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Feeding Dried Distillers Grains with Solubles to Lactating Dairy Cattle: Whole Animal Energy Utilization and Manure Biogas Production

Grant Michael Fincham
University of Nebraska-Lincoln, gfincham2@huskers.unl.edu

FEEDING DRIED DISTILLERS GRAINS WITH SOLUBLES TO LACTATING DAIRY CATTLE; WHOLE ANIMAL ENERGY UTILIZATION AND MANURE BIOGAS PRODUCTION

by

Grant Michael Fincham

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FEEDING DRIED DISTILLERS GRAINS WITH SOLUBLES TO LACTATING DAIRY CATTLE; WHOLE ANIMAL ENERGY UTILIZATION AND MANURE BIOGAS PRODUCTION

Grant M. Fincham, M.S.

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Advisor: Paul J. Kononoff

When DDGS are fed to lactating dairy cattle, the observed response in energy supply, milk production, and methane production varies among studies. One potential reason for these observed discrepancies is the nature of the diet formulation itself. Furthermore, little research has been conducted to examine how dietary changes can affect dairy cattle manure composition and methane production in an anaerobic manure digester. In order to gain a better understanding of these topics, we conducted two experiments.

The first experiment was conducted to test how the manipulation of lactating dairy cattle diets affects energy utilization, milk production, methane production, and manure output. Twelve lactating Jersey cows were arranged in a triplicated 4 × 4 Latin square design consisting of 4 periods of 28 days. Cows were randomly assigned to one of four treatments: CON (0% dried distillers grains with solubles (DDGS)); R-Alf (13% DDGS with alfalfa hay (AH) inclusion reduced from 16.6% to 8.36% of the diet); R-Gc (13% DDGS with ground corn (GC) inclusion reduced from 19% to 9.53% of the diet); R-GcAlf (6.5% DDGS with AH inclusion reduced from 16.6% to 13.9% of the diet and GC inclusion reduced from 19% to 16.3% of the diet). Treatments did not affect milk fat yield or methane production. Compared to CON, both DMI and gross energy intake

increased when cows consumed R-Alf. We observed an increase in both ECM and milk protein yield and concentration compared to CON when cows consumed R-Alf. Manure output increased compared to CON when cows consumed R-Gc. When cows consumed R-Alf, manure volatile solids (VS) output was observed to increase compared to CON.

The second experiment was conducted to test how diet manipulation may affect the chemical composition of manure and its subsequent methane production in an anaerobic digester. Manure samples were collected from lactating dairy cows in the first experiment. A biochemical methane potential test was conducted over two runs averaging 32 d. Observed methane production was corrected for the inoculum methane production. Manure substrate aNDFom was higher when cows consumed R-Gc or CON, and lower when cows consumed R-Alf or R-GcAlf. Compared to CON, Manure VS output increased when feeding R-Alf. Treatments did not affect manure methane or biogas production on a VS basis. Total manure methane potential increased when cows consumed R-Alf and R-Gc compared to CON.

“Be diligent to know the state of your flocks, and attend to your herds; for riches are not forever, nor does a crown endure to all generations.”

-Proverbs 27:23-24

"Farming looks mighty easy when your plow is a pencil, and you're a thousand miles from the corn field."

-Dwight D. Eisenhower

“Commit your works to the Lord, and your thoughts will be established.”

-Proverbs 16:3

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GENERAL INTRODUCTION

Within the United States, agriculture alone is responsible for 10 % of total greenhouse gas (GHG) emissions (US EPA, 2024a). Methane represents approximately 12 % of total greenhouse gas emissions; 25 % of which are from enteric fermentation and 8 % of which are from manure (US EPA, 2024b). In 2020, the United States dairy industry declared the 'Net Zero Initiative' which outlines a voluntary goal to achieve or exceed greenhouse gas neutrality, balancing carbon emissions with carbon absorptions, by the year 2050 (Undeniably Dairy, 2023).

The United States currently has the capacity to produce over 57 billion L of grain-ethanol annually. This volume of production translates into an estimated 44 MMT of dried distillers grains with soluble (DDGS) produced each year (U.S. Grains Council, 2023). When included in lactating dairy cattle diets, DDGS are considered to be a good source of both rumen bypass protein and digestible fiber. As a feed ingredient, DDGS also contribute to the sustainable goals of the dairy sector. Firstly, when they are fed, enteric CH_4 production may be reduced (Benchaar et al., 2013; Foth et al., 2015); and secondly, byproduct feeds such as DDGS are also known to increase human edible feed conversion efficiency of both energy and protein (Karlsson et al., 2018).

Over one hundred MMT of manure are produced by U.S. dairy cattle each year (Pagliari et al., 2020). On average, whole dairy manure emissions amount to 96 kg of CH_4 per head annually. When this estimate is applied to the current U.S. dairy population, it equates to nearly 900,000 metric tons of CH_4 produced by manure (Owen and Silver, 2015; USDA, 2024a). When this methane is released into the atmosphere, it becomes an environmental concern. However, in some cases dairy producers are able to capture methane before it enters the atmosphere and convert it to usable energy. One way this may be accomplished is through the use of an anaerobic manure digester. In the year 2022 alone, the use of anaerobic manure digesters reduced U.S. GHG emissions by 10.43 MMT of CO_2 equivalent in addition to generating around 2.42 million megawatt-hour equivalents of energy (US EPA, 2023).

When DDGS are included in a dairy diet, some studies have observed increases in energy supply and milk production, and reductions in methane production; however, these results are not always consistent (Benchaar et al., 2013; Foth et al., 2015; Reynolds et al., 2019). Furthermore, little research has been conducted to examine how dietary changes can affect dairy cattle manure composition and in turn, methane production in an anaerobic manure digester (Møller et al., 2014; Massé et al., 2016). Therefore, the objectives of this thesis were to 1) examine the effects of feeding DDGS in place of ground corn, alfalfa hay, or a combination of the two on methane production, feed intake, energy and N utilization, milk production, and manure output in lactating Jersey cows; and to 2) test how differences in diet composition can affect the chemical composition of manure and its subsequent methane production in an anaerobic digester.

CHAPTER 1

LITERATURE REVIEW

Ethanol

Ethanol Production

In 2023, the United States produced 58.1 billion L or 49 % of global production of ethanol making it world's largest ethanol producer (IEA, 2023). Currently, fuel ethanol equates to around 10 % of the total volume of fuel consumed by the United States (EIA, 2023). Corn production is closely tied to ethanol production and has seen an increase along with ethanol production in recent years. In the year 2023, the United States produced a record high of 539 million cubic meters of corn (USDA, 2024b). The amount of corn used for ethanol production has risen to nearly 45 %, making it the primary end-use for United States corn (USDA ERS, 2023).

The Corn Kernel and Dry Milling

The corn kernel is made up of 4 main components known as the endosperm, the germ, and the bran and tip cap making up 82, 12, and 6 % of the total kernel, respectively. The endosperm is the largest portion of the corn kernel where the majority of the starch is found. Starch in the kernel is interlocked within a starch protein matrix. The endosperm is composed of approximately 86 % starch and 9 % protein on a dry matter basis. The germ is the second largest portion of the corn kernel and is where most of the oil is found within the kernel. Corn germ is composed of approximately 35 % fat, 19 % protein, and 8 % starch. The bran and tip cap are the smallest portions of the corn kernel, making up only 6 % of the total kernel and containing mostly fiber. The bran is composed of approximately 88 % fiber while the tip cap is composed of 82 % fiber (Anderson and Lamsal, 2011).

Grain ethanol can be produced by two main processes – dry milling and wet milling. Dry milling is the most common way to produce ethanol representing over 90 % of the grain ethanol produced today (RFA, 2023a). The remaining percentage of ethanol is produced through the wet milling process. The main difference between the two is the manner in which corn is treated at the beginning of the process.

The dry grind process of producing ethanol is composed of five basic steps: grinding, cooking, liquification, saccharification, and fermentation. When corn arrives at a dry milling plant, the first step is to grind the entire corn kernel via a hammermill or roller mill. This course flour is then combined with water to make the “mash”. Around 22 gallons of mash is produced from each bushel of corn (Singh et al., 2001). The next step begins the process of converting starch within the corn to glucose. The pH of the mash is first adjusted to 6.0 and alpha amylase is added to it. This enzyme helps to rapidly hydrolyze alpha 1-4 bonds in the starch. The mash is then heated with a jet cooker to above 100° C for several minutes. Starch molecules are broken down and ruptured here as a result of the elevated temperature and mechanical shear. The mash temperature is then lowered to around 80–90° C and liquified for 30 minutes or more. After liquification the pH of the mash is adjusted to approximately 4.5, the temperature is reduced, and glucoamylase is added. This brings us to the saccharification step, where the starch is converted into glucose monomers. Saccharification continues throughout the fermentation process, but it is important that enough glucoamylase is added to prevent it from limiting the rate of fermentation. The next step in the dry milling process is fermentation, which begins when the mash is cooled to 32° C and transferred to the fermenters. Here, yeast is added to the mash along with a nitrogen source such as ammonium sulfate or urea (Bothast and Schlicher, 2005). This fermentation will continue for 48–72 hours before reaching a final ethanol concentration of around 14–20 %. After it is finished fermenting, this product is distilled through the beer column. This produces a solution containing approximately 95 % ethanol, which is later purified to 100 % before the addition of 5 % gasoline for use as fuel ethanol (Kumar and Singh, 2019). Fermentation also produces carbon dioxide that can be sold for use in carbonating beverages or to make dry ice. (Bothast and Schlicher, 2005). After distilling off the ethanol, a product called whole stillage remains. Although this product can be fed as is, it is usually processed further into a product known as distillers grains. Whole stillage is centrifuged to remove the thin stillage and you are left with wet distillers grains. The thin stillage is then further dried and condensed into something known as solubles. These solubles can then be added back to the wet distillers grains to create the feed product known as wet distillers grains with solubles (WDGS) (Bothast and Schlicher, 2005). On average, WDGS contain around 33 % DM. This product can be further dried to either modified distillers grains with solubles (MDGS) at around 49 % DM or dried distillers grains with solubles (DDGS) at around 90 % DM (NASEM, 2021). For each bushel of corn used to produce ethanol, approximately 7.71 kg of DDGS are produced via the dry milling process (Bothast and Schlicher, 2005). This means that the United States currently has the capacity to produce an estimated 44 million metric tons of corn DDGS (U.S. Grains Council, 2023).

Composition of Corn DDGS

In order to use DDGS as a feed product, the chemical composition must first be known. Dried distillers grains with solubles are often fed as a protein source but they are high in both protein and fiber content. One of the issues with DDGS historically is the variability of composition even from within the same ethanol plant. Belyea et al. (2004), collected over 200 samples from a single ethanol plant for 5 consecutive years and reported that the nutrient composition varies significantly across years. Protein content was observed to vary from 28 to 33 % and fat varied from 11 to 13 % DM (Belyea, 2004). Speihs et al. (2002), collected 118 samples from 10 ethanol plants in the upper Midwest to evaluate the nutrient content and variability of DDGS. They observed variability both within and among ethanol plants in addition to significant differences for some nutrients year to year (Spiehs et al., 2002). Variation in the composition of DDGS is due to both the source of raw material and the ethanol production procedure (Liu, 2011). Regular samples for chemical analysis should be taken when feeding DDGS in order to properly balance the diet and avoid underfeeding or overfeeding nutrients.

NDF

According to NASEM (2021), DDGS contain approximately 31 % NDF on a DM basis (NASEM, 2021). Within the literature, reported NDF content ranges from 26 to 44

% DM (Kleinschmit et al., 2006; Christen et al., 2010). When fed to ruminants, the NDF found in DDGS is known to be highly fermentable. Digestibility of NDF is primarily measured in one of two ways in the lab; namely, in vitro and in situ. Current in vitro methods are based on the work of Tilley and Terry (1963) which was later refined by Goering and Van Soest (1970) (Tilley and Terry, 1963; Goering and Van Soest, 1970a). In vitro digestibility is determined within the lab using test tubes which artificially mimic the rumen environment through the use of rumen fluid and a buffer solution. In contrast, in situ digestibility is determined using ruminally canulated animals. For this method, ground feed samples are sealed in polyester bags and placed in the rumen. Bags can be removed at multiple timepoints during digestion to determine the rate of digestibility in addition to extent of digestion (Vanzant et al., 1998). The average 48-hour in vitro NDF digestibility for DDGS is reported to be 63 % according to NASEM (2021). Varga and Hoover (1983) observed an in situ NDF digestibility of 77 % after 24 hours. In comparison, they only observed corn silage and hay NDF to be 32 % digestible after 24 hours in situ (Varga and Hoover, 1983). Krogstad et al. (2020) reported a lower NDF digestibility at 47 % for a 24 hour in situ (Krogstad et al., 2020).

The total tract digestibility of nutrients such as NDF can also be measured in vivo, where both the nutrient’s intake and excretion in the feces are determined using live animals. An increase in apparent NDF digestibility of 53 to 71 % was observed when feeding 0 and 40 % DDGS to feedlot heifers (Walter et al., 2012). Ramirez-Ramirez (2016) also reported an increase in apparent total tract NDF digestibility of 33 to 44 % when feeding 0 and 30 % DDGS to lactating dairy cows (Ramirez-Ramirez et al., 2016). In another study, apparent total-tract NDF digestibility was not affected by DDGS concentration when it was fed at 0 and 18 % of the diet (Ranathunga et al., 2019). Differences in NDF digestibility between studies are likely due to different amounts and types of forage fed along with the DDGS (Walter et al., 2012).

Protein

The NASEM (2021) value for the crude protein (CP) content of DDGS is approximately 31 % DM. Within the literature, reported CP ranged from 28 to 34 % DM (Paz et al., 2013; Morris et al., 2018). This CP can be further categorized into neutral detergent insoluble protein (NDIP) and acid detergent insoluble protein (ADIP). The NDIP fraction can be determined by the N content within the NDF residue multiplied by 6.25 to convert N to CP. Similarly, the ADIP fraction can be determined by the N content of the ADF residue multiplied by 6.25 (Schwab et al., 2003). The NASEM (2021) reports NDIP and ADIP values of approximately 3.9 and 3.0 % DM respectively for DDGS.

Protein can also be broken down into rumen degradable and undegradable components. Rumen degradable protein (RDP) can be measured in situ by calculating the portion of total CP that disappears from the polyester bag after rumen incubation. The portion of rumen undegradable protein (RUP) can then be calculated as 100 – RDP % of CP (Paz et al., 2014). Dried distillers grains with solubles are a good source of rumen undegradable protein (RUP) in cattle diets. The NASEM (2021) lists a RUP value of 47% for all types of DDGS. This RUP fraction is assumed to be 75 % digestible post-ruminally (NASEM, 2021). Castillo-Lopez et al. (2013) reported a greater value for the RUP content of DDGS at 63 % DM. In this experiment, researchers calculated the RUP content of DDGS in vivo. This was done in beef steers fitted with both ruminal and duodenal cannulas fed a either a control diet with no DDGS or a high DDGS diet with 19% DDGS. The RUP concentration of DDGS was calculated as the difference in duodenal CP flow (corrected for microbial CP) between diets divided by the DDGS CP intake (Castillo-Lopez et al., 2013).

There are limitations to both in situ and in vivo methods for determining the RUP content of feeds. In situ methods can underestimate the RUP value for certain feed ingredients due to the polyester bag's porosity and the washout of small feed particles (Schwab et al., 2003). Furthermore, the in vivo method described requires the estimation both duodenal flow and microbial flow using markers (Castillo-Lopez et al., 2013). It is important that these limitations are taken into consideration when deciding which RUP value to use in diet formulation.

One way in which value can be added to conventional DDGS is to increase the protein content. The resulting product is known as high protein DDGS (HPDDGS). The NASEM (2021) reports values for HPDDGS as 39 % CP on a DM basis (NASEM, 2021). Values of up to 46 % CP have been reported in some studies (Hubbard et al., 2009). These HPDDGS are produced by pre-fermentation fractionation. This process separates the corn kernel into its germ, endosperm, and bran components. The endosperm is fermented into ethanol, the germ is used to produce corn oil, and the bran is used to make other animal feeds. This process results in HPDDGS, which will have a lower oil and fiber content, and a higher CP content (Singh et al., 2005). Recently, a new high protein coproduct has been developed from the dry milling process. This product contains 52 % CP on a DM basis. Unlike HPDDGS where the corn fractionation takes place pre-fermentation, this product is the result of post fermentation fractionation. After fermentation, the fibrous particles are separated by sieving and elutriation, resulting in a product rich in both yeast particles from fermentation and kernel protein. As with HPDDGS, this coproduct will also be lower in oil and fiber content, and even higher in CP content (Carroll et al., 2023b).

Fat

The total fatty acid (TFA) content of conventional DDGS is listed as 12 % DM (NASEM, 2021). Reported values for TFA range from 7 to 12 % DM (Ranathunga et al., 2010; Krogstad et al., 2021). There are not many published studies where the TFA content of DDGS is measured and reported. Instead, many manuscripts report the crude fat content of DDGS. According to the NASEM (2021), the crude fat content of conventional DDGS is around 13 % DM (NASEM, 2021). Reported values range from 3 to 17 % for crude fat on a DM basis (Abdelqader et al., 2009; Penner et al., 2009). High fat content is often viewed as a negative when feeding DDGS in dairy diets. It has been shown that dietary inclusions of fat may have a negative impact on ruminal fermentation and fiber digestion. Unsaturated fatty acids especially are known for reducing fiber digestibility and inhibiting the growth of rumen microbial populations (Palmquist and Jenkins, 1980; Jenkins, 1993; Beauchemin et al., 2007). Milk fat depression is also associated with an increase in dietary fat concentration. When fat enters the rumen, polyunsaturated fatty acids (PUFA) are biohydrogenated by ruminal microbes. This process produces bioactive isomers of conjugated linoleic acid (CLA). These CLA's have been shown to be inhibitory to milk fatty acid synthesis within the mammary gland in addition to reducing the uptake of fatty acids from the blood (Chouinard et al., 1999;

Peterson et al., 2003). Milk fat depression has been observed in multiple studies where lactating dairy cattle were fed high levels of conventional DDGS (Abdelqader et al., 2009; Benchaar et al., 2013).

