: Construction, validation and recovery rates of an indirect calorimetry headbox system used to measure heat production of cattle

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ABSTRACT

An indirect calorimetry headbox system was built at the University of Nebraska-Lincoln using a design from USDA Meat Animal Research Center (Clay Center, NE) to collect samples of gas from large ruminants. Three headboxes were constructed with plexiglass sides allowing the animal to stay in visual contact with other animals, and was mounted on wheels to let the animal stay in its normal environment. Three lamp runs were conducted to determine the accuracy of the system. Ethanol (100%) was burned in the sealed headbox and gas samples were collected with oxygen and carbon dioxide recovery rates ranging from 97.5 to 107.4% and 94.2 to 105.5%, respectively. This system has the ability to capture gas and provide accurate results for live animal experiments.
INTRODUCTION

Indirect calorimetry is a method frequently used method to determine energy balance and efficiency of dairy cattle. One of the challenges for any indirect calorimeter is to provide an environment for the animal where it can exhibit normal behavior and to avoid hyperventilation or abnormal behaviors which may influence physical activity and ultimately physiological gas exchange (Place et al., 2011). Respiration calorimeters are artificial environments because animals need to be restrained and their daily routine may be altered during gas collection (Place et al., 2011). As such, to yield results it is important to minimize stress and change for the animal to accommodate normal behaviors. An additional challenge associated with calorimetry is the high cost of construction, and costs associated with operating and maintaining the calorimeters. For example, a recent study outlined a 4 chamber, open-circuit indirect calorimeter system and gas analysis equipment cost approximately $300,000 (Hellwing et al. 2012). The small number of respiration calorimeters that can be found in North America and the world can also lead to the conclusion that cost is a limiting factor.

When designing the indirect calorimeter headbox system used in the current study, these factors were taken into account. The units were designed to be cost effective indirect calorimeters, using many parts from the local hardware stores. In addition, creating a mobile system allowed the indirect calorimeter to be brought to the animal so the animal could stay in its accustomed environment. Also, plexiglass sides provided clear visual access to other animals and its surroundings, resulting in a natural environment (Hellwing et al., 2012). The objective of the experiment was to outline the construction and test gaseous recovery within the system.
SYSTEM DESIGN AND COMPONENTS

Structure

The headboxes were constructed at the University of Nebraska-Lincoln. A complete list of parts, model numbers, manufacturers, and suppliers can be found in Appendix B. The original design was created at the USDA, ARS, US Meat Animal Research Center (Clay Center, NE). Each box measured $1.78 \times 0.81 \times 0.76$ m ($H \times W \times D$) with 0.6 cm thick aluminum angle iron used for the frame, and flat iron for the top and bottom (Figure 2.1a). The backside of the box was also made of aluminum flat iron but contained an opening for the animal’s head ($1.17 \times 0.38$ m). The bottom of the box was partially slanted at a $28.5^\circ$ angle so feed would always be in reach of the cow. All welding work was conducted by Wahoo Metal Products (Wahoo, NE). Three caster wheels (Hamilton Caster & Mtg. Co., Hamilton, OH) were used to mobilize the box with one swiveling wheel in the front and two fixed wheels in the back on either side of the box (Figure 2.1b). Plexiglass (0.48 cm thick) was attached to the frame from the inside of the box on the front and sides to provide the animal with relatively normal view of its surroundings. A door was built into the plexiglass on one side of the box and was used to provide feed to the animal. Weather stripping (3M, St. Paul, MN) was used to seal the door to minimize air leakage, and two latches held it shut during collections. Inside the headbox, a stainless steel waterbowl (DeLaval, Tumba, Sweden) was mounted across from the door to metal support pieces located on the outside (Figure 2.1c). A hole (3.81 cm diameter) was cut in the aluminum sheet metal and 1.90 cm industrial high pressure water hose attached the waterbowl to a water source located outside the box. An
additional hole (3.81 cm diameter) was cut in the bottom of the box to act as an escape for water in the event of a water leak.

A hood was designed to minimize air movement from between the cow and the opening located on the back of the headbox. The hood was made from black military strength tarp material and fit directly into the opening of the headbox (Hastings Canvas, Hastings, NE). The edges of the hood were attached from the inside of the box with 3.18 cm bolts every 0.12-0.14 m through 2.50 cm aluminum straps (0.60 cm thick). The hood tapered down to a 1.32 m circumference at the end that attached to the cow’s neck. Two 0.71 m zippers were built lengthwise into the neck sleeve to move cow in or out of the headbox (Figure 2.1d). Around the hood at the narrow end, a cord was used to tighten it around the cow’s neck and tie it in place. Cows entering the headbox could also be tethered to the inside of the box by an adjustable length chain attached to the far corner. The chain was attached to the cows restraint. This system was designed to allow full movement of the cow to eat, drink, lie down or stand up while remaining in the headbox.

Gas Sampling System

Air was removed from the headbox using a vacuum motor (Model 115923, Ametek Lamb Electric, Kent, OH) using a variable transformer (Figure 2.2a; Model 3PN1010B, Staco Energy Products Co., Dayton, OH) to regulate the air flow rate. The motor was covered with a 15.2 cm diameter tin corn can and mounted to the roof of the box with the aid of a metal support. A standard shop vacuum 15 cm diameter air filter (Craftsman, Hoffman Estates, IL) was fitted over the end of the can to keep feed particles and dust from plugging the motor or getting into the gas sample (Figure 2.2b). PVC pipe
(3.81 cm) was used to divert air from the motor to a gas meter (Model AL425, American Meter, Horsham, PA) and out of the headbox to measure air flow rate (Figure 2.2c). To determine air pressure inside the box to correct for standard temperature and pressure, an air tube (0.64 cm) deflected a sample of air to a U-tube manometer (Item # 1221-8, United Instruments, Westbury, NY) and this was located off a PVC pipe located distal from the gas meter (Figure 2.2d). Another air tube from the same location in the PVC pipe redirected air through a 20.3 cm long Drierite drying tube (Figure 2.3a; WA Hammond Drierite Co. Ltd., Xenia, OH) to remove moisture from the gas and to a glass tube rotameter (Figure 2.3b; Model 1350E Sho-Rate “50”, Brooks Instruments, Hatfield, PA), which allowed for the volume of collected gas to be regulated.

Fresh air entered into the headbox entered from the space between the cow and hood. Air tubes were positioned on the outside of the headbox, close to both the top and bottom of the neck opening where the fresh air was entering, allowing a representative sample of ingoing air to be collected. The air tubes entered a small vacuum pump (Model BP 202-1, Binaca Products Inc., Temecula, CA) and exited as one combined sample (Figure 2.3c). The air was directed through another Drierite drying tube to a second glass tube rotameter.

The samples of gas moving through the rotameters was routed into 44 L sample bags (Figure 2.3d; 61 x 61 cm LAM-JAPCON-NSE) fitted with polypropylene stopcocks (Figure 2.3e; Nalge Nunc 6460-0004, Nalgene Labware) fitted with Teflon resin TFE plugs to control air movement in and out of the bags. Using wire hangers, bags were hung at the top of the bag to a plastic hook on the side of the headbox and out of reach from any neighboring animals. Stopcock plugs were kept closed until gas collection
commenced, opened during collection, and reclosed when the collection period was completed. A probe used to measure temperature and dew point (Figure 2.4a; Model TRH-100, Pace Scientific Inc., Mooresville, NC) within the headbox was positioned close to the top of the box and connected to a pocket logger (Figure 2.4b; Model XR440, Pace Scientific Inc., Mooresville, NC) located in a sealed container on the outside. Temperature and dew point data could be downloaded after collection or be used for real time output (Figure 2.5). Total cost of all components for the headbox was approximately $6,000.

**SYSTEM OPERATION**

*Validation and Recovery Rate*

Previous to any live animal collections, gas recovery using the headbox system was tested. Ethyl alcohol lamps were filled and weighed prior to running the procedure and the headbox was sealed. When ethyl alcohol is burned in the presence of oxygen, carbon dioxide and water are produced. Thus this reaction was used to validate recovery of gases using the headbox system. Four lamps were placed inside the box and the wick was ignited (Figure 2.6). Initial readings of the gas meter were taken and recorded. The vacuum motor was then started and the stopcock plugs were opened to allow gas to be sampled. The system was operated for 2 hours at a consistent rate of air flow as if a live animal was in the headbox. The glass tube rotameters which were used to collect gas samples were opened to a half turn above 65 mm in order to collect a representative sample of air. The volume collected over 2 h was approximately 10 L. After running for 2 hours, the lamps were extinguished by quickly opening the door and capping the wick
of the lamps before resealing the door. The system was operated for an additional 10 minutes to remove all the carbon dioxide produced by the burning alcohol. The stopcocks were then closed and the system was shut down. Final gas meter readings were recorded.

Temperature and pressure of gas exiting the box was recorded to calculate the concentration of carbon dioxide expected to be in the gas samples. The average temperature value from the 2 h collection period was measured directly from the pocket loggers and corrected for standard temperature (Equation 1). Pressure was corrected for vapor, line, and barometric pressure (mmHg; Equation 2). Vapor pressure was calculated from a 2 h average of the dew point (°C) within the headbox from the pocket logger data (Equation 3). Line pressure was measured from the manometer, and barometric pressure of the room was recorded using a barometer (Chaney Instrument Co., Lake Geneva, WI).

\[
\text{Corrected temperature} = 273 \, ^\circ\text{K} + \text{average line pressure} \, [\circ\text{C}] \quad [1]
\]
\[
\text{Corrected pressure} = \left(\text{line pressure} + \text{vapor pressure} + \text{barometric pressure}\right) / 760 \, \text{mmHg} \quad [2]
\]
\[
\text{Vapor pressure} = 0.61078 ^\left(17.27 \times \text{dew point} \, [\circ\text{C}] / (237.3 + \text{dew point} \, [\circ\text{C}])\right) \quad [3]
\]

Flow rates were calculated by the difference in gas meter readings from the beginning to the end of each run and then divided by the number of minutes of the lamp run (Equation 4). The given flow rate was then corrected for the individual gas meter using a pre-determined value (Equation 5), yielding the meter correction factor (MCF), and the overall corrected flow meter (CFM) rate (Equation 6). The total corrected volume of gas flowing through the system was corrected for standard temperature and pressure (Equation 7).
Flow rate = (final meter – initial meter)/minutes [4]

Meter correction factor (MCF) = (0.0002 × flow rate^2) – (slope correction factor × flow rate) + intercept correction factor [5]

Corrected flow meter = flow rate × MCF [6]

Total corrected air volume = (final meter – initial meter [L]) × MCF × 28.32 ft^3/m^3 × (273 K/corrected temperature) × corrected pressure [7]

Determination of the concentration of oxygen and carbon dioxide was performed in duplicate on ambient air and gas collected from inside the headbox, as described by Nienaber and Maddy (1985; Xstream 3channel analyzer, Emerson Process Management, Bloomington, MN). The values for each gas were averaged for each bag and corrected based on the best fit line of the gas tanks. The differences in oxygen concentrations and carbon dioxide concentrations between incoming and outgoing air was converted to volume by multiplying the differences by the total volume of gas corrected for standard temperature and pressure (Equation 8). Because oxygen poses a different density than carbon dioxide a correction factor was used to determine volume of oxygen consumed (Equation 9). With the total volume of oxygen consumed and carbon dioxide produced, the ratio of CO_2 to O_2, also know as the respiratory quotient (RQ) was calculated (Equation 10). The expected ratio was 2:3, or 0.67%. Theoretical oxygen and carbon dioxide were calculated based on the weight of the alcohol burned from the lamps (Equation 11, 12). The ratio of actual to theoretical oxygen or carbon dioxide volumes were calculated as percent of gas recovered (Equation 13, 14). Ratios ranging from 95-105% of gas recovered were accepted as accurate.

Volume O_2/CO_2 = [(outgoing gas – intake gas)/100] × total corrected air volume (L) [8]
Oxygen density correction = volume $O_2 + (\text{volume } O_2 - \text{volume } CO_2) \times [(\text{intake } O_2/100) + (\text{intake } O_2/100)^2 + (\text{intake } O_2/100)^3 + (\text{intake } O_2/100)^4]$ [9]

Respiratory quotient (RQ) = $CO_2$ produced (L)/$O_2$ consumed (L) [10]

Theoretical $O_2 = (96 \times \text{alcohol burned}/100) \times [22.4 \times \% \text{ alcohol}/(46 \times 32)]$ [L] [11]

Theoretical $CO_2 = (88 \times \text{alcohol burned}/100) \times [22.4 \times \% \text{ alcohol}/(46 \times 44)]$ [L] [12]

Percent $O_2$ recovered = $O_2$ consumed/theoretical $O_2$ [13]

Percent $CO_2$ recovered = $CO_2$ produced/theoretical $CO_2$ [14]

Three lamp runs were conducted at the University of Nebraska-Lincoln on each of the three headboxes. Average recovery rates of oxygen were 101.7 ± 1.70, 105.2 ± 1.95 and 98.6 ± 1.22%, for headboxes 1, 2 and 3, respectively. Carbon dioxide recovery rates were 101.3 ± 0.93, 103.7 ± 2.51 and 97.3 ± 3.43% for the same boxes (Table 2.1). Similar results were found in previous validation tests of headbox systems and whole animal chambers. A summary of calorimetry type, gases used, and recovery rates of these experiments are listed in Table 2.2.

When ethanol is burned in the presence of oxygen, carbon dioxide is produced with a ratio of 2 $CO_2$:3 $O_2$. This ratio is considered the RQ. If the RQ value for the lamp run is 0.67, it suggests that gas concentrations are as expected and the headbox is adequately sealed. If the RQ value is different from 0.67, there may be an air leak, or the dessicant may be absorbing $O_2$ or be damp. The overall average of RQ values for all three headboxes is 0.66 ± 0.02 (Table 2.1). This suggests that the headboxes are adequately sealed and gas exchange can occur.
**Live Animal Experiment**

Before gases are collected from animals, the cattle must become accustomed to being in the headbox. This will ensure they are not hyperventilating and will reduce the risk of potential problems occurring so that data collected will be accurate. Cattle that have been adapted to the headboxes could still exhibit signs or behaviors that are not normal to an unstressed cow in a natural habitat, theoretically reducing the validity of the measurements. However, this portable headbox allows the box to be brought to the cow, and the clear plexiglass allows the cow to be aware of its surroundings. These features of the headbox design reduce some of the stress associated with other indirect calorimeter designs.

**CONCLUSIONS**

This headbox system appears to be a valid method of collecting gas samples for analysis. The ability of the system to capture oxygen and carbon dioxide suggests that the headbox is adequate for live animal collection and indirect calorimetry. Mobility and the plexiglass sides of the headboxes make this an ideal system to determine gas concentration of ruminants and provide a lower cost alternative to whole animal chambers. This system has great potential for research in energetics, as well as methane mitigation studies.
REFERENCES


### TABLES AND FIGURES

**Table 2.1.** Lamp run recovery rate means and standard deviations of individual headboxes

<table>
<thead>
<tr>
<th></th>
<th>Headbox 1</th>
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<tr>
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<td>SD</td>
<td>Mean</td>
<td>SD</td>
<td>Mean</td>
<td>SD</td>
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<td>31.3</td>
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<td>Dew point, °C</td>
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<td>10.6</td>
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<td>Alcohol burned, g</td>
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<td>20.7</td>
<td>112.9</td>
<td>31.1</td>
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<td>RQ, CO₂/O₂</td>
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<td>0.66</td>
<td>0.02</td>
<td>0.66</td>
<td>0.02</td>
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<td>O₂ recovered, %</td>
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<td>1.70</td>
<td>105.2</td>
<td>1.95</td>
<td>99.7</td>
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<tr>
<td>CO₂ recovered, %</td>
<td>101.3</td>
<td>0.93</td>
<td>103.6</td>
<td>2.51</td>
<td>98.7</td>
<td>97.3</td>
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Table 2.2. Comparison of previous publications on gas recovery rates from indirect calorimeters

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<th>Author</th>
<th>Calorimeter Type</th>
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<th>Recovery Rates</th>
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<td>Hellwing et al. (2012)</td>
<td>Whole animal chamber</td>
<td>CO₂</td>
<td>101.4 ± 4.0%</td>
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<td></td>
<td></td>
<td>CH₄</td>
<td>98.5 ± 6.6%</td>
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<tr>
<td>Suzuki et al. (2007)</td>
<td>Headbox</td>
<td>CO₂</td>
<td>97.8 ± 1.6%</td>
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<td>Nienaber and Maddy (1985)</td>
<td>Whole animal chamber</td>
<td>O₂</td>
<td>102.3 ± 0.4%</td>
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<tr>
<td></td>
<td></td>
<td>CO₂</td>
<td>99.8 ± 0.8%</td>
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<tr>
<td>Place et al. (2011)</td>
<td>Headbox</td>
<td>Ethanol</td>
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<tr>
<td></td>
<td></td>
<td>CO₂</td>
<td>98.5%</td>
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</table>
Figure 2.1. (a) Headbox built at the University of Nebraska-Lincoln designed with (b) caster wheels, (c) waterbowl, and (d) hood
Figure 2.2. Headbox air flow equipment (a) variable transformer, (b) vacuum motor and air filter, (c) gas meter, and (d) manometer
Figure 2.3. Headbox gas collection equipment (a) Drierite tube, (b) rotameters, (c) vacuum pump, (d) bags, and (e) stopcocks
Figure 2.4. Headbox temperature and dew point recording system (a) probe and (b) pocket logger
Figure 2.5. Example of temperature and dew point data readings taken over 23 h collection period
Figure 2.6. Lamps burning 100% ethyl alcohol in headbox to determine gas recovery of the system
APPENDIX A: EQUATIONS

Corrected temperature = 273 K + average line pressure [°C]  \[1\]

Corrected pressure = (line pressure + vapor pressure + barometric pressure) / 760 mmHg \[2\]

Vapor pressure = 0.61078 \(^{(17.27 \times \text{dew point [°C]})/(237.3 + \text{dew point [°C]})}\) \[3\]

Flow rate = (final meter – initial meter)/minutes \[4\]

Meter correction factor (MCF) = (0.0002 \(\times\) flow rate\(^2\)) – (0.0099 \(\times\) flow rate) + 1.089 \[5\]

Corrected flow meter = flow rate \(\times\) MCF \[6\]

Total corrected air volume = (final meter-initial meter [L]) \(\times\) MCF \(\times\) 28.32 ft\(^3\)/m\(^3\) \(\times\) \(\frac{273 \text{ K}}{\text{corrected temperature}}\) \(\times\) corrected pressure \[7\]

Volume \(\text{O}_2/\text{CO}_2\) = [(intake gas – outgoing gas)/100] \(\times\) total corrected air volume [L] \[8\]

Oxygen density correction = volume \(\text{O}_2\) + (volume \(\text{O}_2\) – volume \(\text{CO}_2\)) \(\times\) [(intake \(\text{O}_2/100\)) \(\times\) \(\text{O}_2\) \(\times\) \(\text{O}_2\) \(\times\) \(\text{O}_2\) \(\times\) \(\text{O}_2\)] \[9\]

Respiratory quotient (RQ) = \(\text{CO}_2\) produced (L)/\(\text{O}_2\) consumed [L] \[10\]

Theoretical \(\text{O}_2\) = (96 \(\times\) alcohol burned/100) \(\times\) [22.4 \(\times\) % alcohol/(46 \(\times\) 32)] [L] \[11\]

Theoretical \(\text{CO}_2\) = (88 \(\times\) alcohol burned/100) \(\times\) [22.4 \(\times\) % alcohol/(46 \(\times\) 44)] [L] \[12\]

Percent \(\text{O}_2\) recovered = \(\text{O}_2\) consumed/theoretical \(\text{O}_2\) \[13\]

Percent \(\text{CO}_2\) recovered = \(\text{CO}_2\) produced/theoretical \(\text{CO}_2\) \[14\]
## APPENDIX B: HEADBOX PARTS