One way to combat milk fat depression while still feeding DDGS, is to reduce the amount of fat found within the byproduct. This process may increase the value of DDGS as a dairy feedstuff, while offering an additional source of revenue for the ethanol plant through the extraction of corn oil. Reduced fat DDGS (RFDDGS) contain approximately 8 % TFA according to NASEM (2021). In the year 2006, only 4 ethanol plants within the USA extracted fat to produce RFDDGS. However, by the year 2012, 90 of the 200 ethanol plants in operation were extracting corn oil (Harangody, 2012). Today this number has grown to include more than 80 % of the USA's corn ethanol plants (AgMRC, 2022). The process of fat extraction occurs in two main ways; either via pre-fermentation fractionation, or by post-fermentation centrifugation of the corn distillers solubles (Lundy and Loy, 2014). Studies have shown that feeding RFDDGS can reduce the risk of milk fat depression. One such study fed a control diet with no DDGS and a RFDDGS diet with 29 % RFDDGS on a DM basis. They observed milk fat yield and concentration to no differ compared to a control while also observing an increase in DMI, milk yield, and both protein yield and concentration. This study also compared conventional DDGS to RFDDGS feeding both at 29 % DM. Conventional DDGS reduced both milk fat yield and concentration and resulted in a lower yield and concentration of de novo fatty acids in the milk (Ramirez-Ramirez et al., 2016). When RFDDGS were fed in other studies, results indicated their ability to either maintain or increase ECM yield while DMI remained constant (Mjoun et al., 2010a; b; Foth et al., 2015).

Starch

The amount of starch found within DDGS is negligible at approximately 5 % DM (NASEM, 2021). During the cooking, liquification, and saccharification process of ethanol production, starch is broken down into glucose monomers. The starch is then fermented to produce ethanol (Bothast and Schlicher, 2005). Because of this, the starch content of DDGS is minimal, ranging from 3 to 10 % on a DM basis (Ranathunga et al., 2010; Reynolds et al., 2019).

Feeding DDGS to Lactating Dairy Cattle

Current Use

In the year 2023, the United States ethanol industry produced over 32 million metric tons of corn milling coproducts via the dry milling process. Cattle were the primary consumers of this product, with beef cattle consuming 42 % and dairy cattle consuming 32 % of total distillers grains produced in the USA. These were followed by swine at 18 % and poultry at 6 % of total consumption. Out of the distillers grains produced in the United States, 43 % were DDGS, 33 % were WDGS, and 11 % were MDGS (RFA, 2023b).

Replacing Forage with DDGS

When DDGS are fed in a ruminant diet, the ingredient they replace could have an effect of the animal’s response. Often, DDGS will be fed in place of some of the forage within the diet. Ranathunga et al. (2018, 2019) conducted two factorial experiments where they fed DDGS at 0 and 18 % DM in both low (41 % forage) and high (60 % forage) forage diets. The DDGS were included in place of some of the soybean meal and ground corn in the diet. They observed an increase in milk production along with a decrease in milk fat concentration when feeding the low forage diet. However, they observed no effect of DDGS concentration or interaction between forage concentration and DDGS concentration (Ranathunga et al., 2018, 2019). Another study tested the effects of feeding 15 % DDGS in low (45 % forage) and high (55 % forage) forage diets (DM basis). Although no significant response in milk yield or composition was observed, rumen pH was significantly lower when cows consumed the low forage diet (Krogstad et al., 2021). One study tested the effectiveness of NDF in whole cottonseed and DDGS compared to that of alfalfa haylage. They fed a basal diet with 30 % haylage and no byproduct, a high fiber diet with 44 % haylage and no byproduct, a cottonseed diet where they fed 31 % haylage and 13 % whole cottonseed, and a DDGS diet where they fed 31 % haylage and 13 % DDGS (DM basis). The researchers found that when the byproduct ingredients (cottonseed and DDGS) were included in place of alfalfa haylage, 4 % fat corrected milk yield increased along with DMI (Clark and Armentano, 1993). In a different study with limit-fed growing dairy heifers, DDGS were increased from 30 to 50 % of the diet DM with grass hay being the only ingredient that was reduced. As DDGS increased, average daily gain (ADG) did not differ between treatments while DMI decreased linearly. This response resulted in an increased gain to feed (G:F) ratio when DDGS concentration was greater (Manthey et al., 2016). The type of forage being fed with DDGS may also have an effect on an animal's response. One study was conducted to determine the effects of feeding corn silage, alfalfa hay, or a 50:50 blend of both as the forage source in diets containing the same amount of DDGS. All diets contained 15 % DDGS and 50 % forage (DM basis). Milk yield increased linearly as alfalfa concentration increased, but fat concentration decreased with increasing alfalfa concentration (Kleinschmit et al., 2007).

Replacing Starch with DDGS

Dried distillers grains with solubles are often fed in place of a portion of ground corn in the diet. This results in a decrease in dietary starch when DDGS are fed. Ranathunga et al. (2010) conducted a feeding trial with lactating dairy cows where they increased DDGS from 0 to 21 % of the diet DM while decreasing starch from 29 to 20 % of the diet via a reduction in ground corn. Results of this study showed that as DDGS increased in place of starch, DMI decreased linearly while milk yield and composition stayed the same. Consequently, as DDGS concentration increased in place of starch, feed efficiency also increased. The decrease in DMI that was observed in this study is likely due to the increase in dietary NDF, as forage concentration was similar for all treatments (Ranathunga et al., 2010). Another study fed DDGS at 0 and 20 % while decreasing ground corn from 29 to 20 % DM in diets fed to lactating dairy cows. Results of this study showed an increase in both milk yield and milk fat yield when DDGS were fed (Kleinschmit et al., 2006).

Within the beef sector, it is common for DDGS to be included in the diet in place of corn. One such study increased DDGS from 20 to 40 % of the diet DM by decreasing ground corn from 60 to 40 % in diets fed to yearling steers. This study showed no effect of DDGS inclusion on ADG. There was however a significant decrease in DMI which resulted in an increased G:F ratio (Swanson et al., 2014). Investigators of another study observed similar results when feeding 65 % DDGS in place of 65 % cracked corn in growing steer diets (DM basis). Results of this study showed an increase in both DMI and ADG when DDGS were fed in place of starch, and this resulted in an increased G:F (Felix et al., 2011). Nuttelman et al. (2013) fed DDGS at 35 % diet DM by replacing 35 % of dietary high moisture and dry rolled corn (50:50 blend) in finishing beef cattle. No difference between treatments was seen for DMI in this study. There was however a significant increase in both ADG and G:F (Nuttelman et al., 2013). Another study fed DDGS at 10 and 20 % by decreasing dry rolled corn from 76 to 66 % DM in finishing diets. This study showed no effect of DDGS inclusion rate on DMI, ADG, G:F, or carcass characteristics (Jenkins et al., 2011).

Manure

Manure Production

In the year 2017, an estimated 1.3 billion metric tons of manure were produced by livestock and poultry species within the United States. Cattle (beef and dairy) produced 1.2 billion metric tons of manure while dairy cattle alone produced 132 million metric tons (Pagliari et al., 2020). A lactating dairy cow producing 69 kg of manure each day will excrete approximately 8.35 kg of total solids (TS) and 7.10 kg of volatile solids (VS) (Varma et al., 2021). Huhtanen et al. (2021) conducted a meta-analysis with 501 total diets from 94 studies. The intake, production, and fecal output data from this meta-analysis are summarized in Table 1.1. The average DMI for this dataset was 19.9 kg/d (range from 10.9 to 26.0). Fecal output of organic matter (OM) was observed to range from 2.36 to 8.11 kg/d with an average output of 5.07 kg/d. Fecal CP output averaged 1.02 kg/d with a range of 0.49 to 1.65 kg/d. Fecal NDF output averaged 3.11 kg/d (range from 1.04 to 5.68 kg/d) while the potentially digestible NDF output averaged only 1.66 kg/d (range from 0.33 to 3.16 kg/d). Neutral detergent solubles measured in the feces ranged from 0.80 to 3.89 kg/d with an average output of 1.95 kg/d (Huhtanen et al., 2021). Nitrogen (N) and phosphorus (P) are notable as they are essential for plant growth and are the most regulated by state and federal natural resource agencies when applied as fertilizer (Caniglia, 2021). A Holstein cow with a total manure output of 56.7 kg/d will produce around 296 g/d of N and 54 g/d of P (Van Horn et al., 1994).

Manure Storage

Manure may be handled as a solid (>20 % TS), semi-solid (13-19 % TS), slurry (8-12 % TS) and liquid (1-7 % TS). A survey was conducted in Wisconsin, where 143 dairy farms provided information on their manure management practices. According to this survey, dairies with less than 99 cows are more likely to handle solid manure, dairies with 100-199 cows are most likely to handle semi-solid manure, dairies with 200-999 cows are more likely to handle slurry and liquid manure, and dairies with over 1000 cows are most likely to handle liquid manure. This survey also showed that while still uncommon, small dairies handling solid manure were the most likely to land-apply daily (Aguirre-Villegas, 2017). Solid manure storage has its advantages in that it takes up less volume, the odor is reduced, and it has a high nutrient retention. Disadvantages with solid manure storage include increased labor cost for manure handling and management of runoff from storage areas. Solid manure storage is usually accomplished with a concrete pad (UMass Extension, 2014). Liquid manure is typically stored in an earthen lagoon due to the larger volume of manure. These lagoons are usually lined with clay although a synthetic liner can be used in some cases. Advantages of lagoon storage include a lower construction cost and increased storage area. However, lagoons are known to increase odor in addition to increasing nitrogen loss and greenhouse gas (GHG) emissions (UMass Extension, 2014). After clay-lined lagoon storage, the most common method for manure storage is below-ground concrete storage. This storage method usually contains either semisolid or slurry manure. All manure storage systems can be covered to reduce runoff potential and minimize nitrogen and GHG losses (Aguirre-Villegas, 2017).

Manure Methane Emissions

Within the United States, agriculture alone is responsible for around 10 % of total GHG production (US EPA, 2024a). Etheric fermentation is agriculture's largest contributor at 25 % of the U.S. methane production. This is followed by manure management which accounts for 8 % of total U.S. methane emissions (US EPA, 2024b). This means that after enteric emissions, manure is the second largest source of GHG emissions on a dairy farm. On average, whole dairy manure emissions amount to 96 kg of CH_4 per head annually (Owen and Silver, 2015). If we apply this to the 9.36 million dairy cows in the U.S. today, that would equate to 898, 272 metric tons of methane produced by manure each year (USDA, 2024a). In the year 2020, the United States dairy sector launched the 'Net Zero Initiative.' This voluntary goal hopes to achieve greenhouse gas neutrality or better by the year 2050 (Undeniably Dairy, 2023).

Manure management practices can have a large impact on the amount of methane produced during storage. For example, depending on the storage temperature liquid manure storage has been shown to produce 4 to 20 times more methane than solid manure storage (Petersen, 2018). Dairies that implement solid-liquid manure separation can reduce their methane emissions by as much as 11 % as this reduces the VS content of the liquid fraction (Aguirre-Villegas, 2017). Frequency of manure pit or lagoon emptying has also been shown to affect manure methane production. Methanogens must adapt to new conditions and because of this, fresh manure can sit for weeks before methanogenesis rate increases to that of an unemptied lagoon (Petersen, 2018). As the dairy industry strives to reduce its GHG emissions from manure, one strategy that is becoming increasingly popular is the implementation of anaerobic digesters. These anaerobic digesters are able to capture methane before it enters the atmosphere and convert it to usable energy.

Anaerobic Digesters

Background

Jain et al. (2015) defines anaerobic digestion as "…a method to decompose organic matter with the help of variety of microorganisms under anaerobic or oxygen-free conditions" (Jain et al., 2015). Products of anaerobic digestion include the produced biogas and organic residue. The process of anaerobic digestion can be summarized in 4 main steps. These include enzymatic hydrolysis, fermentation, acetogenesis, and methanogenesis. Firstly, the substrate being digested must undergo enzymatic hydrolysis where complex polymers of carbohydrate, protein, and fat are broken down into simple sugars, amino acids, and fatty acid chains. Secondly is fermentation, and it is here that these reduced compounds are converted into volatile fatty acids (VFA) such as acetate, propionate, and butyrate by fermentative bacteria. Other minor products such as acetic acid, CO_2, and hydrogen are produced here as well. Thirdly, the volatile fatty acids must then undergo acetogenesis where they are converted to acetate, CO_2, and hydrogen by acetogenic bacteria. This brings us to the fourth and final step known as methanogenesis. Here, the methanogenic bacteria produce methane after consuming the acetate, CO_2, and hydrogen produced during fermentation and acetogenesis (Jain et al., 2015; Wang, 2016).

Current Use

As of September 2023, there were more than 2,400 anaerobic digesters operating within the United States. Of this total, more than half were in operation at water resource recovery facilities with 1,269 digesters. Another 102 were being used to digest food waste and 566 were located in landfills. The remainder were located on farms within the U.S., numbering at 473 anaerobic digesters (American Biogas Council, 2023). As of January 2023, there were 343 manure-based anaerobic digesters operating in the U.S., and another 86 digesters that were currently under construction (US EPA, 2023). In 2023, there were 20,124 operational dairy farms within the United States, meaning only a small percentage of the dairies within the United States utilize anaerobic manure digesters (IBISWorld, 2024). However, this number has grown rapidly in recent years and is expected to continue growing.

Economics

One of the main factors limiting the use of anaerobic manure digesters in the dairy sector is the high capital cost required for construction. Commercial anaerobic digesters can cost between $400,000 and $5,000,000 depending on the size of the digester and technology used. The average farm anaerobic digester costs around $1.2 million to construct (Anaerobic Digestion Community, 2022). Nonetheless, anaerobic manure digestion can increase farm revenue (US EPA, 2024c). A common practice is for a natural gas company to pay for and build a digester on a dairy farm, and to sign a contract to purchase the farm's manure supply. Some contracts may pay up to $80 to $100 per cow annually for the dairy farm's manure (McCully, 2021). Voluntary carbon markets also provide an additional source of revenue in the form of carbon offset credits to dairies who own their own digester. These offset credits can then be sold to outside companies who need to meet their regulatory carbon market targets (Samuels, 2024).

Environmental Impact

Another reason manure digesters are gaining popularity is because of the positive impact they have on the environment. Anaerobic digestion of dairy manure has the potential to reduce GHG emissions both directly and indirectly. Manure digesters indirectly reduce GHG emissions by replacing grid electricity with biogas-based electricity. They also play a significant role in directly reducing GHG emissions by capturing methane gas during digestion before it is released into the atmosphere (Aguirre-Villegas, 2017). In the year 2022 alone, manure-based digestors reduced GHG emissions by 10.4 million metric tons of CO_2 equivalent. They were also responsible for generating around 2.42 million megawatt-hour equivalents of energy in 2022 (US EPA, 2023).

Biochemical Methane Potential Test

The biochemical methane potential (BMP) test is a laboratory batch anaerobic digestion technique that can be used to determine substrate biodegradability and methane potential. This assay was developed by W. F. Owen at Stanford University as a means to measure the bioavailability and potential toxicity of new substrates for anaerobic digestion (Owen et al., 1979). Over the past few decades, improvements have been made to this technique and multiple reviews have been published in an effort to standardize the methods (Angelidaki and Sanders, 2004; Rozzi and Remigi, 2004; Angelidaki et al., 2009; Raposo et al., 2012; Wang, 2016).

Figure 1.1 outlines a BMP test using dairy manure as the substrate. In a typical BMP test, inoculum containing anaerobic microorganisms is collected from an active digester. The substrate being tested is then combined with the inoculum and is incubated under anaerobic conditions in a laboratory digester at a specific temperature and pH. This substrate acts as a source of carbon and energy for the microorganisms within the inoculum. Cumulative biogas is recorded over the duration of the test, which usually continues until daily biogas production is less than 1 % of total gas production. Throughout the experiment, gas samples are collected and tested to determine methane concentration. Inoculum controls (usually referred to as “blanks”) are also included in each BMP test to determine the gas production from organic matter within the inoculum (Raposo et al., 2012).

A number of factors can influence the biodegradability of the BMP assays and their subsequent biogas production. These can be separated into 4 main categories:

substrate, inoculum, digester, and experimental factors. The various methods and protocols which contribute to each factor are outlined in the following sections.

Substrate

One of the most important components to consider when conducting a BMP test is the substrate being used. As previously stated, the substrate serves as the main source of energy and carbon to the anaerobic microorganisms. Because of this, substrate composition is highly related to its subsequent biodegradability. It is critical that the composition of a substrate is known before it is used in a BMP assay.

One of the easiest ways to characterize a substrate is to measure the TS. This process removes the water from the substrate and leaves the solid components. After finding the TS, it is necessary to further characterize the sample by separating it into its organic and inorganic fractions. This is usually done by measuring the VS content. The VS can be determined by igniting the dry sample to remove the organic components. It is important to note that some samples may present more challenges than others when attempting to obtain an accurate value for TS and VS. This is due to a loss in volatile organic matter during the initial drying phase. This loss due to volatilization can be seen even when freeze drying or drying at low temperatures (Buffiere et al., 2008).

The VS portion of the substrate can be further broken down into its four main components. These include fat, protein, carbohydrates, and lignin. Of these components, the fat and protein are generally assumed to be available to the microbes for digestion while the lignin is assumed to be indigestible. The carbohydrate fraction can be further divided into its structural and nonstructural components. The nonstructural carbohydrate fraction is assumed to be readily available to the microbes, while the structural fraction is highly dependent on the crystallinity of cellulose and the lignification of the substrate (Raposo et al., 2012).

Carbon to nitrogen (C/N) ratio also has potential to influence the biogas production of different substrates. If the ratio is too high, VFA concentration can increase causing a decrease in digester pH. Methanogen activity occurs at a pH of 6.2 to 8.0, with optimal conditions around 7.0 to 7.2 (Wang et al., 2012). A decrease in biogas production when the C/N ratio is too low may also be observed. This is due to an increase in digester pH caused by an accumulation of total ammonia nitrogen. In most cases, biogas production in an anaerobic digester can be maximized at a C/N ratio of 20:1 to 30:1 (Wang et al., 2012; Jain et al., 2015).

Particle size has also been shown to impact the biochemical methane potential of a given substrate. As particle size is reduced, the total surface area of the substrate is increased. Since degradation occurs on the surface of the substrate, this has the potential to increase biogas production (Hilkiah Igoni et al., 2008; Menardo et al., 2012). Because of its ability to impact substrate biodegradability, it is recommended that particle size be standardized so that the results can be reproduced (Angelidaki et al., 2009)

In order to maximize substrate biodegradability and microbial activity, it is important to consider the concentration of substrate that is loaded into each digester. When substrate load is too low, microbial activity is reduced and biogas production is very low. In contrast, when substrate load is too high, we see an increase in microbial activity and a buildup of VFA’s. This causes the digester pH to drop too low and will result in microbial inhibition and a drop in biogas production (Raposo et al., 2012).

Inoculum

The inoculum used in a typical BMP test contains the anaerobic microorganisms responsible for digesting the substrate. The inoculum portion of a BMP test may come as an afterthought to researchers, since the focus is often on the substrate being tested. However, finding and using the correct inoculum poses one of the greatest challenges for a BMP test. This is because even when all other protocols are identical, there will still be variability between test results due to the inoculum (Blok et al., 1985). Although some variation is guaranteed, measures should still be taken to minimize the variability due to inoculum in a BMP test.

To begin, it is important that the inoculum is fully characterized before the start of a BMP test. This is usually done by determining the VS content of the inoculum. Although the VS content gives you an idea of the microorganism content of the inoculum, the measure does not distinguish between microbial cells and other organic matter. The VS content also fails to differentiate between microbial cells which are alive from those which are dead (Raposo et al., 2012).

The origin of digester inoculum offers a major source of variation for a BMP test. There is a large degree of variability within the literature relating to inoculum origin (Blok et al., 1985; Moreno-Andrade and Buitrón, 2004; Rozzi and Remigi, 2004). For example, inoculum has been sourced from municipal wastewater treatment plants, industrial treatment plants, soil extracts, manure digesters, and rumen fluid. Raposo et al. (2012), recommended the use of sludge from a municipal wastewater treatment plant as a way to standardize inoculum source for future experiments. Wastewater sludge was chosen due to the diversity of the microbial population, in addition to the fact that wastewater treatment plants can be found worldwide (Raposo et al., 2012).

Another factor to consider when conducting a BMP test is the concentration of inoculum within the digester. A high concentration of inoculum is associated with faster substrate degradation and an overall shorter test. Higher concentration also helps to prevent inhibition by the substrate due to a drop in digester pH. However, having inoculum concentration too high will result in more methane production from the inoculum. When levels are too high, this can affect results for substrate methane production and dilute differences seen between various substrates (Angelidaki and Sanders, 2004). One thing to note when considering inoculum concentration is that it should be determined and reported on a VS basis and not on a volume basis. The German standard VDI guidelines recommend an inoculum concentration of 15 to 20 g VS/L (VDI 4630, 2006)

One of the most important aspects of inoculum to consider is its level of activity (Rozzi and Remigi, 2004). As can be expected, inactive inoculum will result in low biogas production and substrate biodegradability. Although rarely used due to space constraints, it can be a good idea to measure the inoculum activity during a BMP test with a positive control (Hansen et al., 2004). The positive control, or reference substrate, must be completely biodegradable. It also shouldn’t ferment too quickly, as this can result in inhibition due to a drop in pH. For these reasons, cellulose is often used as a reference substrate. If the inoculum is active, the theoretical values for methane production of the positive control should be close to those measured during the experiment. If the measured values are much lower than the theoretical values, you can assume the partial digestion is due to either faulty equipment or inactive inoculum (Raposo et al., 2012).

Another aspect to consider before beginning a BMP test is whether or not pre-incubation of inoculum is necessary for the test. This initial incubation is conducted in an effort to reduce the amount of biogas that is produced by the inoculum during the test. Inoculum is typically incubated until there is no significant methane production. This degassing process usually lasts around 2 to 5 days (Angelidaki et al., 2009). Despite its recommendation by many authors (Birch et al., 1989; ISO 11734, 1995), another study showed that degassing had no effect on net gas production after 3 weeks of preincubation (Battersby and Wilson, 1988). Degassing inoculum is most important in an experiment where the investigator is expecting a small amount of gas production from the substrate or when concentration of inoculum is high.

The amount of time that inoculum is stored has potential to greatly alter the biogas production rate in a BMP test. Although degradation extent isn't significantly affected by storage, the rate at which a substrate is degraded decreases as storage time increases (Shelton and Tiedje, 1984). Fresh inoculum should be obtained for a BMP test if possible as it should have the highest microbial activity (Angelidaki et al., 2009).

Digesters

One of the most basic aspects of a BMP test is the type of digester to be used. Since BMP tests are anaerobic, these digesters will be sealed on day one of the experiment and remain sealed until the test is complete. Digester size must be considered with the variability of the substrate in mind. For a homogeneous substrate, small digesters can be utilized. However, if the substrate is less homogenous, larger digesters should be considered in order to accurately measure the methane potential. Although a wide range of digester volumes have been utilized, most BMP tests are conducted in digesters of less than 1 L (Raposo et al., 2012).

Another way that digesters will differ is in how biogas production is measured. This can be done either volumetrically or manometrically. The first volumetric system for biogas production measurement was published by Owen et al. (1979). This can be done by inserting a gastight syringe with a needle into the cap of the digester. The pressure within the digester will expand the syringe and the volume of gas produced can be read directly from the syringe. Volumetric measurements can also be made via a liquid displacement system. In this system, gas travels to an external vessel where it displaces a liquid barrier solution. This system can be problematic if you are also sampling gas concentrations as components can be lost in the liquid barrier (Rozzi and Remigi, 2004). Another option for volumetric measurement would be to collect the gas produced in a gas sampling bag. The disadvantage for this method is that a gas meter is required to measure the volume of gas produced by the digester (Raposo et al., 2012).

An additional way in which biogas production can be measured is manometrically. In a manometric system, the biogas produced is trapped within the digester. As a result, pressure increases in proportion to the gas being produced. When using this method to measure biogas production, additional analysis is required to measure methane concentration. This can be problematic since CO_2 solubility in the digester liquid can be influenced by headspace pressure (Birch et al., 1989). For this method, pressure, headspace volume, and temperature must be known in order to calculate volume of gas produced. These measures are then used to calculate volume using the Ideal gas law and Avogadro's law.

Gas sampling is another factor to consider for a BMP test. The process of gas sampling usually involves collecting a sample of gas from the headspace of the digester using a gastight syringe. The sample is then injected into a gas chromatograph to determine the concentration of individual gases such as methane. When reporting these concentrations, it is important that they are converted to standard temperature and pressure conditions to aid in comparing results between studies (Angelidaki et al., 2009). Gas sampling frequency should also be considered. Many researchers will begin with a higher sampling frequency but increase the time between samples as the BMP test goes on (Lisboa and Lansing, 2013a; Kafle and Chen, 2016). This is done in an effort to correctly estimate the shape of the methane production curve since the biogas production rate is faster at the beginning of the test.

Experimental Conditions

The experimental conditions of a BMP test also have potential to influence the results of the test. These conditions can be divided into both physical and chemical conditions. One of the most important physical conditions in a BMP test is the temperature the test is performed at. Temperature can be separated into 3 main ranges. These are thermophilic (45-60 ℃), mesophilic (20-45 ℃), and psychrophilic (<20 ℃) (Safley and Westerman, 1992). Psychrophilic temperatures are usually avoided for a BMP test, as lower temperature decreases microbial activity. On the other hand, increasing temperature up to the point where it kills the microorganisms will typically increase substrate degradation rate. It must be noted that this increase in temperature has no effect on biodegradability and that it only increases the digestion rate (Angelidaki and Sanders, 2004). Despite the faster degradation rate, thermophilic temperatures are not used by many due to the increased cost associated with heating the digester. Because of this, most BMP tests are carried out in mesophilic conditions. In addition to operating temperature, one should also consider the amount of fluctuation in temperature the digester will experience. Fluctuating temperatures have been shown to negatively influence methanogen activity. Digesters should be maintained at constant temperature to ensure microorganisms are as active as possible (Wang, 2016).

In order to facilitate the mixing of substrate and microorganisms and allow the release of produced biogas, it is important that the digesters are stirred and agitated periodically. This can be achieved by manually shaking, magnetic stirrers, an orbital shaker, or an agitating water bath. The method of stirring should be reported in the manuscript along with the frequency, intensity, and duration of stirring (Raposo et al., 2012).

The BMP test duration can be affected by a number of factors but is most significantly influenced by substrate degradation rate. In his paper outlining the methodology for a typical BMP test, Owen et al. (1979) advised that the test should last 30 days. Others have recommended extending the time to 50 days in hopes of achieving complete organic matter degradation (Hansen et al., 2004). Within the literature, test duration is highly variable, ranging from 7 to 365 days (Kivaisi and Eliapenda, 1995; Lopes, 2004; Raposo et al., 2008). A general rule of thumb is to terminate the test after the daily gas production is less than 1 % of the total gas production for all digesters on the test (Koch et al., 2016).

Chemical conditions within the digester can also have an impact on substrate degradability. One of the most important environmental conditions is the digester's pH. As previously noted, methanogens are active at a pH of 6.2 to 8.0, with activity maximized around a pH of 7.0 to 7.2 (Wang et al., 2012). If the pH of a specific substrate is outside this range or if the buffering capacity is too low, pH adjustments may need to be made. This can be done via the addition of a base such as NaOH or lime, or by addition of acid such as HCl (Raposo et al., 2012).

Another factor to consider is the type of gas that is used when flushing the digester's headspace prior to sealing. Headspace flushing is crucial as oxygen must be removed in order for the anaerobic microorganisms to survive. Most commonly, a mixture of CO_2 and N gas is used to flush the headspace, although some studies reported just flushing with N (Koch et al., 2015). The level of CO_2 in the flush gas can affect the test results as it has potential to increase the buffering capacity of the digester (Raposo et al., 2012). It is recommended that the concentration of CO_2 in the flush gas be as close as possible to the expected atmosphere at the end of the test (Koch et al., 2016). Flush gas flow rate and the amount of time spent flushing each digester should be reported in the manuscript.

Another aspect to consider when starting a BMP test is whether or not a mineral medium is necessary. Oftentimes, a feedstock solution is added to each digester in addition to the substrate and inoculum. This is done in order to promote microbial growth and reduce inhibition due to limiting nutrients. Calcium, iron, magnesium, nitrogen, phosphorus, potassium, sodium, and sulfur are all inorganic nutrients that have been shown to be necessary for microbial growth (Angelidaki and Sanders, 2004). Multiple trace metals have also been reported to be necessary for proper enzyme function (Demirel and Scherer, 2011). Some studies have shown that a mineral medium may not be necessary if the substrate already contains the required minerals and nutrients (Zhang et al., 2007; Yang et al., 2009). For example, cattle manure has been shown to meet the basic nutrient requirements needed for anaerobic digestion (Labatut et al., 2011a; Lisboa and Lansing, 2013a).

The substrate to inoculum (S:I) ratio is one of the most important factors to consider before starting a BMP test. Despite its importance, there is disagreement within the literature about what ratio is best for a standard BMP test. Many manuscripts do not report their S:I ratio despite its requirement for the reproduction of their BMP test. Experiments that used multiple concentrations of substrate and inocula saw inhibitory effects when the S:I ratio was higher. This is likely due to a decrease in digester pH caused by rapid VFA production from high levels of substrate. On the other hand, if the S:I ratio is too low, it can be more difficult to accurately determine the methane potential of the substrate as the inoculum methane production will be larger compared to that of the substrate (Fernández et al., 2001; Raposo et al., 2006; Yoon et al., 2014; Wang, 2016). Some have recommended using a S:I ratio of 0.5 or less since this concentration has never been shown to be inhibitory (Raposo et al., 2012).

SUMMARY

The United States is the world's largest ethanol producer. Corn production is closely tied to that of ethanol production with ethanol being the primary end use of corn within the United States. The majority of grain ethanol is produced via the dry milling process. This process ferments the starch within the corn and the resulting products include ethanol, CO_2, and distillers grains with solubles. Dried distillers grains with solubles are commonly fed as a source of dietary protein. When fed in cattle diets, they are known to be a good source of RUP. The value of DDGS can be increased by either pre or post fermentation fractionization to produce HPDDGS. Dried distillers grains with solubles are also a good source of digestible fiber. The digestibility of NDF within DDGS is known to be much higher than that of dietary forages such as corn silage or hay. Value can also be added to DDGS by the removal of fat. Reduced fat DDGS can be produced by either pre fermentation fractionization, or by additional centrifugation post fermentation. Studies have shown that RFDDGS reduce the risk of diet-induced milk fat depression when fed to lactating dairy cattle.

Cattle are the primary consumers of DDGS produced within the United States. When DDGS are fed to lactating dairy cattle, the productive responses observed may be affected by the type of ingredient they replace in the diet. Dried distillers grains with solubles are often fed in place of some of the forage in the diet. A reduction in dietary forage often results in an increase in both DMI and milk yield; however, this also increases the risk of a reduction in both rumen pH and milk fat concentration. Often, DDGS will be fed in place of a portion of the ground corn in the diet. When DDGS replace ground corn in dairy cattle diets, milk yield is maintained or increased, while

DMI is often reduced. This results in an increased feed efficiency when DDGS replace dietary starch.

Over one hundred million metric tons of manure are produced by dairy cattle within the United States each year. Manure accounts for around 8 % of the total United States methane emissions, making it the second largest source of GHG emissions on a dairy farm. When this methane is released into the atmosphere, it becomes an environmental concern. However, if dairy producers are able to capture methane before it enters the atmosphere, they are able to convert it to usable energy. This is made possible through the use of an anaerobic manure digester. Anaerobic digestion is the process by which organic matter is decomposed by microorganisms in an oxygen-free environment. Anaerobic manure digesters allow dairy producers to capture methane from their manure and reduce their farm's GHG emissions. In addition, this captured biogas can be sold as natural gas. Overall, manure digesters provide dairy producers with a valuable source of income in addition reducing their carbon footprint.

The amount of methane a substrate may produce in an anaerobic digester can be quantified in the lab by performing a BMP test. This test is commonly used to determine both substrate biodegradability and methane potential. There are many factors which may contribute to variability of results within a BMP test. The substrate's composition, particle size, and concentration along with the inoculum's composition, origin, concentration, and activity can all play a significant role in determining the methane potential determined by the BMP test. Other factors such as the how biogas production is measured, temperature, pH, headspace flush gas, test duration, and substrate to inoculum ratio can also contribute to variability between tests. Although there are many factors which may affect a BMP test's results, these batch anaerobic tests can yield valuable information regarding substrate biodegradability and relative differences in methane potential between substrates.

In closing, DDGS are often fed to lactating dairy cows in place of either forage or starch in the diet. Although some studies have investigated the influence of these dietary substitutions on animal performance, no one has measured its influence on manure composition. It would be beneficial to test how differences in diet composition can affect the chemical composition of manure and its subsequent methane production in an anaerobic digester.

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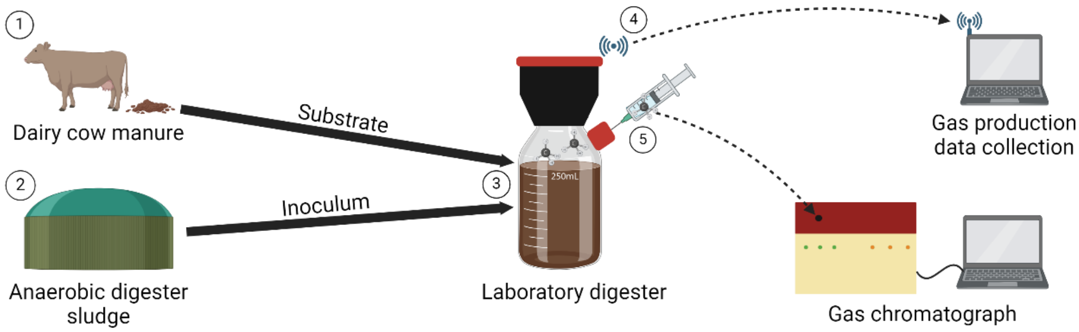
TABLES AND FIGURES

Table 1.1. Intake, production, and fecal output data adapted from a meta-analysis published by Huhtanen et al. (2021)

Item	Mean	SD	Min	Max	N
DMI, kg/d	19.9	2.64	10.9	26.0	501
ECM, kg/d	29.3	4.7	12.8	42.1	501
Fecal output, kg/d					
OM	5.07	1.074	2.36	8.11	501
CP	1.02	0.194	0.49	1.65	477
NDF	3.11	0.849	1.04	5.68	501
pdNDF[2]	1.66	0.532	0.33	3.16	501
Neutral detergent solubles	1.95	0.438	0.80	3.89	501

[1]Data are from Huhtanen et al. (2021).
[2]Potentially Digestible NDF.

Figure 1.1. Biochemical methane potential test using dairy manure as a substrate



1. In this example, manure from a dairy cow is used as the substrate for a biochemical methane potential test. The substrate acts as a source of carbon and energy for the microorganisms within the inoculum.
2. In a typical BMP test, inoculum is collected from an active anaerobic digester. The inoculum contains the anaerobic microorganisms responsible for digesting the substrate.
3. Substrate and inoculum are loaded into the laboratory digester on a VS basis. The digester's headspace is then flushed with N gas to remove oxygen before it is sealed.
4. The digester is equipped with a module to measure the increase in headspace pressure resulting from biogas production. This information is transmitted wirelessly to the gas production software where cumulative pressure is recorded. Cumulative pressure can then be used to calculate the volume of gas produced by the digester.
5. Throughout the experiment, headspace gas samples are taken through the septa port in the digester and analyzed to determine methane concentration using a gas chromatograph.