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<td>Hastings Canvas</td>
<td>230 Eastside Blvd. Hastings, NE 68901</td>
<td>Diane</td>
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<tr>
<td>Welding work</td>
<td>Wahoo Metal Products</td>
<td>130 W. 4th St. Wahoo, NE 68066</td>
<td>Steve Gertz <a href="mailto:wahoo-metal@windstream.net">wahoo-metal@windstream.net</a></td>
<td></td>
<td>402-443-3448</td>
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<td>Gas meters</td>
<td>Central States Group/ Mueller Sales</td>
<td>520 50th Ave Dr. SW Cedar Rapids, IA 52404</td>
<td>Lola Kruse <a href="mailto:Lkruse@Muellersales.com">Lkruse@Muellersales.com</a></td>
<td>American Meter, Horsham, PA AL425-TC 10#</td>
<td>800-332-0159</td>
<td>319-364-1067</td>
<td>$479.80</td>
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<tr>
<td>Vacuum motor</td>
<td>Central Vacuum Factory</td>
<td>P.O. Box 9062 Baskersfield, CA 93389</td>
<td>Ametek Lamb Electric, Kent, OH 115923</td>
<td>2-stage 5.7” vacuum motor 120 volt</td>
<td>877-822-7868</td>
<td>661-391-8826</td>
<td>$93.99</td>
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<td>U-tube manometer</td>
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<td>2727 E. 26th St. Minneapolis, MN 55406</td>
<td>Joel Bain <a href="mailto:joel@parksupplyofamerica.com">joel@parksupplyofamerica.com</a></td>
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<td>Staco Energy Products Co., Dayton, OH 3PN1010B</td>
<td>800-635-1545</td>
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<td>520 50th Ave Dr. SW Cedar Rapids, IA 52404</td>
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<td>PO Box 460 Xenia, OH 45385-0460</td>
<td><a href="mailto:drierite@aol.com">drierite@aol.com</a></td>
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<td>(x2) ¾” o.d. x 8” length hose barbs for ¼” to 3/8” i.d. flexible tubing</td>
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<td>26930 30 g Drierite Max Flow Rate: 300 cm³/min</td>
<td>937-376-2927</td>
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<td>PO Box 4418 Mooresville, NC 28117</td>
<td>Danny Miller <a href="mailto:danny.miller@Pace-sci.com">danny.miller@Pace-sci.com</a></td>
<td>$399</td>
<td>Stores up to 32,256 readings Temp: -40 to 60°C/140F</td>
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<td>Nalge Nunc Stopcocks, polypropylene with Teflon resin TFE plug</td>
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CHAPTER 3

Energy content of reduced-fat dried distillers grains and solubles for lactating dairy cows

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ABSTRACT

Eight Holstein and 8 Jersey multiparous, lactating cows were used to complete 56 energy balances to determine the energy content of reduced-fat distillers grains and solubles (RFDDGS). A repeated switchback design was used to compare treatments with and without RFDDGS. Diets consisted of 24.2 % corn silage, 18.4 % alfalfa hay, 6.94 % brome hay with either 22.9 % rolled corn and 14.8 % soybean meal (Control), or 8.95 % rolled corn, 28.8 % RFDDGS, and 0 % soybean meal (Co-P; DM basis). The inclusion of RFDDGS did not affect ($P = 0.86$) DMI averaging 21.4 ± 0.53 kg DM for all cows but milk production tended ($P = 0.10$) to increase from 29.8 to 30.9 ± 1.46 kg/d for Control and Co-P treatments. There was no difference between treatments in milk fat percentage or ECM ($P = 0.81$ and 0.22, respectively), averaging 4.33 ± 0.14 % and 34.1 kg/d, respectively. Milk protein was decreased ($P < 0.01$) by the Co-P treatment (3.56 and 3.41 ± 0.08 % for Control and Co-P treatments), but protein yield was not affected ($P = 0.51$). Milk energies were 1.40 Mcal/d higher with Co-P ($P = 0.01$). Energy lost as methane was reduced ($P < 0.01$) by 0.31 Mcal/d with the addition of RFDDGS to the diet. Heat loss averaged 29.9 ± 0.55 Mcal/d and was not different between diets ($P = 0.49$). Average energy retained as tissue energy was -2.99 ± 0.93 Mcal/d ($P = 0.73$). Intake of digestible and metabolizable energy were not significantly different ($P = 0.16$ and 0.14 for DE and ME, respectively) between the Control and Co-P treatments, averaging 2.68 and 2.31 Mcal/kg DM, respectively. Net energy of lactation values of Control and Co-P diets were calculated to be 1.43 and 1.47 Mcal/kg DM ($P = 0.10$), respectively. These energy estimates suggest higher energy content of diets containing
RFDDGS than diets containing a mixture of corn and soybean meal in lactating dairy cows.

Key Words: dairy cow, energy balance, headbox, indirect calorimetry, reduced-fat dried distillers grains and solubles
INTRODUCTION

Dry distillers grains and solubles (DDGS), a byproduct of ethanol production from corn grain, is most commonly produced in Midwestern United States and included in dairy rations around the Nation. In recent years, technology has been developed to remove a portion of the oil so that it may be used in biodiesel production. This process results in a reduced-fat dried distillers grains and solubles (RFDDGS; Berger and Singh, 2010). This RFDDGS has been used as a protein and energy source in lactating dairy cow diets, with fat concentrations low enough to reduce the risk of milk fat depression that may be associated with diets high in fat (Bauman and Griinari, 2003). The nutritional value of RFDDGS has not been investigated to the extent that full-fat DDGS has, and the effects of RFDDGS on energy utilization of lactating cows has not yet been evaluated. When replacing forages, corn, soybean meal, and soy products, the inclusion of RFDDGS has been reported to have no effect on milk fat (Castillo-Lopez et al., 2014), or increase milk fat percentage with no negative effect on milk production (Mjoun et al., 2010). Given that the fat content is decreased, it is speculated that the energy content of RFDDG is also less than DDGS. As a consequence, the determination of the energy value of diets containing RFDDGS will allow for more precise formulation of lactating dairy cow diets. The objective of this study was to use total collection and indirect calorimetry techniques to investigate the effect of including RFDDGS in lactating cow diets to replace of corn grain and soybean meal on energy and nitrogen utilization.
MATERIALS AND METHODS

Sixteen multiparous Holstein (8) and Jersey (8) cows averaging 93 ± 20 DIM at the beginning of the experiment with average BW of 693.8 ± 12.9 and 429.2 ± 13.0 kg, respectively. The experimental design and methodology was similar to that of Birkelo et al. (2004) namely 2 treatment 4 period repeated switchback (Cochran and Cox, 1959) within a split-plot design. Cows were randomly assigned 1 of the 2 dietary treatments (Control or Co-P) which alternated over 4 periods; thus, measurements were collected on each animal consuming each treatment during 2 nonconsecutive experimental periods. Animals were blocked by date of calving and the subplot of this study was breed which was duplicated. The objectives of the current study were not to examine and report breed effects, but results will be reported elsewhere (Garcia Gomez et al., 2014). Two diets were formulated which differed in the proportion of RFDDG (Poet Nutrition, Sioux Falls, SD) included in the formulation. A sample of the RFDDGS are illustrated in Figure 3.1. Diets included the Control which did not contain any RFDDG, and Co-P in which the co-product RFDDG was included at 30 % of the diet DM while partially replacing the corn and soybean meal in a similar fashion as Birkelo et al. (2004). Specifically, the proportion of forage was held constant between treatments, but they differed in concentrate formulation. In the Co-P diet, RFDDGS replaced all the soybean meal and approximately half of the ground corn of the Control diet. Diets were balanced to contain similar concentrations of CP and a high protein soybean meal was utilized in the Control diet to accomplish this. The study was conducted over 16 mo and forages varied only by year to reduce variability. Complete diet compositions and nutrient
analysis are presented in Table 3.1. Each experimental period was 35 d in duration with 28 d for ad libitum diet adaptation, followed by 7 d of collection and 95 % ad libitum feeding to minimize refusals, as illustrated in Figure 3.2. During the 28 d diet adaptation, cows were fed for ad libitum consumption to allow for approximately 5 % refusals. All cows were less than 90 d pregnant at the conclusion of the final experimental period. Cows were housed in a temperature-controlled barn at the Dairy Metabolism Facility in the Animal Science Complex of University of Nebraska-Lincoln (Lincoln, NE) in individual tiestalls equipped with rubber mats and milked at 0700 and 1800 h. All animal care and experimental procedures were approved by the University of Nebraska-Lincoln Animal Care and Use Committee. Control and Co-P diets contained corn silage, alfalfa hay, grass hay and concentrate mixed as a total mixed ration (TMR) which was mixed in a Calan Data Ranger (American Calan, Inc., Northwood, NH). Cows were fed once daily at 0900 h.

Individual feed ingredients were sampled (500 g) each day during the collection period and frozen at -20°C. They were later composited by period and a subsample sent to Cumberland Valley Analytical Services Inc. (Hagerstown, MD) for complete nutrient analysis of DM (AOAC, 2000), N (Leco FP-528 N Combustion Analyzer; Leco Corp., St. Joseph, MI), NDF (Van Soest et al. 1991), ADF (method 973.18; AOAC 2000), sugar (DuBois et al., 1956), ether extract (2003.05; 2006), ash (942.05; AOAC 2000), and minerals (985.01; AOAC 2000). Total mixed rations were sampled on each day of collection and used to determine particle size according to Kononoff et al. (2003) using the Penn State Particle Separator. Total fecal and urine outputs were collected from each individual cow during the collection period.
for 2 consecutive days (Figure 3.3). Feces were collected using aluminum pans placed in the gutter behind the stall and urine was collected using a noninvasive urine cup collector (Lascano et al., 2010) and accumulated into a Surge bucket milker (Hinsdale, IL). Urine was deposited 4 times a day into 55-L plastic containers and acidified with 50 mL of concentrated HCl, before subsampling and freezing (−20 °C). Subsamples of milk (100 mL), feces (4 % wet basis), urine (2 % wet basis) and gas (10 to 15 L) were collected. Samples were later thawed and composited for each cow during each period. Likewise, fecal samples were deposited into large containers (Rubbermaid, Wooster, OH), subsampled, and frozen (-20 °C). Samples of feces, orts and each feed ingredient were composited according to cow and period, dried at 55 °C in a forced air oven and ground to pass through a 1-mm screen (Wiley mill, Arthur H. Thomas Co., Philadelphia, PA). Ground samples were analyzed for DM (100°C oven for 24 h). Milk production was measured daily and milk samples (40 mL) were collected during the AM and PM milkings for the 2 d of collection for each animal and preserved using 2-bromo-2-nitropropane-1,3 diol. Milk samples were analyzed for fat, true protein, lactose, SCC and MUN (AOAC, 2000) using a B2000 Infrared Analyzer (Bentley Instruments, Chaska, MN) by Heart of America DHIA (Manhattan, KS).

Feed samples, orts and fecal samples were analyzed at the University of Nebraska-Lincoln for N (Leco FP-528, Leco Corp., St. Joseph, MI), NDF (Van Soest et al., 1991), starch (Megazyme, AOAC method 996.11 and AACC method 76.13), and ash (AOAC, 2000). Heat stable α-amylase (number A3306; Sigma Chemical Co., St. Louis, MO) was included in the NDF procedure (0.5 mL per sample).
Samples were analyzed for ether extract (AOAC, 2000) by Cumberland Valley Analytical Services Inc. (Hagerstown, MD). Urine and milk samples were analyzed for N as previously described. All samples including feed, orts, feces, urine and milk were analyzed for gross energy (Parr 1241 Adiabatic Calorimeter, Moline, IL). Prior to analysis, milk and urine samples were lyophilized (VirTis Freezemobile 25ES, SP Scientific, Gardiner, NY).

Heat production (HP) was determined through the use of a headbox type indirect calorimeters (Chapter 2) which were constructed at the University of Nebraska-Lincoln, and based on indirect calorimetry (Figure 3.4). Prior to collections, 3 headboxes were used to test the rate of recovery of gas by burning 100% ethyl alcohol in the sealed headbox and comparing this measure to calculated gas concentrations. These calculations were based on weight of alcohol burned and a measured volume of gas sample. Three lamp runs were conducted. Recovery rates of \( O_2 \) and \( CO_2 \) averaged 101.8 ± 3.21 and 100.8 ± 3.51%, respectively.

Collection for each cow consisted of 2 consecutive 23-h intervals where gas concentrations were averaged for each interval. Feed was placed in the headbox and ad libitum access to water was available from a waterbowl inside the box. Doors were closed and the vacuum motor turned on 15 min prior to the start of collecting to allow for air equilibrium. Temperature and dew point within the box were recorded every min using a probe (Model TRH-100, Pace Scientific Inc., Mooresville, NC, USA) connected to a data logger (Model XR440, Pace Scientific Inc., Mooresville, NC, USA). Total volume of gas was measured using a gas meter (Model AL425, American Meter, Horsham, PA, USA) and continuous proportional samples of
outgoing and incoming air were diverted to collection bags (61 × 61 cm LAM-JAPCON-NSE; 44L) using glass tube rotameters (Model 1350E Sho-Rate “50”, Brooks Instruments, Hatfield, PA). Gas samples were analyzed (Emerson X-stream 3channel analyzer, Solon, OH) according to Nienaber and Maddy (1985). Heat production was estimated by calculation from oxygen ($O_2$) consumption, and carbon dioxide ($CO_2$) and methane ($CH_4$) production with correction for urinary N loss according to Brouwer (1965) with gases values reported in L and mass of urinary N reported in g (Equation 1). Volume of $CH_4$ formed was multiplied by a constant (9.45 kcal/L) to estimate the amount of energy represented in the formation of gaseous products. Energy balance was adjusted for excess N intake according to Moe et al. (1970) using the following equations:

$$3.866 \times O_2 + 1.200 \times CO_2 - 0.518 \times CH_4 - 1.431 \times N \quad [1]$$

Metabolizable energy ($ME$) = intake energy – fecal energy – urinary energy

− $CH_4$ energy \[2\]

Recovered energy ($RE$) = $ME$ – HP \[3\]

Tissue energy ($TE$) = $RE$ – milk energy \[4\]

Metabolizable energy for recovered energy ($ME_{RE}$) = $ME$

− Metabolizable energy for maintenance ($ME_m$) \[5\]

Metabolizable energy for maintenance was determined by regression of RE on ME, and is the ME at zero RE (Figure 3.4). Lactation energy received from ME of feed ($LE_{ME}$) was defined as milk energy for cows in negative energy balance, and was equal to milk energy plus $TE$ multiplied by a constant estimated by Moe et al.
(1970) for the efficiency of ME use for milk production from tissue energy for lactating animals in positive energy balance (Equation 6).

\[ \text{LE}_{\text{ME}} \text{ (positive energy balance)} = \text{milk energy} + \text{TE} \times 0.84 \]  \[6\]

Metabolizable energy available for lactation (\(\text{ME}_{\text{LE}}\)) was defined as \(\text{ME}_{\text{RE}}\) for cows in positive energy balance, and was equal to \(\text{ME}_{\text{RE}}\) minus TE divided by a constant for the efficiency of body gain from ME (Equation 7; Moe et al., 1970).

Tissue energy in protein was calculated using Equation 8, and was defined as energy used for tissue protein synthesis (Freetly et al., 2006).

\[ \text{ME}_{\text{LE}} \text{ (negative energy balance)} = \text{ME}_{\text{RE}} - \frac{\text{TE}}{0.726} \]  \[7\]

Tissue energy in protein = N balance \(\times (5.88 \text{ kg of protein/kg of N})\)

\(\times (5.7 \text{ Mcal/kg of protein})\)  \[8\]

**Statistical Analysis**

Data were analyzed using the GLIMMIX procedure of SAS (SAS Institute, 2008). Treatment, breed, breed within block and period within block and breed, were modeled as fixed effects while cow within block, based on calving date, was modeled as a random effect. The LSMEANS option was used to generate least square means of treatments listed in this study. Significance was declared at \(P \leq 0.05\) and trends at \(0.05 < P \leq 0.10\).

**RESULTS AND DISCUSSION**

Fifty-six of a possible 64 energy balances were completed. Gas meter calibration was not completed in time and diet composition was altered after the first
collection period of the first block, so the data from those 4 cows were not used for that period. One cow in block 4 died from a non-related source (intestinal intussusception) after the first collection period of that block. During the third collection of block 2, 1 cow became ill and was removed from collections for that period. For a period in block 3, collection was reduced to a single day instead of 2 consecutive days to avoid switching corn silage sources during collections.

*Diet Composition*

Chemical composition of individual ingredients and diet composition is listed in Tables 2 and 3. Diets were formulated to have similar concentrations of CP and was observed to be 18.8 ± 0.23 % CP (DM basis). Ether extract was 1 % higher (DM basis) in the Co-P diet than the Control diet (3.60 ± 0.13 compared to 2.60 ± 0.10 % DM). This was expected because of the greater fat content in RFDDG compared with corn and soybean meal. The NDF content of the Control diet was 30.8 ± 0.69 % (DM basis) which was lower than the Co-P diet at 37.1 ± 0.89 % (DM basis). This is typical of RFDDGS, as in a study by Castillo-Lopez et al. (2014) where NDF content increased by 2.9 % in a diet with 30 % RFDDGS compared to a control diet without RFDDGS. However, Mjoun et al. (2010) observed little difference in NDF content of diets with increasing levels of RFDDGS from 0 to 30 % but this was a function of removing soybean hulls as a source of NDF.

Diet particle size was similar between treatments with 2.85, 20.7, 45.3, and 31.1 % remaining on the > 19.0 mm, 19.0 – 8.0 mm, 8.0 – 1.18 mm, and < 1.18 mm pans, respectively, for the control TMR and 2.87, 19.9, 41.4, and 36.1 % for the
RFDDGS TMR (Table 3.3). According to Kononoff et al. (2003), it is recommended that rations should include 30 to 50% of particles between 8.0 and 19.0 mm and 10 to 20% particles between 1.18 and 8.0 mm in diameter to maximize milk production and to avoid milk fat depression. The proportion of particles in diets between 8.0 and 19.0 mm in the current study is lower than recommended, and particles between 1.18 and 8.0 mm in diameter is greater.

**Intake, Milk Production and Composition**

Dry matter intake did not differ ($P = 0.86$) between treatments and averaged $21.3 \pm 0.53$ kg/d. During collection, animals were offered feed at 95% of their ad libitum intake but refusals averaged $1.49 \pm 1.39$ kg/d (DM basis), or $7.0 \pm 6.5\%$. Hünerberg et al. (2013) also observed a reduction in DMI during gas collection. Similar to the current study, Mjoun et al. (2010) observed no change in DMI with increasing levels of RFDDGS compared to a Control without RFDDGS. However, in a study increasing RFDDGS as a replacement of forage, Castillo-Lopez et al. (2014) observed an increase in DMI from 23.8 kg/d with RFDDGS at 10% of DM to 27.9 kg/d with 30% RFDDGS. In the next experiment, they observed no difference in DMI. A comparison between DDGS from 3 different ethanol plants was made with levels at 20% of dairy cow diets but no difference in intake was observed between sources (Kleinschmit et al., 2006). Benchaar et al. (2013) saw a linear increase in DMI of lactating dairy cows with increasing DDGS from 0 to 30% of the diet. Hünerberg et al. (2013) compared the effects of wheat and corn DDGS and found that DMI was reduced with wheat DDGS compared to corn DDGS. Abdelqader et al.
(2009) compared a 30 % DDGS treatment to diets with other energy sources including a commercial inert fat (2.5 %), corn grain (14 %), and corn oil (2.5 %) and found no difference in DMI between treatments. Another ethanol byproduct, namely wet distillers grains with solubles (WDGS) showed similar variability in DMI responses as DDGS. Gehman and Kononoff (2010) reported DMI was not affected by WDGS when WDGS replaced corn or alfalfa silage. A 10.9 % decrease in DMI was observed with WDGS compared to a control with soybean meal, but was suggested to be due to lower diet DM from adding a wet grain source (Birkelo et al., 2004). Different responses of DMI to inclusion of ethanol byproducts may be explained by differences in DM, NDF or energy content of the diet (Birkelo et al., 2004; Hünerberg et al., 2013), production level (Belyea and Adams, 1990), rumen fill (Tine et al., 2001), or forage:concentrate ratio (Williams et al., 2013). Overall, the lack of change in DMI in the current study is not unexpected and is comparable to many studies with different forms of corn grain and distillers grains.