CHAPTER 2

INTERPRETIVE SUMMARY. Fincham et al. (202X). "Replacing dietary ingredients with DDGS in diets fed to lactating dairy cattle. I. Energy utilization, milk production, methane production, and manure output." Lactating dairy cows were fed diets in which DDGS replaced alfalfa, ground corn, or a combination of the two. Replacing alfalfa with DDGS increased both milk and milk protein yield. Both feed intake and energy intake increased when DDGS replaced alfalfa. Neither milk fat yield nor methane production were affected by the replacement of dietary ingredients with DDGS. Manure output was observed to increase when DDGS replaced ground corn, whereas the output of manure volatile solids increased when DDGS replaced alfalfa. Results of this study suggest that feeding DDGS in place of alfalfa, ground corn, or a combination of the two does not affect energy supply or methane production; but intake and milk production may be increased by replacing alfalfa.

RUNNING HEAD: FEEDING DDGS TO DAIRY COWS

Replacing dietary ingredients with DDGS in diets fed to lactating dairy cattle. I. Energy utilization, milk production, methane production, and manure output

G.M. Fincham[1], A.L. Carroll[1], K.J. Herrick[2], and P.J. Kononoff[1*]

[1]Department of Animal Science, University of Nebraska-Lincoln, Lincoln, NE 68503

[2]POET Nutrition LLC, Sioux Falls, SD 57104

*Corresponding author: P.J. Kononoff, Department of Animal Science C220, Fair St, Lincoln, NE, 68583, Phone number: 402-472-6442, Fax number: 402-472-6362, E-mail: pkononoff2@unl.edu

ABSTRACT

When including dried distillers grains with solubles (DDGS) in a dairy diet, some studies observe increases in energy supply and milk production, and reductions in methane production. The objective of this experiment was to examine the effects of feeding DDGS in place of ground corn (GC), alfalfa hay (AH), or a combination of the two on energy utilization with indirect calorimetry, milk, and methane production. Twelve multiparous Jersey cows (98 ± 6.5 DIM and weighing 441 ± 26.9 kg) were arranged in a triplicated 4 × 4 Latin square design consisting of 4 periods of 28 days. Cows were randomly assigned to one of four treatments: CON (0% DDGS); R-Alf (13% DDGS with AH inclusion reduced from 16.6% to 8.36% of the diet); R-Gc (13% DDGS with GC inclusion reduced from 19% to 9.53% of the diet); R-GcAlf (6.5% DDGS with AH inclusion reduced from 16.6% to 13.9% of the diet and GC inclusion reduced from 19% to 16.3% of the diet). Diets were balanced to be isonitrogenous. Diet aNDFom was 28.6 26.9, 31.9, 29.2 and starch was 28.2, 29.7, 22.8, 27.2 % DM for CON, R-Alf, R-Gc, and R-GcAlf, respectively. Compared to CON, DMI increased when cows consumed R-Alf (18.2 vs 19.4 ± 0.60 kg/d for CON and R-Alf, respectively), but cows consuming R-Gc or R-GcAlf (average 18.6 ± 0.60 kg/d) did not differ compared to CON. Energy corrected milk yield increased with R-Alf (37.4 vs 39.7 ± 1.07 kg/d for CON and R-Alf, respectively), but R-Gc or R-GcAlf (average 37.5 ± 1.07 kg/d) did not differ from CON. Cows consuming R-Alf or R-GcAlf produced more milk protein than CON (1.01, 1.13, 1.06 ± 0.038 kg/d for CON, R-Alf, and R-GcAlf, respectively), but milk protein yield in cows consuming R-Gc was did not differ (1.04 ± 0.038 kg/d). Treatments did not affect milk fat yield (average 1.55 ± 0.067 kg/d) or methane production (average 396.6 ± 19.96

L/d). No differences were observed for DE, ME, or NEL intake (54.3 ± 1.75, 48.3 ± 1.60, 34.2 ± 1.20 Mcal/d, respectively). Compared to CON, manure output increased when cows consumed R-Gc (59.1 vs 63.2 ± 1.89 kg/d for CON and R-Gc, respectively), but no difference was seen for R-Alf or R-GcAlf (average 60.2 ± 1.89 kg/d). Manure volatile solids (VS) output increased when feeding R-Alf (5.35 vs 5.70 ± 0.169 kg/d for CON and R-Alf, respectively), but no difference was seen when feeding R-Gc or R-GcAlf (average 5.46 ± 0.169 kg/d). Results indicate that feeding DDGS in place of AH, GC, or a combination of the two does not affect enteric methane production, but that intake and milk production may be increased by replacing AH.

Key Words: DDGS, energy, milk production

INTRODUCTION

The United States currently has the capacity to produce over 57 billion liters of ethanol every year, resulting in an estimated 44 million metric tons of dried corn distillers grains with solubles (DDGS) (U.S. Grains Council, 2023). Dried distillers grains with solubles continue to be a feed ingredient that is commonly used by the U.S. Dairy Industry (NASEM, 2021). In this setting, it is seen as an ingredient that supplies both digestible fiber and protein to the lactating cow. Within the United States, 25% of all methane emissions are produced by enteric fermentation (US EPA, 2024b). In 2020, the ‘Net Zero Initiative’ was launched by the United States dairy industry. This initiative outlined a voluntary goal to achieve greenhouse gas neutrality or better by the year 2050 (Undeniably Dairy, 2023). The NASEM (2021) model predicts an increase in enteric methane production as DMI and dietary digestible NDF increases, and a decrease in enteric methane production as dietary fatty acids increase. Assuming DMI is not affected, DDGS may influence enteric methane production due to changes in both fatty acids and digestible NDF supplied in the diet.

Several studies have observed increases in energy supply and milk production, and reductions in methane production when DDGS are fed (Benchaar et al., 2013; Foth et al., 2015); however, this is not always observed (Reynolds et al., 2019). One potential reason for these observed discrepancies is the nature of the diet formulation itself. For example, if DDGS are used to replace a source of fiber which is lower in digestibility, energy supply and milk production may increase (Clark and Armentano, 1993; Ranathunga et al., 2018, 2019). However, if DDGS are fed in place of a low fiber ingredient such as ground corn, feed efficiency may be increased through a reduction in DMI (Ranathunga et al., 2010).

The objective of this experiment was to examine the effects of feeding DDGS in place of ground corn, alfalfa hay, or a combination of the two on enteric methane production, feed intake, energy and N utilization, manure output, and milk production in lactating Jersey cows. Additionally, samples of feces and urine were collected to be used in a companion experiment to test how diet affects manure methane production in anaerobic digesters (Fincham et al., 2024b). We hypothesized that feeding DDGS in place of alfalfa hay would increase DMI, energy supply, and milk production, while feeding DDGS in place of ground corn would decrease DMI without changing energy supply or milk production. Additionally, we hypothesized that feeding DDGS in place of ground corn would increase fecal NDF content and that feeding DDGS in place of alfalfa would increase both manure and manure VS output.

MATERIALS AND METHODS

Animals and Treatments

The University of Nebraska – Lincoln Animal Care and Use Committee approved animal care and all experimental procedures. Twelve multiparous Jersey cows averaging 98 ± 6.5 DIM and weighing 441 ± 26.9 kg were utilized in this study. Cows were housed in individual tie stalls in a climate-controlled environment (20° C) at the University of Nebraska-Lincoln Dairy Metabolism Facility in the Animal Science Complex. Water was provided ad libitum and cows were milked at 0700 and 1800 h. All cows were less than 156 d pregnant at the conclusion of the experiment.

The experimental design was a triplicated 4 × 4 Latin square with 4 periods of 28 days including 24 days of diet adaptation followed by 4 days of collection. Prior to the start of the study, cows were blocked by milk yield and then assigned to one of four experimental treatments. Treatments were as follows: CON [0% DDGS]; R-Alf [13% DDGS with alfalfa hay inclusion reduced from 16.6% to 8.36% of the diet]; R-Gc [13% DDGS with ground corn inclusion reduced from 19% to 9.53% of the diet]; R-GcAlf [6.5% DDGS with alfalfa hay inclusion reduced from 16.6% to 13.9% of the diet and ground corn inclusion reduced from 19% to 16.3% of the diet]. The R-GcAlf diet was formulated to more closely represent a commercial dairy diet with a lower inclusion level of DDGS. Diets were balanced to contain similar concentrations of CP and NEL using the NASEM (2021) ration formulation software. All TMR ingredients (corn silage, alfalfa hay, and concentrate mix) were mixed in a Calan Data Ranger (American Calan, Inc. Northwood NH) and delivered to cows at 0930 h. Refusals were targeted at 5% during the 24 d adaptation of each period. Cattle were fed at 100% of the previous week's average intake during the 4 d collection period in order to minimize refusals.

Sample Collection and Analysis

During the collection periods, individual feed ingredients were sampled daily and frozen at -20° C. All feed ingredients were then composited and dried at 60° C and ground to pass through a 1 mm screen (Wiley Mill; Arthur A. Thomas Co., Philadelphia, PA). A subsample was then sent to Cumberland Valley Analytical Services Inc. (Waynesboro, PA) for analysis of DM (method 930.15, AOAC, 2000), N (Leco FP-528 Nitrogen Combustion Analyzer. Leco, 3000 Lakeview Avenue, St. Joseph, MI 49085, method 990.03, AOAC, 2000), ADICP and NDICP (Van Soest et al., 1991 coupled with Leco FP-528 Nitrogen Combustion Analyzer. Leco, 3000 Lakeview Avenue, St. Joseph, MI 49085), ADF (method 973.18, AOAC, 2000), NDF and NDF with sodium sulfite and α amylase corrected for ash contamination (aNDFom) (Van Soest et al., 1991), lignin (Goering and Van Soest, 1970b), sugar (DuBois et al., 1956), starch (Hall, 2009), ash (method 942.05, AOAC, 2000), minerals (method 985.01, AOAC, 2000), TFA (Sukhija and Palmquist, 1988). Ingredients were also analyzed for gross energy (GE) content at the University of Nebraska-Lincoln using a bomb calorimeter (Parr 6400 Calorimeter, Moline, IL). Samples of TMR were taken on d 1 of each collection period and analyzed for particle size using the Penn State Particle Separator (Heinrichs and Kononoff, 2002) and reported on a DM basis (60º C for 48 h). Refusals were sampled daily during each collection period and were composited on a weight basis. They were then analyzed for DM, CP, NDF, aNDFom, starch, ash, fatty acids, and GE according to the methods used for feeds described above.

Total urine and fecal output were collected from individual cows during the 4 days of each collection period and composited as described by McLain et al. (2021). After collection, feces were dried at 60° C for 48 h and ground to pass through a 1mm screen (Wiley Mill; Aurthur A. Thomas Co., Philadelphia, PA). Ground feces were analyzed for DM, CP, NDF, aNDFom, ash, fatty acids, GE, and urine was analyzed for CP using the same methods as described for feeds. Manure volatile solids (VS) output was calculated as follows: Manure VS output = Manure output (kg/d) × Manure VS %/100. Manure VS % was determined according to standard methods (2540 SOLIDS, 2017) and is reported in Fincham et al. (2024b).

Milk production was measured daily, and samples were collected during the morning and evening milking of collection periods as described by McLain et al. (2021). Milk samples were then sent to Heart of America DHIA (Kansas City, Mo.) for NIR analysis of milk fat, protein, and lactose percentage. Composited milk samples were also analyzed for N as previously described for feeds. Body weight was recorded on the first day of each collection period at 0800 h and on the last day at 1000 h before feeding.

Heat Production and Energy Utilization

Heat production was determined indirectly through 23-hour composite gas samples in headbox-type indirect calorimeters described by McLain et al. (2021) equipped with mass flow meters as described by Carroll et al. (2023b). Prior to experiment initiation animals were trained and acclimated to headboxes as described by Carroll et al. (2023b). System efficiency (head box and gas analyzer) was determined by burning 100% ethyl alcohol and measuring gas recoveries. Recoveries of O_2 and CO_2 were (average ± SD) 100.7 ± 2.55% and 99.5 ± 1.57%, respectively.

Energy Calculations

Heat production was estimated as follows (Brouwer, 1965):

Equation 2.1. Heat production (HP, kcal/d) = 3.866 × O_2 (L/d) + 1.200 × CO_2 (L/d) – 0.518 × CH_4 (L/d) – 1.431 × Urinary N excretion (g/d)

The respiratory quotient (RQ) was calculated using the ratio of carbon dioxide produced to oxygen consumed (L/L). Methane energy was estimated by multiplying CH_4 production by its enthalpy (9.45 kcal/L). Calculations to estimate digestible energy (DE), metabolizable energy (ME), and NEL were as follows:

Equation 2.2. DE intake (Mcal/d) = GE intake (Mcal/d) – fecal energy (Mcal/d)

Equation 2.3. ME (Mcal/d) = DE intake (Mcal/d)–urine energy (Mcal/d)–methane energy (Mcal/d)

Unaccounted for energy was assumed to represent tissue energy retention or mobilization which was corrected to an NEL basis as follows:

Equation 2.4. Tissue energy (Mcal/d) = ME (Mcal/d) – heat production (Mcal/d) – milk energy (Mcal/d) – fetal energy (Mcal/d)

Equation 2.5. Adjusted tissue energy (TE; Mcal of NEL/d) = positive tissue energy × k_L/k_G or negative tissue energy × k_T

Tissue energy was adjusted according to NRC (1989) where k_T is the efficiency of utilizing body reserve energy for milk production, k_G is the efficiency of utilizing ME intake for tissue gain, and k_L is the efficiency of utilizing ME for milk synthesis (Moe et al., 1971). Values of 0.66 and 0.74, and 0.89 were used for k_L, k_G, and k_T, respectively (Moraes et al., 2015; NASEM, 2021; Chris Reynolds, personal communication)

Energy utilization for pregnancy (fetal energy) was calculated according to NASEM (2021). These estimates were then used to calculate NEL using the equation:

Equation 2.6. Net energy of lactation (NEL; Mcal/d) = $0.10 \times BW^{0.75}$ + Milk E (Mcal/d) + Adjusted TE (NEL Mcal/d) + Fetal E (Mcal/d)

Statistical Analysis

Data were analyzed using the PROC GLIMMIX procedure in SAS (version 9.4, SAS Institute Inc.). The model included fixed effects of treatment, square, and period nested in square as well as the random effects of cow nested in square. All data are presented as least-squares means ± largest standard error. When the overall F test was observed to be significant, a pairwise comparison was conducted using LSMEANS statement of SAS. Significance was declared with a P-value ≤ 0.05 and trends at a P-value > 0.05 but ≤ 0.15.

RESULTS

Data Collection

Out of the 48 total planned observations, there were 44 complete collections. In period 3, one cow consuming the R-GcAlf treatment and one cow consuming the R-Alf treatment habitually physically manipulated the water bowls situated within the headboxes. Another cow from the R-Gc treatment did this as well during period 4. Because of this, the water intake data from these observations was unusable and was excluded from calculations for mean free water intake. One cow consuming the R-Alf treatment refused to drink normal volumes of water during period 4 and as a result, her water intake was much less than 2.5 standard deviations from the mean water intake. Because of this, her data from period 4 were not used to calculate mean free water intake for the R-Alf treatment. Additionally, one cow consuming the R-Gc diet during period 3 had a low RQ value that was greater than 3 standard deviations from the mean RQ value for this study. We were unable to identify a reasonable explanation for this, so the observation was not used.

Chemical Composition of Diets

The chemical composition of the diets is summarized in Table 1. Crude protein was observed to be similar across treatments (16.6 ± 0.17 % DM (Average ± SD)). The TFA content of the diet was highest when DDGS replaced alfalfa, intermediate when replacing ground corn or both ground corn and alfalfa, and lowest for the zero control (6.08, 5.93, 5.96, 5.36 % DM for R-Alf, R-Gc, R-GcAlf, and CON, respectively). The aNDFom was highest when DDGS replaced ground corn and lowest when replacing alfalfa (31.9 and 26.9 % DM for R-Gc and R-Alf, respectively). Nutrient predicted milk production was observed to be similar between treatments (31.7 ± 0.15 % DM (Average ± SD)). The chemical composition of corn silage, alfalfa hay, concentrate mixes, and

DDGS is summarized in Table 2. On a dry matter basis, the DDGS used in this study contained 35.0 % NDF, 30.5 % CP, and 7.44 % TFA.

Energy Utilization

Table 3 summarizes gas production, gas consumption, and energy utilization. The replacement of alfalfa, ground corn, or both ground corn and alfalfa was not observed to affect RQ averaging 1.03 ± 0.006. Similarly, no difference was observed in enteric CH_4 production averaging 396.6 ± 19.96 L/d across all treatments. Compared to the zero control, when DDGS replaced alfalfa or when DDGS replaced ground corn, gross energy (GE) intake increased (79.0, 84.4, 83.3 ± 2.67 Mcal/d for CON, R-Alf, and R-Gc, respectively). Intake of GE was not affected when DDGS replaced both ground corn and alfalfa. Compared to the zero control, digestible energy (DE) intake tended to increase when DDGS replaced alfalfa (52.8 vs 56.1 ± 1.75 Mcal/d for CON and R-Alf, respectively), but no difference was observed when replacing ground corn or both ground corn and alfalfa. Similarly, compared to the zero control, metabolizable energy (ME) intake tended to increase when DDGS replaced alfalfa (46.9 vs 50.1 ± 1.60 Mcal/d for CON and R-Alf, respectively); however, no difference was observed when replacing ground corn or both ground corn and alfalfa. The replacement of alfalfa, ground corn, or both ground corn and alfalfa was not observed to affect NEL intake, averaging 34.2 ± 1.20 Mcal/d. Similarly, the replacement of alfalfa, ground corn, or both ground corn and alfalfa was not observed to affect the ratio of ME to DE or the ratio of NEL to ME (averaging 0.889 ± 0.0035 and 0.708 ± 0.0075, respectively).