Milk yield tended ($P = 0.10$) to increase from 29.8 ± 1.46 kg/d to 30.9 ± 1.45 kg/d with the addition of RFDDGS to the diet. There was no difference ($P = 0.81$ and 0.14) in milk fat percentage or yield, and no difference between treatments was observed ($P = 0.22$) for energy corrected milk (ECM), averaging 34.1 kg/d. Benchaar et al. (2013) reported a linear increase in milk production but a decrease in milk fat percentage with increasing levels of DDGS. This resulted in a quadratic effect tendency for FCM and ECM to increase with DDGS up to 20 % of diet DM, but then decrease at 30 %. Abdelqader et al. (2009) also observed that the inclusion of DDGS reduced milk fat with 30 % DDGS in the diet when compared to corn grain
at 14 %, potentially due to a difference in physical form and a reduction in effective fiber. It is also possible that the fat contained corn grain may be less available in the rumen than found in DDGS and thus may have a lesser effect on rumen fermentation. Schingoethe et al. (2009) suggests the greater volume of milk produced is due to the higher energy content of DDGS. In the current study, the greater energy with the Co-P treatment, or more available energy may be an explanation for increased production and FCM.

In a review on the use of distillers grains in lactating cow diets, Schingoethe et al. (2009) suggested that milk protein is seldom affected unless dietary protein is limiting. Additionally, Paz et al. (2013) reported that diets with 20 % DDGS delivered sufficient protein and amino acids to maintain or increase milk protein synthesis. Contrary to this, in the current study, milk protein was significantly \( (P < 0.01) \) reduced from 3.56 to 3.41 ± 0.08 % with the addition of RFDDGS but yield of protein was not affected \( (P = 0.51) \) because of increased milk production (1.04 and 1.02 ± 0.03 kg/d for Control and Co-P treatments, respectively). This suggests protein in RFDDGS is less available for milk production than in DDGS. Another possible explanation is a diet deficient in lysine which is possible for diets that rely on corn-based ingredients (Paz et al., 2013). In a meta-analysis, Paz et al. (2013) reported a positive trend in milk protein concentration with increasing lysine as metabolizable protein compared to diets deficient in lysine, such as diets with a high proportion of DDGS.
Gas Consumption and Production

Oxygen consumption was similar ($P = 0.88$) between treatments ($5,911.6 \pm 110.5$ L/d) but CO$_2$ production ($6,291.4 \pm 108.4$ L/d) and CH$_4$ ($488.2 \pm 11.9$ L/d) production was reduced ($P \leq 0.01$) with RFDDGS in the diet (Table 3.5). Methane production was reduced from $504.2 \pm 11.9$ L/d with the Control diet to $472.1 \pm 11.6$ L/d with the Co-P diet, a 7% reduction. The volume of CH$_4$ produced per kg milk yield was also significantly reduced by Co-P from $15.6 \pm 0.54$ to $14.1 \pm 0.53$ L CH$_4$/kg milk ($P < 0.01$). Similarly, Benchaar et al. (2013) reported a linear decrease in CH$_4$ production per kg milk produced from 15.6 to 13.2 g/kg with an increasing rate of DDGS in the diet. This suggests that at least a portion of energy retained from reduced CH$_4$ loss was utilized for milk production, implying it is possible to increase milk production directly by reducing energy loss as CH$_4$. Others have reported a reduction in CH$_4$ production with DDGS in dairy and beef cattle (Benchaar et al., 2013; McGinn et al., 2009; Hünerberg et al. 2013). The high level of fat affecting the rumen environment and altering fermentation by suppressing methanogens and utilizing hydrogen is the most likely cause of reduced CH$_4$. The effect of added fat to ruminant diets has been shown to reduce CH$_4$ energy losses (van Zijderveld et al., 2011; Grainger et al., 2010; Holter et al., 1992; Andrew et al., 1991). In the current study, total dietary fat of the Co-P treatment was 3.22% on a DM basis, and we believe it likely was not high enough to suppress CH$_4$ production. However, in a review by Knapp et al. (2014), they suggest a 2% increase in diet ether extract may reduce CH$_4$ emissions by 10% from reduced DMI, suppression of protozoa and methanogen populations, or alternative hydrogen sinks from biohydrogenation. In the
current study there is also a possibility that the increased proportion of RFDDGS increased the extent of hind gut fermentation which may increase enteric CH$_4$ production would not be captured by the headbox system.

**Energy Partitioning**

Gross energy intake (GEI) was greater ($P = 0.04$) with the Co-P treatment, but digestible energy (DE) and metabolizable energy (ME) did not differ ($P = 0.22$ and 0.24, respectively) by treatment (Table 3.6). Energy lost as feces was significant ($P = 0.05$) and urine tended ($P = 0.08$) to be 2.06 and 0.31 Mcal/d greater with RFDDGS, respectively. Energy lost as CH$_4$ was significantly ($P < 0.01$) reduced from 4.77 ± 0.11 Mcal/d to 4.46 ± 0.11 Mcal/d with Co-P treatment, but HP did not differ ($P = 0.49$) at 30.0 ± 0.55 and 29.7 ± 0.53 Mcal/d between animals consuming the Control and Co-P diets. Total RE was determined by adding milk and tissue energy, but did not differ ($P = 0.18$) by treatment. Milk energy was 1.39 Mcal/d higher with Co-P and was significantly ($P = 0.01$) greater due to higher milk production. Tissue energy, or energy balance, did not differ ($P = 0.73$). In a similar study by Birkelo et al. (2004) comparing wet corn distillers grains and solubles replacing corn grain and soybean meal, a decrease in GEI was reported, along with no difference in milk energy, resulting in a lower energy balance. This observation is contrary to our results, however they also reported a reduction in DMI with wet distillers grains. In the current study, there was no difference in DMI between treatments but higher energy content in the Co-P diet, resulting in higher GEI with RFDDGS inclusion.
When expressed as a percent of total GEI, partitioning of DE and ME did not differ \((P \geq 0.26)\) between treatments. Fecal and urinary energies as a percent of GEI also did not differ \((P \geq 0.26)\), suggesting the increased energy outputs were solely due to higher energy intakes. Methane energy was significantly lower with Co-P when expressed as a percent GEI and was reduced \((P < 0.01)\) from 5.72 to 5.13 ± 0.14 %. Similar to the current study, Birkelo et al. (2004) reported energy lost as CH\(_4\), when expressed as a percent of GEI was reduced by 14 % with the inclusion of wet distillers grains and solubles. However, they did observe an increase in urinary energy as a percent of GEI, contrary to our findings, potentially due to greater protein metabolism.

Energy estimates of diets are listed in Table 3.6. Gross energy content of the diet was significantly \((P < 0.01)\) higher at 4.11 ± 0.01 Mcal/kg DM for the Co-P treatment compared to the Control diet at 3.96 ± 0.01 Mcal/kg DM. This is a result of higher energy content of the diet and higher DMI with RFDDGS inclusion. There were no differences \((P \geq 0.14)\) in DE or ME content of diets. Net energy for lactation \((\text{NE}_L)\) for Control and Co-P treatments tended \((P = 0.10)\) to be higher for cows consuming RFDDGS and were 1.43 and 1.47 Mcal/kg DM, for Control and Co-P respectively. These values are lower than those calculated by Birkelo et al. (2004), with 1.82 Mcal/kg DM for a diet with wet distillers grains included at 30 %. Lower values for RFDDGS are expected when compared to full fat distillers grains because of the reduced fat and energy. It is interesting to note that with a lower inclusion rate of ground corn in the diet in the Co-P compared to the Control treatment, similar
levels of DE, ME, and NE_L were achieved. This may indicate an economic benefit for greater utilization of energy while feeding a low starch diet.

Based on the energy content of the diet, we were able to calculate the energy content of RFDDGS by assuming energy values from the NRC (2001) for DE, ME, and NE_L of 3.53, 3.12, and 2.01 Mcal/kg DM, respectively, for corn and 3.0, 3.29, and 1.94 Mcal/kg DM for soybean meal. Estimated values for RFDDGS were calculated by difference and were 3.82 Mcal/kg DE at 1 × maintenance, 3.41 Mcal/kg ME at 1 × maintenance, and 2.03 Mcal/kg NE_L at 3 × maintenance. These values are lower than values determined for wet distillers grains by Birkelo et al. (2004), but similar to NRC (2001) values for ground corn. The energy content of RFDDGS was expected to be lower than wet distillers grains because of the removed oil and energy, but similar values to corn grain was unexpected because of lower starch.

Estimation of maintenance energy requirements were determined through regression of ME and RE scaled for MBW and solving for ME when RE equals zero (Figure 3.5). Maintenance was calculated to be 208 kcal/MBW with an efficiency of ME use for lactation ($k_l$) of 0.76. These values are higher than previous estimates of maintenance energy requirements and efficiencies of lactation for mature lactating dairy cows (136.2 kcal/MBW, Birkelo et al., 2004; 121 kcal/MBW, Vermorel et al., 1982). Yan et al. (1997) reported maintenance estimates ranged from 146 to 179 kcal/MBW, with a mean of 160 kcal/MBW in a meta-analysis of energy metabolism trials in Northern Ireland and determined the $k_l$ to range from 0.61 to 0.68. This is lower than that observed in the current study, suggesting our animals had greater maintenance energy requirements and were more efficient at converting ME to milk.
Maintenance requirements have been shown to be higher for first lactation heifers (Freetly et al., 2006; Xue et al., 2011), which could explain the higher values calculated in by Yan et al. (1997) with an unknown distribution of primiparous and multiparous animals. Animals in the current study were all multiparous, suggesting the high maintenance energy was not due to young age. Nonetheless, it is reasonable to accept maintenance estimates of the current study (208 kcal/MBW) because of the high level milk production which would result in increased organ function to support milk synthesis, and therefore increased maintenance.

**Nitrogen Balance and Digestibilities**

There were no significant differences ($P \geq 0.63$) in nitrogen partitioning or nitrogen balances (intake nitrogen minus fecal, urinary, and milk nitrogen production) between treatments (Table 3.7). Nitrogen intakes were 641.6 ± 17.6 g/d, and balances were 60.5 ± 11.4 g/d. Others have found differences in nitrogen partitioning with diet changes. However, responses may differ between studies. Gehman and Kononoff (2010) evaluated the effects of WDGS on nitrogen balance and found an increase in urinary and milk nitrogen excretion with the inclusion of distillers grains, but also higher nitrogen balances. Contrary to these findings, Birkelo et al. (2004) reported WDGS reduced fecal and milk nitrogen, and increased urinary nitrogen, resulting in similar nitrogen balances. In a study with increasing levels of DDGS, Benchaar et al. (2013) observed intake, fecal, urinary, and milk nitrogen increased linearly, resulting in higher nitrogen balances. Feeding DDGS to growing steers has also resulted in linear increases of nitrogen intakes and urinary nitrogen, but decreasing fecal nitrogen
excretion (Walter et al., 2012). It has been suggested that when used as an energy source, the high proportion of CP in DDGS may result in greater nitrogen excretion, but greater fecal nitrogen may also be the result of a greater extent of hind gut fermentation. Consequently this would result in an overestimation of fecal nitrogen excretion, or a greater amount of microbial nitrogen exiting the rumen from a higher digestible feed (McGinn et al., 2009; Tine et al., 2001). However, sampling error may also be a major factor in determining nitrogen partitioning from loss of feed, through the volatile loss of nitrogen from urine or drying fecal samples, or nitrogen gas production (Walter et al., 2012).

Dry matter (DMD) and organic matter digestibilities (OMD) were reduced ($P < 0.01$) by 2.68 % with the inclusion of RFDDGS in the diet (Table 3.8). There was no difference ($P = 0.92$) in CP digestibility averaging 69.2 ± 0.64 %. Digestibility of NDF tended ($P = 0.09$) to increase from 49.3 ± 1.22 to 52.3 ± 1.18 % with RFDDGS inclusion, and EE digestibility was significantly improved ($P < 0.01$) by 5.20 %. There were no differences ($P = 0.29$ and 0.59) in starch or NFC digestibilities between treatments, and values were similar for those components. Castillo-Lopez et al. (2014) fed diets with increasing increments of RFDDGS from 0 to 30 % to lactating dairy cows and reported no difference in DMD or NDF digestibilities. Nitrogen and NFC digestibilities tended to increase linearly with RFDDGS. However, balance of forage, corn, cottonseed, and soy-based feeds were altered to maintain similar CP, potentially resulting in different digestibility responses compared to the current study with only corn grain and soybean meal inclusion changing. Another potential reason for the different DMD responses is differences in
processing or heating (Hünerberg et al., 2013). Also, Benchaar et al. (2013) reported a decrease in DMD and OMD with DDGS, and suggested the cause was the high concentration of fat in DDGS. Responses of NDF digestibility tended to be quadratic, increasing from 0 to 20 % DDGS and then decreasing at 30 % DDGS. The increase in NDF digestibility was suggested to result from highly digestible fiber in DDGS, but small particle size increased rumen passage rate at 30 % DDGS which reduced digestibility. This is not the case for the current study, even with the fine particle size NDF digestibility was improved. Fat content of RFDDGS was relatively low compared to DDGS, so the reduction in DMD is most likely not a result of high fat, but of less available nutrients for fermentation. The reduction in DMD and OMD with an increase in NDF and EE digestibilities could be explained by a reduction in digestibility of other nutrients. However, there was no decrease in digestibility of any other nutrients tested. Either a decrease in DMD and an increase in NDF digestibility may be expected with RFDDGS, but is unknown why both occurred.

**CONCLUSIONS**

Replacement of corn and soybean meal with RFDDGS was able to increase efficiency of milk production by reducing energy lost as CH\textsubscript{4}. A greater NE\textsubscript{L} value for the Co-P diet was a function of increased DMI and greater energy content. Dry matter digestibility and OMD were reduced with RFDDGS inclusion by 4 %, but NDF digestibility was increased by 6 %. The reduction in DMD, OMD and CH\textsubscript{4} production by Co-P indicate an alteration of rumen fiber digestion which is the most likely explanation for improved milk production. The addition of RFDDGS to the
diet did not affect nitrogen partitioning, balance, or excretion. Milk production may be improved without negative effects on milk fat yield with RFDDGS, but the concentration of milk protein may be reduced. Future research should evaluate at the relationship between RFDDGS intake and rumen microbial populations present which may be causing the reduction in CH$_4$ production.
REFERENCES


Vermorel, M., B. Remond, J. Vernet, and D. Liamadis. 1982. Utilization of body reserves by high-producing cows in early lactation; effects of crude protein and amino-acid supply. Pages 18-21 in Energy Metabolism of farm animals.


### Table 3.1. Composition and analysis of Control, and Co-Product (Co-P) with 28.8 % reduced-fat dried distillers grains and solubles (RFDDGS) diets

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>Co-P</th>
</tr>
</thead>
<tbody>
<tr>
<td>% of DM</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Corn silage</td>
<td>24.5</td>
<td>24.5</td>
</tr>
<tr>
<td>Alfalfa hay</td>
<td>18.4</td>
<td>18.4</td>
</tr>
<tr>
<td>Brome hay</td>
<td>6.94</td>
<td>6.94</td>
</tr>
<tr>
<td>Ground corn</td>
<td>22.9</td>
<td>8.95</td>
</tr>
<tr>
<td>RFDDGS</td>
<td>--</td>
<td>28.8</td>
</tr>
<tr>
<td>Soybean meal</td>
<td>14.8</td>
<td>--</td>
</tr>
<tr>
<td>Ground soybean hulls</td>
<td>7.93</td>
<td>7.93</td>
</tr>
<tr>
<td>Soypass(^1)</td>
<td>2.01</td>
<td>2.01</td>
</tr>
<tr>
<td>Calcium carbonate</td>
<td>0.89</td>
<td>0.89</td>
</tr>
<tr>
<td>Sodium bicarbonate</td>
<td>0.65</td>
<td>0.65</td>
</tr>
<tr>
<td>Calcium diphosphate</td>
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<td>0.30</td>
</tr>
<tr>
<td>Salt</td>
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<td>0.22</td>
</tr>
<tr>
<td>Magnesium oxide</td>
<td>0.18</td>
<td>0.18</td>
</tr>
<tr>
<td>Trace mineral premix(^2)</td>
<td>0.12</td>
<td>0.12</td>
</tr>
<tr>
<td>Vitamin premix(^3)</td>
<td>0.12</td>
<td>0.12</td>
</tr>
<tr>
<td>Chemical Composition, % DM (^4)</td>
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<td></td>
</tr>
<tr>
<td>CP</td>
<td>18.6 (0.77)</td>
<td>19.0 (1.00)</td>
</tr>
<tr>
<td>Ether extract(^3)</td>
<td>2.26 (0.11)</td>
<td>3.22 (0.18)</td>
</tr>
<tr>
<td>NDF</td>
<td>36.7 (1.91)</td>
<td>43.4 (1.37)</td>
</tr>
<tr>
<td>Ash</td>
<td>7.66 (0.57)</td>
<td>8.38 (0.62)</td>
</tr>
<tr>
<td>Starch</td>
<td>26.4 (1.47)</td>
<td>17.9 (1.31)</td>
</tr>
<tr>
<td>NFC(^6)</td>
<td>34.9 (2.00)</td>
<td>26.1 (2.41)</td>
</tr>
<tr>
<td>Gross energy, cal/g</td>
<td>3970.8 (77.9)</td>
<td>4114.8 (92.4)</td>
</tr>
</tbody>
</table>

\(^1\)LignoTech, Overland Park, KS

\(^2\)Contains 13.9 % Ca, 0.03 % P, 0.42 % Mg, 0.20 % K, 4.20 % S, 0.08 % Na, 0.03 % Cl, 445 ppm Fe, 60,021 ppm Zn, 17,375 ppm Cu, 43,470 ppm Mn, 287 ppm Se, 527 ppm Co, and 870 ppm I

\(^3\)Formulated to supply approximately 120,000 IU/d vitamin A, 24,000 IU/d of vitamin D, and 800 IU/d Vitamin E in total ration

\(^4\)Determined from composite samples collected throughout the experiment and analyzed at the University of Nebraska-Lincoln, mean (SD)