Digestibility Data

Table 4 summarizes digestibility data. Compared to the zero control, when DDGS replaced ground corn, DM digestibility tended to decrease (67.5 vs 65.7 ± 0.48 % for CON and R-Gc, respectively); but no difference was observed when DDGS replaced ground corn or both ground corn and alfalfa. Compared to the zero control, when DDGS replaced ground corn, OM digestibility decreased (69.5 vs 67.6 ± 0.48 % for CON and R-Gc, respectively). In comparison, OM digestibility was not affected when DDGS replaced alfalfa or both ground corn and alfalfa. Decreased aNDFom digestibility was observed when DDGS replaced alfalfa compared to the zero control (47.5 vs 43.9 ± 0.81 % for CON and R-Alf, respectively), but no difference was observed when replacing ground corn or both ground corn and alfalfa. The replacement of alfalfa, ground corn, or both ground corn and alfalfa was not observed to affect the digestibility of CP, Starch, or TFA (averaging 68.4 ± 0.66 % , 95.4 ± 0.41 % , and 74.9 ± 1.21 %, respectively).

Manure Output and Nitrogen Utilization

Fecal output, urine output, manure output, and N utilization are listed in Table 5. Increased fecal output was observed when DDGS replaced ground corn compared to the zero control (37.5 vs 41.0 ± 1.43 kg/d for CON and R-Gc, respectively), but no difference was observed when replacing alfalfa or both ground corn and alfalfa. When compared to the zero control, no difference was observed for urine output when DDGS replaced alfalfa, ground corn, or both ground corn and alfalfa. Manure output was observed to increase when DDGS replaces ground corn compared to the zero control (59.1 vs 63.2 ± 1.89 kg/d for CON and R-Gc, respectively); however, no difference in manure output was seen when replacing alfalfa or both ground corn and alfalfa. Manure volatile solids (VS) output increased when DDGS replaced alfalfa (5.35 vs 5.70 ± 0.169 kg/d for CON and R-Alf, respectively), but no difference was seen when replacing ground corn or both ground corn and alfalfa compared to the zero control. The replacement of alfalfa, ground corn, or both ground corn and alfalfa was not observed to affect fecal N, averaging 158.1 ± 6.37 g/d across all treatments. Urinary N was observed to increase compared to the zero control when DDGS replaced alfalfa or when DDGS replaced ground corn (130.5, 159.0, 149.3 ± 6.92 g/d for CON, R-Alf, and R-Gc, respectively). Urinary N was not affected when DDGS replaced both ground corn and alfalfa. An increase in manure N excretion was observed when DDGS replaced alfalfa or when DDGS replaced ground corn when compared to the zero control (284.4, 317.1, 311.1 ± 11.44 g/d for CON, R-Alf, and R-Gc, respectively). No difference in manure N excretion was observed when DDGS replaced both ground corn and alfalfa.

Chemical Composition of Feces

The chemical composition of feces is listed in Table 6. When DDGS replaced alfalfa, fecal DM was observed to increase compared to the zero control (15.8 vs 16.3 ± 0.17 % for CON and R-Alf, respectively); however, no difference was observed when DDGS replaced ground corn or both ground corn and alfalfa. Compared to the zero control, when DDGS replaced alfalfa or ground corn, fecal CP was observed to decrease (16.3, 15.4, 15.6 ± 0.21 % DM for CON, R-Alf, and R-GC, respectively). No difference was observed when DDGS replaced both ground corn and alfalfa. When DDGS replaced ground corn, fecal aNDFom was observed to increase when compared to the zero control

(45.1 vs 47.9 ± 0.49 % DM for CON and R-Gc, respectively). The replacement of alfalfa or both ground corn and alfalfa was not observed to affect fecal aNDFom content. Fecal starch was observed to decrease compared to the zero control when DDGS replaced ground corn (4.01 vs 2.94 ± 0.304 % DM for CON and R-Gc, respectively), but no difference was observed when DDGS replaced alfalfa or both ground corn and alfalfa. The replacement of alfalfa, ground corn, or both ground corn and alfalfa was not observed to affect fecal OM content, averaging 85.6 ± 0.39 % DM across all treatments.

Dry Matter Intake, Milk Yield, and Milk Composition

Production data are summarized in Table 7. Compared to the zero control, when DDGS replaced alfalfa, DMI increased (18.2 vs 19.4 ± 0.60 kg/d for CON and R-Alf, respectively). In comparison, DMI was not affected when replacing ground corn or both ground corn and alfalfa. Increased milk yield was observed when DDGS replaced alfalfa compared to the zero control (29.5 vs 31.5 ± 0.80 kg/d for CON and R-Alf, respectively), but no difference was observed when replacing ground corn or both ground corn and alfalfa. Compared to the zero control, when DDGS replaced alfalfa, ECM yield increased (37.4 vs 39.7 ± 1.07 kg/d for CON and R-Alf, respectively). The replacement of ground corn or both ground corn and alfalfa was not observed to affect ECM. Protein yield was observed to increase when DDGS replaced alfalfa or both ground corn and alfalfa compared to the zero control. The greatest response in milk protein yield was observed when replacing alfalfa alone (1.01, 1.13, 1.06 ± 0.038 kg/d for CON, R-Alf, and R-GcAlf, respectively). The replacement of ground corn did not affect milk protein yield.

The replacement of alfalfa, ground corn, or both ground corn and alfalfa was not observed to affect milk fat yield, averaging 1.55 ± 0.067 kg/d across all treatments.

DISCUSSION

The objective of this experiment was to examine the effects of feeding DDGS by replacing ground corn, alfalfa hay, or a combination of the two on methane production, feed intake, energy and N utilization, manure output, and milk production in lactating Jersey cows. Additional samples of feces and urine were collected for a follow-up experiment to test how diet manipulation affects manure methane production in laboratory anaerobic digesters (Fincham et al., 2024b). We hypothesized that feeding DDGS in place of alfalfa hay would increase DMI, energy supply, and milk production, while feeding DDGS in place of ground corn would decrease DMI without changing energy supply or milk production. Additionally, we hypothesized that feeding DDGS in place of ground corn would increase fecal NDF content and that feeding DDGS in place of alfalfa would increase both manure and manure VS output.

Diet Composition

When DDGS are included in a dairy diet, they often replace either forage or starch within the diet (Clark and Armentano, 1993; Kleinschmit et al., 2006; Ranathunga et al., 2010, 2018, 2019). The DDGS used in this experiment contained 35.0 % NDF, 30.5 % CP, 7.2 % starch, and 7.4 % TFA (DM basis). Compared to alfalfa hay, DDGS are typically higher in CP, starch, and TFA content while also containing less NDF. Although lower in NDF, the 48 hour in vitro NDF digestibility of DDGS is higher than that of alfalfa hay (62.6 and 52.4 %;NASEM, 2021). Ruminal protein degradability is expected to be affected as RUP is also higher in DDGS than in alfalfa hay (47 % and 22 %; NASEM, 2021). When compared to DDGS, ground corn is notably lower in NDF and CP, however higher in starch (NASEM, 2021). We suggest that this difference in starch at least in part explains why the predicted and observed NEL content was lowest when DDGS replaced ground corn (1.80 vs 1.79 Mcal/kg DM for CON and R-Gc, respectively).

Dry Matter Intake, Energy Intake, and Milk Yield

When DDGS replaced a portion of the alfalfa in the diet, we observed an increase in DMI (18.2 vs 19.4 kg/d for CON and R-Alf, respectively). The NASEM (2021) model includes two equations to predict DMI (Equations 2-1 and 2-2, p. 12, 13). The first of these predicts DMI based on animal factors alone, while the second equation uses both animal and dietary factors; the latter of which includes forage NDF (fNDF), ADF/NDF, and fNDF digestibility. We compared our observed response in DMI to the NASEM (2021) equation which uses dietary factors to predict intake. This equation predicted that DMI would increase 0.25 kg/d when replacing a portion of dietary alfalfa with DDGS. Although the direction of the effects was similar, we observed a greater response of 1.2 kg/d. In this case, the increase in DMI we observed was likely due to a decrease in dietary fNDF when DDGS replaced alfalfa. This is because fNDF is believed to illicit a filling effect which is mostly attributed to its longer particle size and slower NDF digestibility. Additionally, this increase in observed DMI may have been further increased by the high palatability of corn milling coproducts such as DDGS (Buse et al., 2023; Carroll et al., 2023a). When DDGS replaced a portion of the ground corn in the diet, we did not observe a response in DMI. The NASEM (2021) model also predicted this to be similar with a difference in DMI of only 0.14 kg/d expected.

When DDGS replaced ground corn or alfalfa in the diet, we observed an increase in GE intake. Similarly, intake of DE and ME tended to increase when DDGS replaced alfalfa along with a numeric increase in NEL intake. When DDGS replaced alfalfa, we observed the concentration of GE, DE, ME, and NEL to not differ from the zero control. Since the conversion efficiencies of DE/GE, ME/DE, and NEL/ME did not differ between treatments, we can conclude that the increase in energy intake was primarily the result of an increase in DMI when DDGS replaced alfalfa. We suggest that this increase in energy intake is at least in part what caused the observed increase in ECM and both protein yield and concentration when DDGS replaced alfalfa. This is consistent with other studies, which demonstrated that increasing energy intake by feeding DDGS showed a significant increase in both milk and protein yield (Benchaar et al., 2013).

Enteric Methane Production

When DDGS replaced alfalfa, ground corn, or a combination of the two, enteric methane production was not observed to differ from the zero control (averaging 396.6 ± 19.96 L/d). In contrast, other studies have observed a decrease in enteric methane production when feeding DDGS to lactating dairy cattle (Benchaar et al., 2013; Foth et al., 2015). One potential reason for this discrepancy in results is the inclusion level of DDGS within the diet. In the current study, we fed a maximum of 13 % DDGS when DDGS replaced alfalfa or ground corn. Benchaar et al. (2013) and Foth et al. (2015) both fed DDGS at a higher percentage of the diet, including them at 30 and 29 % DM, respectively. Another factor known to affect enteric methane production is dietary fat concentration. The DDGS fed in this study contained 5.8 % crude fat while the DDGS fed by Benchaar et al. (2013) and Foth et al. (2015) contained 16.3 and 6.2 % crude fat, respectively. Since dietary fat has been shown to decrease enteric methane production, the low fat concentration of the DDGS fed in this study may have contributed to the lack of response observed for enteric methane production.

Manure Output and Composition

Compared to the zero control, when DDGS replaced ground corn we observed an increase in manure output (59.1 vs 63.2 kg/d for CON and R-Gc, respectively). The NASEM equation for estimating manure output in lactating cows is predicted from DMI alone (Equation 14-15c, p. 308). As a consequence, increasing response in manure output was also predicted by the NASEM (2021) model, which predicted wet manure output to be 55.3 and 58.0 kg/d for CON and R-Gc, respectively. This equation predicted manure output to be greatest when DDGS replaced alfalfa due to this treatment having the highest DMI. In contrast, we observed manure output to be highest when replacing ground corn, while the manure output when DDGS replaced alfalfa was not significantly different from the zero control. We speculate that the discrepancy between predicted and observed manure output is at least in part due to other factors which are known to influence manure output including diet digestibility, CP, and Na and K mineral intake (NASEM, 2021). When DDGS replaced ground corn, DM digestibility tended to decrease, which may have contributed to this treatment's increase in manure output.

When DDGS replaced alfalfa, we observed an increase in manure VS output compared to the control (5.35 vs 5.70 kg/d for CON and R-Alf, respectively). This increase in VS output was expected due to the increased DMI and fecal DM when DDGS replaced alfalfa. This observation was consistent with the NASEM model estimate, which predicted manure VS output to be 6.12 and 6.52 kg/d for CON and R-Alf, respectively. NASEM (2021) predicted manure VS output increases as DMI and NDF percentage in the diet increase and decreases as the CP content of the diet decreases (Equation 14-3, p. 302). As the diets were similar in CP content and the NDF content was lower than that of the control, the increase in manure VS output observed when DDGS replaced alfalfa was most likely a result of the increase in DMI. When DDGS replaced ground corn, we observed an increase in the concentration of fecal NDF. We originally expected that this would translate to an increase in manure VS output, however this was not observed. When DDGS replaced ground corn, fecal starch concentration decreased. Because of this decrease in fecal starch concentration, we observed OM concentration to be similar between treatments; despite the increase in fecal NDF concentration when DDGS replaced ground corn.

CONCLUSIONS

In this study, lactating dairy cows were fed diets in which DDGS replaced alfalfa, ground corn, or a combination of the two. Replacing alfalfa with DDGS increased ECM and both milk protein yield and concentration. However, milk fat yield and concentration were not affected. The increase in ECM was most likely supported by an increase in DMI caused by a reduction in fNDF when DDGS replaced alfalfa. Additionally, TFA content in the diet was elevated when DDGS replaced alfalfa and this likely helped increase GE intake along with an increased DMI. Manure output was observed to increase when DDGS replaced ground corn, whereas the output of manure VS increased when DDGS replaced alfalfa. Overall, our results indicate that productive responses to feeding DDGS are largely dependent on what ingredient in the diet is replaced. Feeding DDGS in place of alfalfa, ground corn, or a combination of the two does not affect enteric methane production, but DMI and milk production may be increased by replacing alfalfa.

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TABLES

Table 2.1. Ingredient inclusion and chemical composition of experimental diets of lactating Jersey cattle fed DDGS replacing alfalfa, ground corn, or a mixture of the two

	Treatment[1]			
Item	CON	R-Alf	R-Gc	R-GcAlf
Ingredient, % DM				
Corn silage	40.5	41.5	40.5	40.5
Alfalfa hay[2]	16.6	8.36	16.6	13.9
Ground corn[2]	19.0	19.0	9.53	16.3
DDGS[3]	-	13.0	13.0	6.50
Soybean meal	7.64	5.14	4.14	6.14
Non-enzymatically browned soybean meal (NEBSBM)[4]	3.83	2.33	1.33	3.83
Soybean hulls	4.48	2.17	7.13	4.93
Calcium soaps[4]	3.02	3.02	3.02	3.02
Blood meal	1.65	1.65	1.65	1.65
Salt	0.40	0.40	0.40	0.40
Sodium bicarbonate	0.50	0.50	0.50	0.50
Calcium carbonate	0.85	1.56	1.20	1.16
Calcium phosphate (di)	0.45	0.15	0.20	0.30
Magnesium oxide	0.30	0.25	0.25	0.25
Potassium chloride	-	0.40	-	0.05
Calcium sulfate (2H2O)	0.20	-	-	0.09
Rumen protected Met[6]	0.15	0.15	0.15	0.15
Rumen protected Lys[7]	0.30	0.30	0.30	0.30
Trace mineral premix[8]	0.045	0.045	0.045	0.045
Trace vitamin premix[9]	0.045	0.045	0.045	0.045
Chemical composition, % DM (SD)				
DM	53.8 (0.41)	52.4 (0.53)	53.3 (0.76)	53.0 (0.89)
CP	16.4 (0.53)	16.6 (0.10)	16.7 (0.27)	16.8 (0.39)
NDICP[10]	1.31 (0.489)	1.16 (0.337)	1.38 (0.417)	1.48 (0.180)
ADICP[10]	0.82 (0.440)	0.79 (0.337)	0.88 (0.431)	0.84 (0.260)
Total fatty acids	5.36 (0.167)	6.08 (0.142)	5.93 (0.225)	5.96 (0.253)
16C fatty acids	1.71 (0.077)	1.73 (0.077)	1.77 (0.099)	1.82 (0.108)
18C fatty acids	3.50 (0.089)	4.21 (0.084)	4.00 (0.125)	3.99 (0.134)
Starch	28.2 (0.91)	29.7 (0.78)	22.8 (0.75)	27.2 (0.67)
NDF	29.3 (1.26)	27.6 (0.76)	32.6 (0.84)	29.9 (1.18)
aNDFom[11]	28.6 (1.34)	26.9 (0.89)	31.9 (0.86)	29.2 (1.16)
fNDF	22.1 (0.63)	18.9 (0.54)	22.1 (0.63)	20.9 (0.59)
ADF	18.5 (0.77)	15.9 (0.11)	20.6 (0.79)	18.0 (0.42)
Lignin	2.91 (0.163)	2.51 (0.210)	3.15 (0.192)	2.95 (0.422)
Ash	8.73 (1.625)	9.26 (1.437)	8.74 (1.328)	8.73 (1.421)
Na	0.31 (0.051)	0.38 (0.063)	0.35 (0.038)	0.33 (0.022)
K	1.47 (0.087)	1.44 (0.079)	1.50 (0.088)	1.44 (0.080)
NEL, Mcal/kg[12]	1.80	1.84	1.79	1.81

Nutrient predicted milk production[13]	31.8	31.8	31.5	31.8
Particle Size, %DM (SD)				
>19.0 mm	3.3 (0.84)	1.9 (0.43)	2.8 (0.22)	2.7 (0.41)
19.0 to 8.00 mm	32.3 (1.22)	31.3 (1.34)	33.0 (2.04)	32.7 (0.86)
8.0 to 1.18 mm	13.3 (1.38)	12.7 (0.66)	13.1 (1.15)	13.0 (0.71)
<1.18 mm	51.1 (1.10)	54.0 (1.24)	51.1 (1.62)	51.6 (1.05)

[1]Treatments: CON = 0% DDGS; R-Alf = 13% DDGS with alfalfa hay inclusion reduced from 16.6% to 8.36% of the diet; R-Gc = 13% DDGS with ground corn inclusion reduced from 19% to 9.53% of the diet; R-GcAlf = 6.5% DDGS with alfalfa hay inclusion reduced from 16.6% to 13.9% of the diet and ground corn inclusion reduced from 19% to 16.3% of the diet.
[2]Alfalfa was ground through a 20.3 cm screen and corn was ground using a 9.53 mm and 12.7 mm screen.
[3]Dakota Gold (POET Bioproducts, Sioux Falls, SD)
[4]Soypass (LignoTech, Overland Park, KS).
[5]Megalac (Church and Dwight Co., Princeton, NJ).
[6]Smartamine-M (Adisseo, Antony, France).
[7]AjiPro-L (Ajinomoto Co. Inc., Tokyo, Japan)
[8]Contained per kilogram of premix (DM basis): 339 g of Manganese Sulfate, 329 g Zinc Sulfate, 4 g Ferrous Sulfate, 100 g Copper Sulfate, 72 g Manganese Oxide, 85 g Zinc Oxide, 42 g Sodium Selenate, 7 g Cobalt Sulfate, 3 g EDDI, and 20 g Corn Oil.
[9]Contained per kilogram of premix (DM basis): 314 g Corn Dist Ethanol Z, 476 g Calcium Carbonate, 23 g Vitamin A, 8 g Vitamin D, and 180 g Vitamin E.
[10]ADICP = acid detergent insoluble crude protein; NDICP = neutral detergent insoluble crude protein.
[11]Amylase-treated NDF on organic matter basis.
[12]NEL, Mcal/kg prediction values are from NASEM, 2021.
[13]Nutrient predicted milk prediction values are from NASEM, 2021.