\(^5\)Analyzed by Cumberland Valley Analytical Services, Hagerstown, MD

\(^6\)NFC = Nonfiber carbohydrate calculated by difference 100 – (% NDF + % CP + % Fat + % Ash)
<table>
<thead>
<tr>
<th>Chemical, % DM</th>
<th>Corn Silage</th>
<th>Alfalfa Hay</th>
<th>Brome Hay</th>
<th>Control Concentrate</th>
<th>Co-P Concentrate</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean</td>
<td>SD</td>
<td>Mean</td>
<td>SD</td>
<td>Mean</td>
<td>SD</td>
</tr>
<tr>
<td>DM</td>
<td>38.5</td>
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<td>86.6</td>
<td>1.85</td>
<td>86.7</td>
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<tr>
<td>CP</td>
<td>7.76</td>
<td>0.49</td>
<td>20.5</td>
<td>1.48</td>
<td>14.7</td>
</tr>
<tr>
<td>Soluble protein</td>
<td>4.02</td>
<td>0.53</td>
<td>4.59</td>
<td>0.65</td>
<td>3.53</td>
</tr>
<tr>
<td>ADICP(^2)</td>
<td>0.86</td>
<td>0.11</td>
<td>2.74</td>
<td>1.39</td>
<td>1.42</td>
</tr>
<tr>
<td>NDICP(^3)</td>
<td>1.17</td>
<td>0.32</td>
<td>6.09</td>
<td>2.42</td>
<td>5.35</td>
</tr>
<tr>
<td>ADF</td>
<td>25.3</td>
<td>0.97</td>
<td>32.5</td>
<td>4.01</td>
<td>38.3</td>
</tr>
<tr>
<td>NDF</td>
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<td>43.9</td>
<td>5.13</td>
<td>66.3</td>
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<tr>
<td>Lignin</td>
<td>3.11</td>
<td>0.47</td>
<td>7.62</td>
<td>1.18</td>
<td>4.2</td>
</tr>
<tr>
<td>NFC</td>
<td>45.6</td>
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<td>28.5</td>
<td>2.78</td>
<td>11.2</td>
</tr>
<tr>
<td>Starch</td>
<td>35.7</td>
<td>2.62</td>
<td>2.59</td>
<td>0.67</td>
<td>0.88</td>
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<tr>
<td>Sugar</td>
<td>0.92</td>
<td>0.27</td>
<td>2.70</td>
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<tr>
<td>Ether extract</td>
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<td>1.99</td>
<td>0.36</td>
<td>2.33</td>
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<tr>
<td>Ash</td>
<td>5.08</td>
<td>0.65</td>
<td>11.2</td>
<td>0.39</td>
<td>10.3</td>
</tr>
<tr>
<td>Ca, %</td>
<td>0.30</td>
<td>0.13</td>
<td>1.32</td>
<td>0.13</td>
<td>0.38</td>
</tr>
<tr>
<td>P, %</td>
<td>0.23</td>
<td>0.03</td>
<td>0.33</td>
<td>0.04</td>
<td>0.35</td>
</tr>
<tr>
<td>Mg, %</td>
<td>0.14</td>
<td>0.03</td>
<td>0.23</td>
<td>0.02</td>
<td>0.14</td>
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<tr>
<td>K, %</td>
<td>1.05</td>
<td>0.09</td>
<td>3.54</td>
<td>0.34</td>
<td>3.35</td>
</tr>
<tr>
<td>S, %</td>
<td>0.13</td>
<td>0.02</td>
<td>0.27</td>
<td>0.03</td>
<td>0.19</td>
</tr>
<tr>
<td>Na, %</td>
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<td>0.00</td>
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<tr>
<td>Cl, %</td>
<td>0.17</td>
<td>0.03</td>
<td>0.33</td>
<td>0.06</td>
<td>1.32</td>
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<tr>
<td>Fe, ppm(^4)</td>
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<td>83.0</td>
<td>212.5</td>
<td>42.9</td>
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<td>Cu, ppm</td>
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<td>Mn, ppm</td>
<td>27.0</td>
<td>8.49</td>
<td>33.8</td>
<td>5.00</td>
<td>31.3</td>
</tr>
</tbody>
</table>

\(^1\)Values determined by Cumberland Valley Analytical Services, Hagerstown, MD

\(^2\)Acid detergent insoluble crude protein

\(^3\)Neutral detergent insoluble crude protein

\(^4\)Parts per million
Table 3.3. Chemical composition and particle distribution of Control, and Co-Product (Co-P) with 28.8 % reduced-fat dried distillers and solubles (RFDDGS) diets

<table>
<thead>
<tr>
<th>Chemical, % DM</th>
<th>Control</th>
<th>Co-P</th>
<th>Mean</th>
<th>SD</th>
<th>Mean</th>
<th>SD</th>
</tr>
</thead>
<tbody>
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<td>DM</td>
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<td>76.3</td>
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<td>0.35</td>
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<tr>
<td>CP</td>
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<td>18.8</td>
<td>0.23</td>
<td>0.21</td>
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<td></td>
</tr>
<tr>
<td>Soluble protein</td>
<td>4.32</td>
<td>3.88</td>
<td>0.18</td>
<td>0.13</td>
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<td></td>
</tr>
<tr>
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<td>1.60</td>
<td>0.12</td>
<td>0.14</td>
<td></td>
<td></td>
</tr>
<tr>
<td>NDICP</td>
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<td>3.42</td>
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<td>37.1</td>
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<td>Lignin</td>
<td>3.14</td>
<td>3.81</td>
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<td>0.65</td>
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<td></td>
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<td>18.9</td>
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<td>Sugar</td>
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<td>0.13</td>
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<tr>
<td>Ash</td>
<td>8.21</td>
<td>8.41</td>
<td>0.16</td>
<td>0.12</td>
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</tr>
<tr>
<td>Ca, %</td>
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<td>1.02</td>
<td>0.04</td>
<td>0.06</td>
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<td></td>
</tr>
<tr>
<td>P, %</td>
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<td>0.59</td>
<td>0.01</td>
<td>0.03</td>
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<tr>
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<td>0.33</td>
<td>0.00</td>
<td>0.01</td>
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</tr>
<tr>
<td>K, %</td>
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<td>1.75</td>
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<td>0.04</td>
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<tr>
<td>S, %</td>
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<td>0.40</td>
<td>0.00</td>
<td>0.01</td>
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<td></td>
</tr>
<tr>
<td>Na, %</td>
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<td>0.40</td>
<td>0.01</td>
<td>0.01</td>
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<tr>
<td>Cl, %</td>
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<td>0.41</td>
<td>0.02</td>
<td>0.01</td>
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</tr>
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<td>311.0</td>
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<td>28.0</td>
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<td>119.9</td>
<td>4.32</td>
<td>3.80</td>
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<td>1.32</td>
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</tr>
<tr>
<td>Mn, ppm</td>
<td>83.2</td>
<td>96.4</td>
<td>3.18</td>
<td>3.74</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Particle Size, %²</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&gt; 19.0 mm</td>
<td>2.85</td>
<td>2.87</td>
<td>0.66</td>
<td>0.74</td>
<td></td>
<td></td>
</tr>
<tr>
<td>19.0 – 8.0 mm</td>
<td>20.7</td>
<td>19.9</td>
<td>2.88</td>
<td>3.06</td>
<td></td>
<td></td>
</tr>
<tr>
<td>8.0 – 1.18 mm</td>
<td>45.3</td>
<td>41.4</td>
<td>4.86</td>
<td>6.15</td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt; 1.18 mm</td>
<td>31.1</td>
<td>36.1</td>
<td>5.58</td>
<td>4.91</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

¹Values determined by Cumberland Valley Analytical Services, Hagerstown, MD
²Determined using the Penn State Particle Separator on wet basis (Heinrichs and Kononoff, 2002)
Table 3.4. DMI, milk production and composition, BW and BCS\textsuperscript{1} of Control, and Co-Product (Co-P) with 28.8 % reduced-fat dried distillers grains and solubles (RFDDGS) treatments

<table>
<thead>
<tr>
<th>Diet</th>
<th>Control</th>
<th>Co-P</th>
<th>SEM\textsuperscript{2}</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>DMI, kg/d</td>
<td>21.3</td>
<td>21.4</td>
<td>0.53</td>
<td>0.86</td>
</tr>
<tr>
<td>Milk yield, kg/d</td>
<td>29.8</td>
<td>30.9</td>
<td>1.46</td>
<td>0.10</td>
</tr>
<tr>
<td>ECM\textsuperscript{3}</td>
<td>33.7</td>
<td>34.5</td>
<td>1.22</td>
<td>0.22</td>
</tr>
<tr>
<td>Fat, %</td>
<td>4.32</td>
<td>4.34</td>
<td>0.14</td>
<td>0.81</td>
</tr>
<tr>
<td>Fat yield, kg/d</td>
<td>1.24</td>
<td>1.28</td>
<td>0.05</td>
<td>0.14</td>
</tr>
<tr>
<td>Protein, %</td>
<td>3.56</td>
<td>3.41</td>
<td>0.08</td>
<td>&lt; 0.01</td>
</tr>
<tr>
<td>Protein yield, kg/d</td>
<td>1.04</td>
<td>1.02</td>
<td>0.03</td>
<td>0.51</td>
</tr>
<tr>
<td>MUN\textsuperscript{4}, mg/dl</td>
<td>16.9</td>
<td>16.6</td>
<td>0.43</td>
<td>0.58</td>
</tr>
<tr>
<td>Body weight, kg</td>
<td>564.0</td>
<td>559.0</td>
<td>9.32</td>
<td>0.14</td>
</tr>
<tr>
<td>BCS</td>
<td>3.30</td>
<td>3.29</td>
<td>0.06</td>
<td>0.81</td>
</tr>
</tbody>
</table>

\textsuperscript{1}BCS = Body Condition Score 1-5 scale according to Wildman et al. (1982)
\textsuperscript{2}Highest standard error of treatment means is shown
\textsuperscript{3}Energy corrected milk = 0.327 \times \text{milk yield [kg]} + 12.95 \times \text{fat [kg]} + 7.20 \times \text{protein [kg]} adjusted for 3.5 % fat and 3.2 % total protein (DHI Glossary, 2014)
\textsuperscript{4}Milk urea nitrogen
Table 3.5. Daily consumption of oxygen and production of carbon dioxide and methane for Control, and Co-Product (Co-P) with 28.8 % reduced-fat dried distillers grains and solubles (RFDDGS) treatments

<table>
<thead>
<tr>
<th>Diet</th>
<th>Control</th>
<th>Co-P</th>
<th>SEM(^1)</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>O(_2) consumption, L/d</td>
<td>5,917.2</td>
<td>5,906.1</td>
<td>110.5</td>
<td>0.88</td>
</tr>
<tr>
<td>CO(_2) production, L/d</td>
<td>6,379.9</td>
<td>6,202.9</td>
<td>108.4</td>
<td>0.03</td>
</tr>
<tr>
<td>CH(_4) production, L/d</td>
<td>504.2</td>
<td>472.1</td>
<td>11.9</td>
<td>&lt; 0.01</td>
</tr>
<tr>
<td>CH(_4)/kg milk produced</td>
<td>15.6</td>
<td>14.1</td>
<td>0.54</td>
<td>&lt; 0.01</td>
</tr>
<tr>
<td>Heat production, Mcal/d(^2)</td>
<td>29.5</td>
<td>29.3</td>
<td>0.55</td>
<td>0.62</td>
</tr>
</tbody>
</table>

\(^1\)Highest standard error of treatment means is shown

\(^2\)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 \* O\(_2\) + 1.200 \* CO\(_2\) – 0.518 \* CH\(_4\) – 1.431 \* N)
Table 3.6. Energy partitioning of Control, and Co-Product (Co-P) with 28.8 % reduced-fat dried distillers grains and solubles (RFDDGS) treatments in Mcal/d