Table 2.2. Chemical composition of corn silage, alfalfa hay, concentrate mixes, and DDGS fed to lactating Jersey cattle fed DDGS replacing alfalfa, ground corn, or a mixture of the two

	Corn Silage		Alfalfa Hay		CON Concentrate		R-Alf Concentrate		R-Gc Concentrate		R-GcAlf Concentrate		DDGS[2]
Item, % DM	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean
DM, as is	33.2	1.47	89.9	1.87	92.8	0.66	91.9	0.63	92.1	0.64	92.4	0.98	90.5
CP	7.80	0.34	18.8	0.78	23.7	0.82	23.5	0.21	24.3	0.49	24.1	0.66	30.5
ADF	22.2	0.90	33.2	2.13	9.48	1.35	7.83	0.73	14.4	1.29	9.65	0.52	9.50
NDF	37.1	1.28	42.8	2.85	16.8	1.68	17.2	0.87	24.5	1.07	19.6	1.92	35.0
aNDFom[3]	36.0	1.64	42.3	2.34	16.4	1.62	16.8	0.72	24.1	0.79	19.2	1.63	32.4
ADICP[4]	0.64	0.20	1.03	0.54	0.90	0.65	0.87	0.41	1.05	0.61	0.96	0.24	1.55
NDICP[4]	0.77	0.23	2.09	0.72	1.53	0.67	1.34	0.41	1.69	0.53	1.94	0.03	3.54
Lignin	2.79	0.17	7.29	0.16	1.32	0.33	1.48	0.31	1.88	0.47	1.77	0.81	1.88
Sugar	0.58	0.40	5.48	0.48	3.50	1.50	2.78	0.57	2.45	0.75	2.88	0.81	1.70
Starch	36.1	1.42	0.98	0.33	31.3	0.93	29.2	0.83	18.8	0.81	27.4	0.41	7.20
Total fatty acids	2.82	0.19	1.31	0.05	8.91	0.40	9.24	0.23	10.3	0.57	9.74	0.53	7.44
Ash	6.35	1.96	10.9	2.37	10.2	1.15	11.4	0.97	10.2	0.43	10.2	0.94	4.88
Ca	0.21	0.02	1.21	0.10	2.20	0.16	2.39	0.19	2.25	0.16	2.04	0.20	0.04
P	0.25	0.01	0.34	0.04	0.50	0.12	0.56	0.03	0.28	0.02	0.56	0.03	0.83
Mg	0.15	0.01	0.23	0.01	0.51	0.04	0.45	0.04	0.53	0.02	0.48	0.01	0.35
K	1.10	0.06	3.42	0.29	1.06	0.05	1.39	0.07	1.13	0.06	1.13	0.05	1.28
S	0.10	0.00	0.24	0.02	0.34	0.03	0.47	0.02	0.50	0.02	0.42	0.01	1.11
Na	0.02	0.00	0.03	0.00	0.70	0.12	0.73	0.13	0.78	0.09	0.70	0.05	0.19
GE, kcal/g	4.33	0.053	4.29	0.057	4.38	0.032	4.39	0.080	4.53	0.024	4.49	0.054	4.84

[1]Mean and SD (n=4) for corn silage, alfalfa hay, and concentrate based on samples of feedstuff collected during each period and analyzed by commercial feed laboratory (Cumberland Valley Analytical Services, Waynesboro, PA.).
[2]Dakota Gold (POET Bioproducts, Sioux Falls, SD)
[3]Amylase-treated NDF on organic matter basis.
[4]ADICP = acid detergent insoluble crude protein; NDICP = neutral detergent insoluble crude protein.

Table 2.3. Oxygen consumption, carbon dioxide and enteric methane production, respiratory quotient, and energy utilization of lactating Jersey cattle fed DDGS replacing alfalfa, ground corn, or a mixture of the two

	Treatments[1,2]				SEM	*P*-value
Item	CON	R-Alf	R-Gc	R-GcAlf		
Gases, L/d						
O_2 consumption	4,660[c]	4,906[a]	4,846[ab]	4,680[bc]	129.7	0.01
CO_2 production	4,807[b]	5,044[a]	4,915[ab]	4,808[b]	142.6	0.04
CH_4 production[4]	391.7	403.3	404.8	386.6	19.96	0.58
RQ	1.03	1.03	1.02	1.03	0.006	0.44
Components, Mcal/d						
Feces	26.2[c]	28.3[ab]	28.8[a]	27.0[bc]	1.05	0.01
Urine	2.19	2.23	2.26	2.24	0.078	0.81
Methane	3.70	3.81	3.83	3.65	0.188	0.57
Heat	23.4[b]	24.6[a]	24.2[ab]	23.5[b]	0.66	0.02
Milk	25.7[b]	27.4[a]	25.8[b]	25.9[b]	0.75	0.03
Tissue	-2.02	-1.72	-1.38	-1.54	0.898	0.96
Fetal[5]	0.04	0.04	0.04	0.03	0.011	0.52
Fraction, Mcal/d						
GE	79.0[c]	84.4[a]	83.3[ab]	80.6[bc]	2.67	0.02
DE	52.8	56.1	54.6	53.6	1.75	0.15
ME	46.9	50.1	48.5	47.7	1.60	0.15
NEL[6]	33.4	35.4	34.0	34.0	1.20	0.41
Fraction, Mcal/kg of DM						
GE	4.35[c]	4.35[c]	4.41[a]	4.39[b]	0.004	<0.01
DE	2.91	2.90	2.89	2.92	0.022	0.72
ME	2.58	2.58	2.56	2.60	0.025	0.71
NEL	1.84	1.83	1.80	1.86	0.033	0.58
Efficiencies						
DE/GE	0.669	0.666	0.657	0.666	0.0051	0.36
ME/DE	0.888	0.891	0.888	0.890	0.0035	0.70
NEL/ME	0.712	0.707	0.701	0.713	0.0075	0.49
Feed efficiency[7]	0.506	0.508	0.501	0.510	0.0094	0.89

[1]Treatments: CON = 0% DDGS; R-Alf = 13% DDGS with alfalfa hay inclusion reduced from 16.6% to 8.36% of the diet; R-Gc = 13% DDGS with ground corn inclusion reduced from 19% to 9.53% of the diet; R-GcAlf = 6.5% DDGS with alfalfa hay inclusion reduced from 16.6% to 13.9% of the diet and ground corn inclusion reduced from 19% to 16.3% of the diet.

[2] Least squares means; largest SEM is listed.

[4]Standard temperature and pressure (0º C and 101.3 kPa); The conversion from L/d to g/d = (CH_4 L/d / 22.4 L per mol at standard temperature and pressure (0º C and 101.3 kPa)) * 16.04 grams per mol of CH_4.

[5]Calculated with the average birth weight for Jersey cattle at 26 kg (NASEM, 2021)

[6]NEL= $0.10 \times BW^{0.75}$+ Milk E (Mcal/d) + TE (Mcal NEL) + Fetal E (Mcal/d)

[7]Feed efficiency = (Milk energy + Tissue energy) / ME intake (Equation 3-21, NASEM 2021

[a,b,c]Least squares means within rows with different superscripts differ based on LINES means comparison ($P < 0.05$) of SAS (version 9.4, SAS Institute Inc., Cary, NC).

Table 2.4. Apparent total-tract digestibility of nutrients of lactating Jersey cattle fed DDGS replacing alfalfa, ground corn, or a mixture of the two

	Treatments[1,2]				SEM	*P*-value
Item	CON	R-Alf	R-Gc	R-GcAlf		
DM	67.5	67.0	65.7	67.1	0.48	0.06
OM	69.5[a]	69.0[ab]	67.6[b]	69.1[a]	0.48	0.05
NDF	45.9[a]	41.9[b]	46.9[a]	46.1[a]	0.92	<0.01
aNDFom[3]	47.5[a]	43.9[b]	48.1[a]	48.1[a]	0.81	<0.01
CP	68.1	69.3	68.0	68.0	0.66	0.36
Starch	95.4	95.1	95.6	95.6	0.41	0.49
Total fatty acids	75.5	74.3	74.7	75.1	1.21	0.79
16C fatty acids	75.6	74.2	74.7	74.5	1.23	0.75
18C fatty acids	77.1	75.7	76.2	76.8	1.25	0.70
Energy	66.9	66.6	65.5	66.6	0.50	0.21

[1]Treatments: CON = 0% DDGS; R-Alf = 13% DDGS with alfalfa hay inclusion reduced from 16.6% to 8.36% of the diet; R-Gc = 13% DDGS with ground corn inclusion reduced from 19% to 9.53% of the diet; R-GcAlf = 6.5% DDGS with alfalfa hay inclusion reduced from 16.6% to 13.9% of the diet and ground corn inclusion reduced from 19% to 16.3% of the diet.

[2] Least squares means; largest SEM is listed.

[3]Amylase-treated NDF on organic matter basis.

[a,b,c]Least squares means within rows with different superscripts differ based on LINES means comparison ($P < 0.05$) of SAS (version 9.4, SAS Institute Inc., Cary, NC).

Table 2.5. Fecal output, urine output, manure output, and N utilization of lactating Jersey cows fed DDGS replacing alfalfa, ground corn, or a mixture of the two

	Treatments[1,2]				SEM	*P*-value
Item	CON	R-Alf	R-Gc	R-GcAlf		
Output, kg/d (as is)						
Feces	37.5[b]	39.4[ab]	41.0[a]	37.9[b]	1.43	0.01
Urine	21.6[ab]	22.8[a]	22.2[a]	20.3[b]	0.68	0.03
Manure	59.1[bc]	62.2[ab]	63.2[a]	58.2[c]	1.89	0.01
Manure VS[3]	5.35[b]	5.70[a]	5.59[ab]	5.33[b]	0.169	0.03
Mass, g/d						
N intake	482.9	515.2	505.8	493.8	16.28	0.09
Fecal N	153.9	158.1	161.8	158.6	6.37	0.46
Urinary N	130.5[c]	159.0[a]	149.3[ab]	139.8[bc]	6.92	0.01
Manure N	284.4[b]	317.1[a]	311.1[a]	298.4[ab]	11.44	0.01
Milk N	198.2	204.1	202.4	207.6	9.21	0.78
N balance	0.33	-6.09	-7.63	-12.2	8.519	0.77
As a proportion of total N intake, %						
Fecal N	32.0	30.7	32.0	32.0	0.66	0.37
Urinary N	27.1	31.1	29.5	28.5	1.19	0.11
Milk N	41.2	39.8	40.2	42.1	1.59	0.63
N balance	-0.31	-1.51	-1.76	-2.59	1.795	0.84

[1]Treatments: CON = 0% DDGS; R-Alf = 13% DDGS with alfalfa hay inclusion reduced from 16.6% to 8.36% of the diet; R-Gc = 13% DDGS with ground corn inclusion reduced from 19% to 9.53% of the diet; R-GcAlf = 6.5% DDGS with alfalfa hay inclusion reduced from 16.6% to 13.9% of the diet and ground corn inclusion reduced from 19% to 16.3% of the diet.
[2]Least squares means; largest SEM is listed.
[3]Manure VS = Manure output (kg/d) × Manure VS%/100.
[a,b,c]Least squares means within rows with different superscripts differ based on LINES means comparison ($P < 0.05$) of SAS (version 9.4, SAS Institute Inc., Cary, NC).

Table 2.6. Chemical composition of feces produced by lactating Jersey cattle fed DDGS replacing alfalfa, ground corn, or a mixture of the two

	Treatments[1,2]					
Item, % DM	CON	R-Alf	R-Gc	R-GcAlf	SEM	*P*-value
DM, as is	15.8	16.3	15.9	16.0	0.17	0.09
CP	16.3[a]	15.4[b]	15.6[b]	16.4[a]	0.21	<0.01
NDF	47.6[b]	48.0[b]	50.1[a]	48.4[b]	0.55	<0.01
aNDFom[3]	45.1[b]	45.1[b]	47.9[a]	45.4[b]	0.49	<0.01
Starch	4.01[ab]	4.42[a]	2.94[c]	3.68[b]	0.304	<0.01
Ash	14.5	14.8	14.0	14.4	0.39	0.47
OM	85.5	85.2	86.0	85.6	0.39	0.47
Total fatty acids	4.08[b]	4.78[a]	4.42[ab]	4.56[a]	0.210	0.01
16C fatty acids	1.29	1.35	1.32	1.42	0.064	0.21
18C fatty acids	2.49[c]	3.13[a]	2.81[b]	2.85[b]	0.146	<0.01

[1]Treatments: CON = 0% DDGS; R-Alf = 13% DDGS with alfalfa hay inclusion reduced from 16.6% to 8.36% of the diet; R-Gc = 13% DDGS with ground corn inclusion reduced from 19% to 9.53% of the diet; R-GcAlf = 6.5% DDGS with alfalfa hay inclusion reduced from 16.6% to 13.9% of the diet and ground corn inclusion reduced from 19% to 16.3% of the diet.
[2]Least squares means; largest SEM is listed.
[3]Amylase-treated NDF on organic matter basis.
[a,b,c]Least squares means within rows with different superscripts differ based on LINES means comparison ($P < 0.05$) of SAS (version 9.4, SAS Institute Inc., Cary, NC).

Table 2.7. Feed intake, milk production and composition, water intake, BW and BCS of lactating Jersey cattle fed DDGS replacing alfalfa, ground corn, or a mixture of the two

	Treatments[1,2]				SEM	*P*-value
Item	CON	R-Alf	R-Gc	R-GcAlf		
DMI, kg/d	18.2[b]	19.4[a]	18.9[ab]	18.3[b]	0.60	0.03
Milk yield, kg/d	29.5[b]	31.5[a]	30.0[b]	30.1[b]	0.80	<0.01
ECM, kg/d	37.4[b]	39.7[a]	37.4[b]	37.6[b]	1.07	0.03
Protein, %	3.41[c]	3.59[a]	3.46[bc]	3.53[ab]	0.047	<0.01
Protein, kg/d	1.01[c]	1.13[a]	1.04[bc]	1.06[b]	0.038	<0.01
Fat, %	5.25	5.15	5.09	5.05	0.248	0.56
Fat, kg/d	1.55	1.60	1.52	1.52	0.067	0.31
Lactose, %	4.71	4.73	4.71	4.74	0.039	0.50
Lactose, kg/d	1.39[b]	1.49[a]	1.41[b]	1.43[b]	0.040	<0.01
MUN, mg/dL	13.4	13.0	13.4	13.0	0.49	0.58
Free water intake, L/d	82.1	87.0	87.4	87.5	5.06	0.49
BW, kg	438.7	445.7	439.8	440.1	8.50	0.18
BCS[4]	3.0	2.9	2.9	2.9	0.04	0.12
ECM/DMI	2.07	2.05	1.98	2.05	0.045	0.34

[1]Treatments: CON = 0% DDGS; R-Alf = 13% DDGS with alfalfa hay inclusion reduced from 16.6% to 8.36% of the diet; R-Gc = 13% DDGS with ground corn inclusion reduced from 19% to 9.53% of the diet; R-GcAlf = 6.5% DDGS with alfalfa hay inclusion reduced from 16.6% to 13.9% of the diet and ground corn inclusion reduced from 19% to 16.3% of the diet.
[2]Least squares means; largest SEM is listed.
[3]ECM= 0.327 × milk yield (kg) + 12.95 × fat (kg) + 7.20 × true protein (kg) (Tyrrell and Reid, 1965).
[4]Scored 1-5 by 2 independent observations.
[a,b,c]Least squares means within rows with different superscripts differ based on LINES means comparison ($P < 0.05$) of SAS (version 9.4, SAS Institute Inc., Cary, NC).

CHAPTER 3

INTERPRETIVE SUMMARY. Fincham et al. (202X). "Replacing dietary ingredients with DDGS in diets fed to lactating dairy cattle: II. Manure composition and resulting biogas and methane production in an anaerobic digestor." Manure samples were collected from lactating Jersey cows fed diets in which DDGS replaced alfalfa, ground corn, or a combination of the two. These samples were then placed in laboratory anaerobic digesters and methane production was measured. Although methane production per unit of volatile solids (VS) was not affected by dietary treatment, manure volatile solids output increased when DDGS replaced alfalfa. We estimated total manure methane potential by multiplying VS output by methane produced per unit of VS; this increased when DDGS replaced alfalfa or ground corn. Results of this study indicate that feeding DDGS in place of other dietary ingredients does not affect manure methane production in an anaerobic digester, but it may influence total manure methane potential.

RUNNING HEAD: DAIRY COW MANURE AND METHANE

Replacing dietary ingredients with DDGS in diets fed to lactating dairy cattle: II. Manure composition and resulting biogas and methane production in an anaerobic digestor

G.M. Fincham[1], A.L. Carroll[1], K.J. Herrick[2], and P.J. Kononoff[1*]

[1]Department of Animal Science, University of Nebraska-Lincoln, Lincoln, NE 68503

[2]POET Nutrition LLC, Sioux Falls, SD 57104

*Corresponding author: P.J. Kononoff, Department of Animal Science C220, Fair St, Lincoln, NE, 68583, Phone number: 402-472-6442, Fax number: 402-472-6362, E-mail: pkononoff2@unl.edu

ABSTRACT

Diet has an effect on how much enteric methane a dairy cow produces, but little is known about how diet affects methane production when manure is used in an anaerobic digester. The objective of this experiment was to examine how dietary factors affect manure output and its subsequent methane production from anaerobic digestion. Manure samples were collected from a feeding trial with 12 lactating dairy cows in a triplicated 4 × 4 Latin square. Treatments were as follows: CON (0% dried distillers grains with solubles (DDGS)); R-Alf (13% DDGS with alfalfa hay (AH) inclusion reduced from 16.6% to 8.36% of the diet); R-Gc (13% DDGS with ground corn (GC) inclusion reduced from 19% to 9.53% of the diet); R-GcAlf (6.5% DDGS with AH inclusion reduced from 16.6% to 13.9% of the diet and GC inclusion reduced from 19% to 16.3% of the diet). Diets were balanced to be isonitrogenous. Diet aNDFom was 31.9, 29.2, 28.6, 26.9 % DM for R-Gc, R-GcAlf, CON, and R-Alf, respectively. A biochemical methane potential test was conducted over two runs averaging 32 d. Observed methane production was corrected for the inoculum methane production. Manure substrate aNDFom was higher when cows consumed R-Gc or CON, and lower when cows consumed R-Alf or R-GcAlf (43.5, 42.9, 40.4, 39.2 for R-Gc, CON, R-Alf, and R-GcAlf, respectively). Manure volatile solids (VS) output increased when feeding R-Alf (5.35 vs 5.70 ± 0.169 kg/d for CON and R-Alf, respectively), but no difference was seen when feeding R-Gc or R-GcAlf (average 5.46 ± 0.169 kg/d). Treatment did not affect manure methane (average 279.7 ± 10.76 mL/g VS) or biogas production (average 444.2 ± 19.96 mL/g VS). Total manure methane potential increased when cows consumed R-Alf or R-Gc (1464.4, 1642.0, 1571.7 ± 48.11 L/d for CON, R-Alf, and R-Gc, respectively), but no difference was observed for R-GcAlf (1466.1 ± 48.11 L/d). Results of this study indicate that feeding DDGS in place of other dietary ingredients has no effect on manure methane production in an anaerobic digester, but it may influence manure VS output and thus total manure methane potential.

Key Words: anaerobic digester, biochemical methane potential, manure

INTRODUCTION

The average dairy cow produces around 64 kg of manure each day (Varma et al., 2021). This means that within the U.S., 218.6 million metric tons of manure are produced by dairy farms every year. On average, whole dairy manure emissions amount to 96 kg of CH_4 per head annually (Owen and Silver, 2015). With 9.36 million dairy cows in the U.S. today, this would equate to 898, 272 metric tons of methane produced by manure each year (USDA, 2024a). Manure-based anaerobic digesters represent an opportunity to capture the energy in this methane before it can negatively impact the environment (US EPA, 2023). In the year 2022 alone, the use of anaerobic manure digesters reduced U.S. greenhouse gas emissions by 10.4 million metric tons of CO_2 equivalent. They were also responsible for generating around 2.42 million megawatt-hour equivalents of energy. As of January 2023, there were 343 manure-based anaerobic digesters operating in the U.S., and another 86 digesters under construction (US EPA, 2023). Anaerobic digestors represent an opportunity to increase farm revenue. In many cases, construction funds to build a digester are provided by a natural gas company and an agreement between that company and dairy producer is made to purchase the manure the farm produces. It is estimated that these contracts can return approximately $80 to $100 per cow annually to the dairy farm (McCully, 2021).

Many studies have been conducted in the field of nutrition to examine how diets fed to cattle affect enteric methane production (Benchaar et al., 2013; Foth et al., 2015; Drehmel et al., 2018; Reynolds et al., 2019). However, little research has been conducted to examine how changes in the diet affect manure composition and how it affects methane production in an anaerobic digester. Dried distillers grains with solubles (DDGS) are a coproduct of the dry milling process of ethanol production and several studies have reported that inclusion of this feed may reduce enteric methane production (Benchaar et al., 2013; Foth et al., 2015). This coproduct is commonly fed to dairy and beef cattle, as it supplies both digestible fiber and protein in ruminant diets (NASEM, 2021). When DDGS are included in a dairy diet, they often replace either forage or starch within the diet (Clark and Armentano, 1993; Kleinschmit et al., 2006; Ranathunga et al., 2010, 2018, 2019). While some studies have investigated the change in manure composition when DDGS are fed (Hao et al., 2009; Benke et al., 2010; Lee et al., 2020), it would be useful to know how these formulation practices can affect methane production in a manure-based anaerobic digester.

The objective of this study was to test how differences in diet composition can affect the chemical composition of manure and resulting methane production in an anerobic digester. Lactating Jersey cows were fed four different diets (Fincham et al., 2024a), and manure was collected and chemically characterized. We then placed these manure samples in laboratory anaerobic digesters to test for differences in methane production between dietary treatments. The NASEM (2021) model predicts an increase in enteric methane production as dietary digestible NDF increases. Because of this, we hypothesized that as diets resulted in an increase in manure NDF content, methane production in the anaerobic digester would also increase.

MATERIALS AND METHODS

Manure and Inoculum

Manure samples were collected from a feeding study with 12 lactating Jersey cows in a triplicated 4 × 4 Latin square. Animals were fed diets in which DDGS replaced alfalfa, ground corn, or a combination of the two. Dietary treatments along with the chemical composition of ingredients are published in a companion paper (Fincham et al., 2024a). Total fecal output was collected and composited for each cow according to Fincham et al. (2024a). At the end of each period an additional 400 g of feces were collected from composites and frozen at -20° C for use in this experiment. Unacidified urine was collected via spot sampling on d 3 and 4 of each collection period 10 hours after feeding. To do so 100 mL of urine was drawn from each cow’s catheter and the samples were stored at -20° C. For each of the 4 treatments, feces and unacidified urine were weighed into a single composite according to the proportions by which they were produced by each cow in each treatment. They were then homogenized using a food blender (Ninja model BN701). After mixing, each manure composite was sealed in a 7.6 L airtight bucket, flushed with N gas, and frozen at -20° C.

Inoculum was obtained from two different anaerobic manure digesters from two different commercial dairy farms near Rock Valley, IA. The first of these (Inoc A) was a farm with Jersey cattle while the second was farm with Holstein cattle (Inoc B). Both digesters were manufactured by DVO brand (Chilton, WI) and operated in the plug flow style at 38° C. Digester sludge was collected from the effluent pit of the digester. Inocula were stored at 4° C in 19 L airtight buckets flushed with N gas both prior to the experiment and between runs.

Test Setup and Design

A biochemical methane potential (BMP) test was conducted using the ANKOM RF Gas Production System (ANKOM Technology, Macedon, NY). This system consists of 250 mL glass septa bottles (hereafter referred to as digesters) with a working volume of 150 mL. Each digester was equipped with a module that communicates continuously with the system software to record cumulative pressure and temperature. The system is also equipped with a zero-control module that measures ambient pressure.

Prior to loading the digesters, equal volumes of inoculum A and B were mixed together to be used that day. The substrate to inocula ratio was 1:1 on a volatile solids (VS) basis in all digesters. Maintaining this ratio, digesters were loaded with 100 g of combined manure and inocula. The manure was weighed into individual weigh boats to within 0.01 g and then rinsed into the digester with 10 mL deionized distilled water (DDW). Inoculum was weighed into a 100 mL beaker to within 0.01 g and rinsed with 10 mL DDW. After adding both manure and inoculum, the digesters were filled with DDW to their working volume of 150 mL. Five digesters were loaded with inoculum and DDW to account for methane production of the inoculum. The average methane production (ml/g VS) of the inoculum from each run was later subtracted from the treatment methane production. No culture media was added to the digesters as previous studies reported manure to contain adequate levels of nutrients and trace elements for anaerobic microorganisms (Labatut et al., 2011b; Lisboa and Lansing, 2013b).

The headspace of each digester was purged with N gas for 20 seconds and immediately sealed to ensure anaerobic conditions. To ensure digesters were sealed, petroleum jelly was applied to the lip of each bottle before the module top was fastened. The digesters were maintained in an agitating water bath at 38° C and continuously shaken at 80 RPM (Thermo Scientific Precision Shaking Water Bath SWB 27; Thermo Fisher Scientific, Inc., Waltham, MA). Global release pressure was set at 13.8 kPa and the valve open time was set at 250 milliseconds. Cumulative pressure and temperature were recorded each hour. The BMP tests were concluded when each digester's daily gas production was less than 1% of their total gas production, which occurred on d 31 and 33 for runs 1 and 2, respectively.

The BMP test was carried out over two separate runs. In run 1, each treatment was replicated in four digesters; two of which were used to measure biogas production, and two which were used for headspace gas sampling to determine methane concentration. We also included two inoculum blanks in run 1 with one to measure biogas production and one to sample headspace gas. Run 2 was carried out in similar fashion with the same number of digesters used to measure headspace gas concentration. However, an additional digester was added to each treatment and inoculum blank to measure biogas production. In total, 41 digesters were utilized over two separate runs.

Biogas and Methane Measurement and Calculations

To calculate total gas production, the ideal gas law (PV = nRT) and Avogadro's law were used. For the ideal gas law, n = gas produced in moles (mol), p = pressure in kilopascal (kPa), V = headspace volume in glass digester bottle in litters (L), T = temperature in Kelvin (K) and R = gas constant (8.314472 $L \cdot kPa \cdot K^{-1} \cdot mol^{-1}$). Using Avogadro's law, at atmospheric pressure measured in psi (1 psi = 6.894757293 kilopascal) 1 mole will occupy 22.4 L at 273.15°K and 101.325 kPa. Gas measured in moles can be converted to gas measured in mL as follows: gas produced (mL) = n × 22.4 × 1000. To measure methane concentration, an SRI 8610C Gas Chromatograph (GC) (SRI Instruments, Torrance, CA) was utilized. The GC was programmed to measure the concentration of methane in two-minute intervals and helium was used as a carrier gas. The GC was calibrated using a certified gas containing 0.08502% methane, 0.8462% carbon dioxide, 20.0840% oxygen, and 78.98478% nitrogen. Gas was sampled using a 25 mL gas tight syringe (Hamilton Co., Reno, Nevada) from each digester's headspace through the septa port on each bottle. To prepare for analysis, each sample was diluted to a 1/10 concentration. To do this, we drew 2.5 mL of biogas from the headspace of the digester, filled the syringe to 25 mL with ambient air, and injected 10 mL of this diluted sample into the GC. The methane concentration was measured daily on days 1-7, every other day on days 7-13, every 3 days on days 13-28, and then once more on the final day of the experiment.

Analytical Methods

Triplicate samples of each manure treatment and inoculum source were analyzed for total solids (TS) and VS before the experiment began. TS and VS were determined according to standard methods (2540 SOLIDS, 2017). The pH was measured before the experiment started using a pH meter (Fisher Science Education pH 510 pH/vM/Temperature meter; Thermo Fisher Scientific, Inc., Waltham, MA). Samples of each manure treatment and inoculum were also sent to Cumberland Valley Analytical Services Inc. (Waynesboro, PA) for analysis of DM (method 930.15, AOAC, 2000), N (Leco FP-528 Nitrogen Combustion Analyzer. Leco, 3000 Lakeview Avenue, St. Joseph, MI 49085, method 990.03, AOAC, 2000), crude fat (method 954.02, AOAC, 2000), NDF and NDF with sodium sulfite and α amylase corrected for ash contamination (aNDFom) (Van Soest et al., 1991), ADF (method 973.18, AOAC, 2000), lignin (Goering and Van Soest, 1970b), ash (method 942.05, AOAC, 2000), and carbon (Leco SC832 Sulfur and Carbon Analyzer). Samples of each cow's feces were analyzed according to methods described by Fincham et al. (2024a).

Statistical Analysis

Data were analyzed using the PROC GLIMMIX procedure in SAS (version 9.4, SAS Institute Inc.). When analyzing the chemical composition of feces, manure output, and total manure methane potential the model included fixed effects of treatment, square, and period nested in square as well as the random effects of cow nested in square. When analyzing biogas and methane production, the model included the fixed effect of treatment as well as the random effect of run replication. All data are presented as least-squares means ± largest standard error. When the overall F test was observed to be significant, a pairwise comparison was conducted using LSMEANS statement of SAS.

Significance was declared with a P-value ≤ 0.05 and trends at a P-value > 0.05 but ≤ 0.15.

RESULTS

Data Collection

In run 1, a digester from the control treatment malfunctioned on day 1; thus, data from this digester was not used to calculate average headspace methane concentration. In run 2, a digester from the control treatment also malfunctioned so from this observation were not used to calculate headspace methane concentration for the control treatment after d 19. Additionally, the pressure sensor of a digester containing the control treatment malfunctioned on day 11 of run 2. As a result, this digester's biogas production was not used in calculating mean biogas production for the control treatment.

Chemical Composition of Substrate

The chemical composition of the manure substrates and inocula are summarized in Table 2. Total solids concentration was observed to be similar among manure substrates averaging 11.22 ± 0.183 (Average ± SD). Volatile solids concentration of manure substrates was highest when DDGS replaced alfalfa or both alfalfa and ground corn, intermediate for the zero control, and lowest when DDGS replaced ground corn (9.15 ± 0.047, 9.15 ± 0.062, 9.04 ± 0.030, 8.86 ± 0.026 (Average ± SD) for R-Alf, R-GcAlf, CON, and R-Gc, respectively). The crude protein content of the substrates was highest when DDGS replaced both ground corn and alfalfa or alfalfa alone, intermediate for the zero control, and lowest when replacing ground corn (17.6, 17.4, 16.4, 15.3 for R-

GcAlf, R-Alf, CON, and R-Gc, respectively). Carbon to nitrogen ratio was highest when DDGS replaced ground corn, intermediate for the zero control, and lowest when DDGS replaced both ground corn and alfalfa or alfalfa alone (18.3, 17.3, 15.5, 15.5 for R-Gc, CON, R-GcAlf, and R-Alf, respectively). Manure substrate aNDFom was higher when DDGS replaced ground corn or in the zero control, and lower when DDGS replaced alfalfa or both ground corn and alfalfa (43.5, 42.9, 40.4, 39.2 for R-Gc, CON, R-Alf, and R-GcAlf, respectively). The lignin concentration of manure substrates was highest in the zero control and when DDGS replaced both ground corn and alfalfa, intermediate when DDGS replaced ground corn, and lowest when DDGS replaced alfalfa (8.43, 8.10, 6.87, 5.11 for CON, R-GcAlf, and R-Gc, and R-Alf, respectively).

Feces, Urine, and Manure Output

Fecal, urine, manure, and manure VS outputs are reported in Table 3. Increased fecal output was observed when DDGS replaced ground corn compared to the zero control (37.5 vs 41.0 ± 1.43 kg/d for CON and R-Gc, respectively), but no difference was observed when replacing alfalfa or both ground corn and alfalfa. When compared to the zero control, no difference was observed for urine output when DDGS replaced alfalfa, ground corn, or both ground corn and alfalfa. Manure output was observed to increase when DDGS replaces ground corn compared to the zero control (59.1 vs 63.2 ± 1.89 kg/d for CON and R-Gc, respectively); however, no difference in manure output was seen when replacing alfalfa or both ground corn and alfalfa. Manure VS output (calculated as manure output kg/d × Manure VS%/100) increased when DDGS replaced alfalfa (5.35 vs

5.70 ± 0.169 kg/d for CON and R-Alf, respectively), but no difference was observed when replacing ground corn or both ground corn and alfalfa compared to the zero control.

Methane Production, Biogas Production, and Manure Methane Potential

Methane and biogas production along with total manure methane potential results are listed in Table 4. The replacement of alfalfa, ground corn, or both ground corn and alfalfa was not observed to affect manure methane production averaging 279.7 ± 10.76 mL/g VS across all treatments. Similarly, when DDGS replaced alfalfa, ground corn, or both ground corn and alfalfa we observed no effect on biogas production averaging 444.2 ± 19.96 mL/g VS across all treatments. Compared to the zero control, total manure methane potential (calculated as manure VS kg/d × CH_4 L/kg VS) increased when DDGS replaced alfalfa or when DDGS replaced ground corn (1464.4, 1642.0, 1571.7 ± 48.11 L/d for CON, R-Alf, and R-Gc, respectively). No difference in total manure methane potential was observed when DDGS replaced both ground corn and alfalfa.

DISCUSSION

To test how diet manipulation affects manure methane production, we conducted an in vitro experiment with manure collected from cows fed DDGS in place of other common dietary ingredients (Fincham et al., 2024a). The in vitro experiment was conducted in the ruminant nutrition lab at University of Nebraska – Lincoln. Feces and urine were composited by treatment and inoculum originated from two different dairy methane digesters. A BMP test was replicated over two different runs averaging 32 d in length. In total, there were 5 digesters for each of the 4 treatments along with 3 inoculum blanks. Methane concentration was measured using a gas chromatograph and pressure was also constantly measured within each digester. Observed methane production was converted to CH_4 mL/g VS and also corrected for the inoculum methane production. The objective of this experiment was to test how differences in diet composition can affect the chemical composition of manure and its subsequent methane production. We hypothesized that as manure NDF content increased, so would methane production in an anaerobic digester.