<table>
<thead>
<tr>
<th>Component</th>
<th>Control</th>
<th>Co-P</th>
<th>SEM¹</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gross energy intake</td>
<td>84.3</td>
<td>88.1</td>
<td>2.26</td>
<td>0.04</td>
</tr>
<tr>
<td>DE</td>
<td>56.7</td>
<td>58.3</td>
<td>1.48</td>
<td>0.24</td>
</tr>
<tr>
<td>ME</td>
<td>48.9</td>
<td>50.4</td>
<td>1.40</td>
<td>0.22</td>
</tr>
<tr>
<td>Feces</td>
<td>27.8</td>
<td>29.9</td>
<td>1.03</td>
<td>0.05</td>
</tr>
<tr>
<td>Methane</td>
<td>4.77</td>
<td>4.46</td>
<td>0.11</td>
<td>&lt; 0.01</td>
</tr>
<tr>
<td>Urine</td>
<td>3.05</td>
<td>3.36</td>
<td>0.13</td>
<td>0.08</td>
</tr>
<tr>
<td>Heat</td>
<td>30.0</td>
<td>29.7</td>
<td>0.55</td>
<td>0.49</td>
</tr>
<tr>
<td>Retained</td>
<td>19.1</td>
<td>20.7</td>
<td>1.11</td>
<td>0.18</td>
</tr>
<tr>
<td>Milk</td>
<td>22.1</td>
<td>23.5</td>
<td>0.96</td>
<td>0.01</td>
</tr>
<tr>
<td>Tissue</td>
<td>-3.20</td>
<td>-2.78</td>
<td>0.93</td>
<td>0.73</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Component</th>
<th>% of GE</th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Feces</td>
<td>33.1</td>
<td>34.1</td>
<td>0.65</td>
<td>0.26</td>
</tr>
<tr>
<td>Methane</td>
<td>5.72</td>
<td>5.13</td>
<td>0.14</td>
<td>&lt; 0.01</td>
</tr>
<tr>
<td>Urine</td>
<td>3.62</td>
<td>3.83</td>
<td>0.17</td>
<td>0.35</td>
</tr>
<tr>
<td>DE</td>
<td>66.9</td>
<td>65.9</td>
<td>0.65</td>
<td>0.26</td>
</tr>
<tr>
<td>ME</td>
<td>57.6</td>
<td>57.0</td>
<td>0.66</td>
<td>0.51</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th></th>
<th>Mcal/kg DM</th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>GE</td>
<td>3.96</td>
<td>4.11</td>
<td>0.01</td>
<td>&lt; 0.01</td>
</tr>
<tr>
<td>DE</td>
<td>2.65</td>
<td>2.71</td>
<td>0.03</td>
<td>0.16</td>
</tr>
<tr>
<td>ME</td>
<td>2.28</td>
<td>2.34</td>
<td>0.03</td>
<td>0.14</td>
</tr>
<tr>
<td>NE_L</td>
<td>1.43</td>
<td>1.47</td>
<td>0.02</td>
<td>0.10</td>
</tr>
</tbody>
</table>

¹Highest standard error of treatment means is shown
Table 3.7. Nitrogen partitioning of Control, and Co-Product (Co-P) with 28.8% reduced-fat dried distillers grains and solubles (RFDDGS) treatments in g/d and as a percentage of nitrogen intake in %

<table>
<thead>
<tr>
<th>Diet</th>
<th>Control</th>
<th>Co-P</th>
<th>SEM(^1)</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mass</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>N intake</td>
<td>637.4</td>
<td>645.9</td>
<td>17.6</td>
<td>0.63</td>
</tr>
<tr>
<td>Fecal N</td>
<td>194.9</td>
<td>198.9</td>
<td>6.29</td>
<td>0.53</td>
</tr>
<tr>
<td>Urine N</td>
<td>200.3</td>
<td>215.8</td>
<td>7.24</td>
<td>0.13</td>
</tr>
<tr>
<td>Milk N</td>
<td>178.1</td>
<td>173.1</td>
<td>7.71</td>
<td>0.50</td>
</tr>
<tr>
<td>N balance(^1)</td>
<td>63.4</td>
<td>57.7</td>
<td>11.4</td>
<td>0.71</td>
</tr>
<tr>
<td>N intake</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fecal N</td>
<td>30.9</td>
<td>30.8</td>
<td>0.64</td>
<td>0.92</td>
</tr>
<tr>
<td>Urine N</td>
<td>31.7</td>
<td>33.6</td>
<td>1.14</td>
<td>0.21</td>
</tr>
<tr>
<td>Milk N</td>
<td>28.2</td>
<td>27.0</td>
<td>1.02</td>
<td>0.27</td>
</tr>
<tr>
<td>N balance(^1)</td>
<td>9.15</td>
<td>8.53</td>
<td>1.67</td>
<td>0.78</td>
</tr>
</tbody>
</table>

\(^1\)Highest standard error of treatment means is shown

\(^2\)Nitrogen balance = Intake N – Fecal N – Urine N – Milk N
Table 3.8. Apparent digestibilities of Control, and Co-Product (Co-P) with 28.8 % reduced-fat dried distillers grains and solubles (RFDDGS) treatments

<table>
<thead>
<tr>
<th>Component</th>
<th>Control</th>
<th>Co-P</th>
<th>SEM(^1)</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>DM</td>
<td>69.5</td>
<td>66.8</td>
<td>0.49</td>
<td>&lt; 0.01</td>
</tr>
<tr>
<td>OM</td>
<td>71.7</td>
<td>69.0</td>
<td>0.47</td>
<td>&lt; 0.01</td>
</tr>
<tr>
<td>Ash</td>
<td>42.0</td>
<td>43.3</td>
<td>1.85</td>
<td>0.58</td>
</tr>
<tr>
<td>CP</td>
<td>69.1</td>
<td>69.2</td>
<td>0.64</td>
<td>0.92</td>
</tr>
<tr>
<td>NDF</td>
<td>49.3</td>
<td>52.3</td>
<td>1.22</td>
<td>0.09</td>
</tr>
<tr>
<td>Ether extract</td>
<td>73.3</td>
<td>78.5</td>
<td>0.83</td>
<td>&lt; 0.01</td>
</tr>
<tr>
<td>Starch</td>
<td>96.0</td>
<td>96.6</td>
<td>0.43</td>
<td>0.29</td>
</tr>
<tr>
<td>NFC(^2)</td>
<td>95.8</td>
<td>95.2</td>
<td>0.94</td>
<td>0.59</td>
</tr>
</tbody>
</table>

\(^1\)Highest standard error of treatment means is shown

\(^2\)NFC = Nonfiber carbohydrate calculated by difference 100 – (% NDF + % CP + % Fat + % Ash)
Figure 3.1. Reduced-fat dried distillers grains and solubles
Figure 3.2. Timeline of each period, including 28 d of diet adaptation, followed by 7 d of collection and sampling.
Figure 3.3. Urine collection cups (a) electronic drawing, (b) attachment to cow, and (c) fecal and urine collection system into an aluminum pan and a Surge milk can, respectively
Figure 3.4. Collection of gas from a live animal using the indirect calorimeter headbox system
Figure 3.5. Regression of recovered energy (milk + tissue energy) on metabolizable energy (intake energy – fecal energy – urinary energy – methane energy) in kcal/metabolic body weight (MBW; \( y = 0.7614x - 158 \); \( R^2 = 0.86 \)). Recovered energy = 0 at 158 kcal/MBW and efficiency of converting metabolizable energy to lactation energy, \( k_l = 76\% \).
APPENDIX A: EQUATIONS

\[ HP = 3.866 \times O_2 + 1.200 \times CO_2 - 0.518 \times CH_4 - 1.431 \times N \]  
[1]

Metabolizable energy (ME) = intake energy – fecal energy – urinary energy – CH\(_4\) energy  
[2]

Recovered energy (RE) = ME – HP  
[3]

Tissue energy (TE) = RE – milk energy  
[4]

Metabolizable energy for recovered energy (ME\(_{RE}\)) =  

\[ ME – \text{Metabolizable energy for maintenance (ME\(_m\))} \]  
[5]

\( LE_{ME} \) (positive energy balance) = milk energy + TE \times 0.84  
[6]

\( ME_{LE} \) (negative energy balance) = ME\(_{RE}\) – TE/0.726  
[7]

Tissue energy in protein = N balance \times (5.88 \text{ kg of protein/kg of N}) \times (5.7 \text{ Mcal/kg of protein})  
[8]
OBSERVATIONS, PRACTICAL LIMITATIONS, AND RECOMMENDATIONS FOR FUTURE STUDIES

The headbox style indirect calorimeter appeared to be a practical method to determine gas exchange and CH₄ production. A larger vacuum motor may be beneficial for future headbox designs for use with large Holstein cows to avoid running fans close to their maximum power. This may reduce the risk of headboxes breaking down or shutting off during collection. Headbox design allowed for easy use and maintenance and was a great tool for this study.

Visually speaking animals appeared to behave normally while in the headbox, and they appeared relatively comfortable. However, it should be noted that they did appear to be reluctant to lie down.

Total fecal and urine collection was difficult and not 100% accurate, but there is more confidence in this method than the use of a fecal marker. Some feces were lost when splattered or dried on to the pan. The urine cups did not always remain attached to the animal or became filled with feces, resulting in lost urine, or contaminating of urine and feces. However, avoiding an invasive procedure such as urinary catheterization was a better choice for the cows. Smaller urine cups should be designed for future use with Jersey cows. Lyophilizing of urine is a difficult and messy process, and may be improved by boiling the subsample prior to freeze drying. This will avoid sample loss in the lyophilizer, and provide a more accurate estimate of sample dry matter. Even though fecal and urine collection was difficult, we are confident in our results because of the effort that was put into total collection.
For future energetics research, it would be beneficial to take measurements of gas exchange and energy losses for 4 to 6 d per animal for each period. This would allow a more accurate average of energy partitioning because of the typical drop in DMI when headbox collections occur. However, the number of replications in this trial made up for the lack of repetition of collection days. Also, reducing feed offered to 90 or 85 % ad libitum may avoid refusal accumulation, and therefore reduce laboratory sample analysis and simplify calculations.