Manure Methane Production

The replacement of dietary ingredients with DDGS did not significantly affect manure methane production (CH_4 ml/g VS). This was contrary to our hypothesis, which believed that an increase in manure NDF content would result in an increase in methane production. We speculate that the differences in chemical composition were too small to affect methane production in the digestor. Kafle and Chen (2016) conducted a BMP experiment with manure from 5 different livestock species including cattle (dairy), horse, goat, chicken, and swine. The chemical composition of these manure samples varied greatly with crude fiber ranging from 15.6 to 38.5 % TS and lignin concentration ranging from 3.8 to 18.1 % TS. This in turn resulted in major differences in manure methane production between treatments. The authors also developed linear regression models to predict methane potential using the chemical composition of manure. The variables tested included total carbohydrate, CP, total fat, lignin, and ADF. They tested models using up to 3 variables and concluded that the best model to predict methane potential included CP, lignin, and ADF. According to this model, manure methane potential increases as CP and ADF concentration increase and decreases as lignin increases. Authors reported that the best single variable model to predict manure methane potential used lignin. Since lignin limits fiber degradation by microbes and is completely undegradable, it impedes manure methane production among substrates even with similar VS concentrations (Kafle and Chen, 2016). One factor that these authors did not consider for their regression model was lignin as a percentage of fiber in the manure. Fiber is partially composed of lignin and its degradability is reduced when lignin concentration increases. Because of this, it would be useful to investigate the relationship between lignin as a percentage of fiber and manure methane potential in future experiments. Although not statistically significant, we did observe a numerical increase in methane production when lignin as a percentage of manure aNDFom decreased from 20.7 to 12.6 % aNDFom.

In the current study, we did not observe dietary treatments to affect manure methane production (CH_4 ml/g VS). However, we did observe an increase in manure VS output compared to the control when DDGS replaced alfalfa (5.35 vs 5.70 kg/d for CON and R-Alf, respectively). This increase in VS output was a result of differences in DMI and fecal DM. Specifically, DMI and fecal DM increased when DDGS replaced alfalfa, and this resulted in an increase in VS output. Appuhamy et al. (2018) evaluated factors that affect VS output. These authors observed that manure VS output increases as OM intake and NDF percentage in the diet increase and decreases as the CP content of the diet decreases. Among those variables tested, OM intake has the greatest effect on VS output (Appuhamy et al., 2018). When DDGS replaced alfalfa, we observed this positive relationship between OM intake and manure VS output. Treatment diets were entered into the NASEM (2021) ration formulation software which also predicted an increase in manure VS output from 6.1 to 6.5 kg/d when DDGS replaced alfalfa. This response is discussed in greater detail by Fincham et al. (2024a). When DDGS replaced a portion of alfalfa or ground corn in the diet of lactating Jersey cows, we observed an increase in total manure methane potential (1464.4, 1642.0, 1571.7 ± 48.11 L/d for CON, R-Alf, and R-Gc, respectively). This was calculated by multiplying manure VS output in kg/d times the CH_4 production in L/kg VS. Since manure methane production did not differ between treatments, total manure methane potential was primarily influenced by manure VS output.

When designing this study, we assumed that diet differences would have a greater impact on manure composition and, as a result, the treatments would influence manure methane production in an anaerobic digester. However, we speculate that the differences observed in manure composition were not large enough to influence the methane production of treatments on a VS basis. Future research should further explore how to manipulate the digestible VS portion of dairy manure and thus influence its methane production in an anaerobic digester.

CONCLUSIONS

In this study, manure samples were collected and analyzed from lactating Jersey cows fed diets in which DDGS replaced alfalfa, ground corn, or a combination of the two. When these manure samples were placed in laboratory anaerobic digesters, dietary treatments did not affect manure methane or biogas production. However, manure VS output was observed to increase when DDGS replaced alfalfa. Total manure methane potential was also observed to increase when DDGS replaced alfalfa or ground corn. Results of this study indicate that feeding DDGS in place of other dietary ingredients does not affect manure methane production in an anaerobic digester, but it may change the output of manure VS and thus influence total manure methane potential.

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TABLES

Table 3.1. Abbreviations, units, and definitions for uncommon terms used in this paper

Term	Abbreviation	Units	Definition
Total Solids	TS	%	The material residue left after evaporation and subsequent drying of a sample
Volatile Solids	VS	%	The portion of solids lost after ignition of dry solids at 550° C
Biochemical Methane Potential	BMP	ml/g VS	The maximum amount of methane that can be produced by anaerobic digestion of a specific substrate
Substrate	-	-	The main source of energy and carbon for the anaerobic microorganisms
Inoculum	-	-	The source of microorganisms that start the substrate degradation process
Headspace	-	-	The non-liquid volume in a sealed digester

Table 3.2. Chemical composition of manures collected from lactating Jersey cows fed DDGS replacing alfalfa, ground corn, or a mixture of the two, and inocula collected from commercial anaerobic manure digesters

		Treatments[1]					
Item	Units	CON	R-Alf	R-Gc	R-GcAlf	Inoc-A	Inoc-B
Total Solids (TS)	%	11.2 (0.03)[3]	11.4 (0.07)	11.0 (0.03)	11.3 (0.07)	4.04 (0.022)	3.32 (0.029)
Volatile Solids (VS)	%	9.04 (0.030)	9.15 (0.047)	8.86 (0.026)	9.15 (0.062)	2.74 (0.010)	2.28 (0.031)
pH	-	7.41	7.30	7.38	7.05	7.58	7.68
Crude Protein	% TS	16.4	17.4	15.3	17.6	-	-
Crude Fat (EE)	% TS	5.34	6.76	4.76	4.74	-	-
NDF	% TS	45.0	42.3	44.9	40.8	-	-
aNDFom[2]	% TS	42.9	40.4	43.5	39.2	-	-
ADF	% TS	28.9	25.1	30.1	30.5	-	-
Lignin	% TS	8.43	5.11	6.87	8.10	-	-
Ash	% TS	18.8	20.8	19.55	18.5	-	-
Carbon	% TS	45.5	43.0	44.9	43.8	-	-
C:N	-	17.3	15.5	18.3	15.5	-	-

[1]Treatments: CON = 0% DDGS; R-Alf = 13% DDGS with alfalfa hay inclusion reduced from 16.6% to 8.36% of the diet; R-Gc = 13% DDGS with ground corn inclusion reduced from 19% to 9.53% of the diet; R-GcAlf = 6.5% DDGS with alfalfa hay inclusion reduced from 16.6% to 13.9% of the diet and ground corn inclusion reduced from 19% to 16.3% of the diet.

[2]Amylase-treated NDF on organic matter basis.

[3]Values in parenthesis are standard deviation.

Table 3.3. Fecal, urine, manure, and manure VS output of lactating Jersey cows fed DDGS replacing alfalfa, ground corn, or a mixture of the two

	Treatments[1,2]				SEM	*P*-value
Item	CON	R-Alf	R-Gc	R-GcAlf		
Output, kg/d (as is)						
Feces	37.5[b]	39.4[ab]	41.0[a]	37.9[b]	1.43	0.01
Urine	21.6[ab]	22.8[a]	22.2[a]	20.3[b]	0.68	0.03
Manure	59.1[bc]	62.2[ab]	63.2[a]	58.2[c]	1.89	0.01
Manure VS[3]	5.35[b]	5.70[a]	5.59[ab]	5.33[b]	0.169	0.03

[1]Treatments: CON = 0% DDGS; R-Alf = 13% DDGS with alfalfa hay inclusion reduced from 16.6% to 8.36% of the diet; R-Gc = 13% DDGS with ground corn inclusion reduced from 19% to 9.53% of the diet; R-GcAlf = 6.5% DDGS with alfalfa hay inclusion reduced from 16.6% to 13.9% of the diet and ground corn inclusion reduced from 19% to 16.3% of the diet.

[2]Least squares means; largest SEM is listed.

[3]Manure VS = Manure output (kg/d) × Manure VS%/100.

[a,b,c]Least squares means within rows with different superscripts differ based on LINES means comparison ($P < 0.05$) of SAS (version 9.4, SAS Institute Inc., Cary, NC).

Table 3.4. Digester biogas and methane production, and total manure methane potential of lactating Jersey cattle fed DDGS replacing alfalfa, ground corn, or a mixture of the two

	Treatments[1,2]				SEM	*P*-value
Item	CON	R-Alf	R-Gc	R-GcAlf		
CH_4 mL/g VS[3]	274.2	288.3	280.6	275.6	10.76	0.41
Biogas mL/g VS[3]	433.2	457.9	446.4	439.3	19.96	0.48
Total Manure CH_4 Potential L/d[4]	1464.4[b]	1642.0[a]	1571.7[a]	1466.1[b]	48.11	<0.01

[1]Treatments: CON = 0% DDGS; R-Alf = 13% DDGS with alfalfa hay inclusion reduced from 16.6% to 8.36% of the diet; R-Gc = 13% DDGS with ground corn inclusion reduced from 19% to 9.53% of the diet; R-GcAlf = 6.5% DDGS with alfalfa hay inclusion reduced from 16.6% to 13.9% of the diet and ground corn inclusion reduced from 19% to 16.3% of the diet.
[2]Least squares means; largest SEM is listed.
[3]Milliliters of gas produced per gram of volatile solids with the inoculum gas production subtracted from each digester.
[4]Total Manure CH_4 Potential L/d = Manure VS (kg/d) × CH_4 L/kg VS.
[a,b,c]Least squares means within rows with different superscripts differ based on LINES means comparison ($P < 0.05$) of SAS (version 9.4, SAS Institute Inc., Cary, NC).

GENERAL SUMMARY AND CONCLUSIONS

When DDGS are fed to lactating dairy cattle, the observed response in energy supply, milk production, and methane production varies among studies. One potential reason for this is the nature of the diet formulation itself. Furthermore, little research has been conducted to examine how dietary changes may affect dairy cattle manure composition and methane production in an anaerobic manure digester. In response to these assertions, we conducted two experiments. Their objectives were to 1) examine the effects of feeding DDGS in place of ground corn, alfalfa hay, or a combination of the two on methane production, feed intake, energy and N utilization, milk production, and manure output in lactating Jersey cows; and to 2) test how differences in diet composition can affect the chemical composition of manure and its subsequent methane production in an anaerobic digester.

Energy utilization, milk production, methane production, and manure output. A feeding trial was conducted in which lactating Jersey cows were fed DDGS in place of dietary alfalfa, ground corn, or a combination of the two. Diets were balanced to contain similar concentrations of CP and NEL using the NASEM (2021) ration formulation software. The replacement of dietary ingredients with DDGS did not affect enteric methane production or milk fat yield. We did observe an increase in both DMI and GE intake when DDGS replaced some of the alfalfa in the diet. This increase in intake was likely caused by a decrease in dietary fNDF when DDGS replaced alfalfa. We also observed an increase in both ECM and milk protein yield when DDGS replaced alfalfa. We suggest that this increase was caused by an increase in energy intake when

DDGS replaced alfalfa. Additionally, total manure output was observed to increase when DDGS replaced ground corn. Manure volatile solids (VS) output was also observed to increase when DDGS replaced alfalfa. This was likely the result of the increase in DMI observed when DDGS replaced alfalfa.

Manure composition and resulting biogas and methane production in an anaerobic digestor. To test how diet manipulation affects manure methane production, we conducted an in vitro experiment with manure collected from cows in the study above. Total feces and urine were collected and composited by treatment and inoculum was collected from two different dairy methane digesters. A biochemical methane potential test was replicated over two runs. Methane concentration was measured using a gas chromatograph and pressure was also continually measured within each digester. The replacement of dietary ingredients with DDGS did not affect manure methane production when expressed per kg of VS. We speculate that this observation was due to the similarity in chemical composition of manures. Although methane production was not affected by dietary treatments, manure VS output increased when DDGS replaced alfalfa as a result of an increase in DMI. This increase in manure VS output resulted in an increase in total manure methane potential when DDGS replaced alfalfa.

APPENDIX A: FECES, URINE, AND INOCULUM COLLECTION, STORAGE, AND CHARACTERIZATION FOR ANAEROBIC DIGESTER EXPERIMENT

I. Feces and Urine Collection

- **A.** Feces Collection: 400 g of feces were collected from each cow's treatment composite at the end of each period and frozen at -20° C
- **B.** Urine Collection: 100 ml of unacidified urine were collected from the catheter hose of each cow on days 3 and 4 of each period 10 hours after feeding (Lee et al., 2019) and frozen at -20° C

II. Feces and Urine Treatment Composite and Storage

- **A.** Feces samples from each cow were weighed into a ninja blender (Model BN701) in their appropriate percentages for the treatment
- **B.** After being blended, feces were placed in a 7.6 L airtight bucket
- **C.** Urine samples from each cow were weighed into the same bucket in their appropriate percentages for the treatment
- **D.** Feces and urine were then mixed together using an electric hand mixer
- **E.** Bucket headspace was flushed with Nitrogen gas
- **F.** The buckets were then sealed and frozen at -20° C

III. Inoculum Collection and Storage

- **A.** Inoculum was obtained from two different anaerobic manure digesters from two different commercial dairy farms near Rock Valley, IA
- **B.** Using a small plastic bucket and paracord, digested sludge was collected from the effluent pit of each digester
- **C.** This process was repeated as many times as necessary to fill a 19 L airtight bucket
- **D.** After filling, buckets were promptly sealed to prevent oxygen contamination
- **E.** Bucket headspace was flushed with Nitrogen gas upon returning to UNL
- **F.** The buckets were then sealed and stored in a fridge at 4° C

IV. Total and Volatile Solids Determination

A. Total Solids

- **i.** 25g of manure or 30g of inoculum were weighed into a clean crucible
- **ii.** Samples were initially dried at 40° C for 24 hours
- **iii.** Samples were then dried at 105° C in 1-hour increments until the observed weight change was less than 50 mg (0.05g)

B. Volatile Solids

- **i.** Dry Samples were initially ignited in a 550° C furnace for 1 hour
- **ii.** Samples were then ignited in 30 minute increments at 550° C until the observed weight change was less than 50 mg (0.05g)

APPENDIX B: BIOCHEMICAL METHANE POTENTIAL TEST SETUP

I. Inoculum Preparation

- **A.** Stir both buckets of inocula using a spatula and a gloved arm
- **B.** Add 1000 ml of Inoculum A to a 3.8 L plastic container
- **C.** Add 1000 ml of Inoculum B to the same 3.8 L plastic container
- **D.** Purge the container's headspace with N gas
- **E.** Seal the container and shake until mixed
- **F.** Take a sample of inoculum and store it in the freezer at -20° C
- **G.** Use as much as is needed for the BMP test within the day and discard the remainder

II. Loading Digesters

- **A.** Maintaining a substrate to inoculum ratio of 1:1 on a VS basis, calculate the amount of each needed to achieve a combined weight of 100 g
- **B.** Weigh the appropriate amount of manure substrate into a clean weigh boat to within 0.01 g
- **C.** Record all weights on data paper
- **D.** Pour manure into an ANKOM digester bottle
- **E.** Rinse the residue with 10 ml of deionized distilled water (DDW) using a clean syringe
- **F.** Weigh the appropriate amount of inoculum into a 100 ml beaker to within 0.01 g
- **G.** Pour inoculum into an ANKOM digester bottle
- **H.** Rinse the residue with 10 ml of DDW using a clean syringe
- **I.** Fill all bottles to the working volume of 150 ml with DDW

III. Sealing Digesters

- **A.** Apply petroleum jelly to both sides of each digester's gasket and septum
- **B.** Flush each digester's headspace with N gas for 20 seconds
- **C.** Promptly seal each digester by hand tightening the ANKOM module on top

IV. Water Bath and Gas Production System Settings

A. Water bath settings:

i. 38° C

ii. 80 RPM

B. ANKOM RF Gas Production System settings:

i. Global release set to 13.8 kPa

ii. Valve open time set to 250 milliseconds

iii. Recording interval set to 60 minutes

iv. Live interval set to 10 seconds

V. Daily Digester Care

A. The following tasks need done a minimum of twice a day:

i. Check ANKOM batteries and replace any that are below 6.5 V

ii. Add distilled water to the water bath till it is at the fill mark

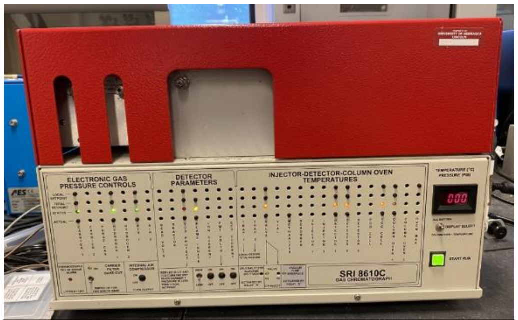
iii. Check recorded data for pressure, temperature, and battery voltage abnormalities

Digesters loaded with substrate, inoculum, and DDW before topping with ANKOM modules

Sealed digesters in the agitating water bath

APPENDIX C: GAS SAMPLING PROTOCOL

1. At least 1 hour before gas sampling, turn on the GC and open the gas tanks
2. Remove the correct modules from the water bath and roll them to the GC using a cart
3. Open Peaksimple on the GC laptop
4. Go to: Edit>Channels>Postrun
5. In "add to results log" change the log file name to show the correct date
6. Change the run file name to show the correct date and module #
7. Hit "OK" twice
8. Open a new 25-gauge needle and attach it to the gas sampling syringe
9. Pull 2.5 ml of gas into the syringe from the correct ANKOM bottle septum
10. Remove the needle from bottle and draw back the plunger to fill the syringe with 25 ml of ambient air
11. Push plunger forward to the 10 ml mark
12. Inject this gas into GC port
13. Hit the "space bar" on the laptop to start analyzing
14. After 2 minutes, the GC will sound to signal it is done analyzing that sample
15. Check to see if the results look normal (call Grant if you have questions)
16. Repeat steps 4-15 (you don't need to rename the results log file) until all bottles are analyzed
17. Check the log file to see if all of the results are there
18. Email the log file to Grant with the date in the subject line
19. When you are finished sampling:
    a) Shut off the GC
    b) Close the gas tanks
    c) Return each digester to its place in the water bath



Gas Chromatograph

Sampling gas from digester

Injecting gas sample into the GC

APPENDIX C: NASEM DIET REPORTS

Control

Report 1. Animal Inputs

1.1 Physiological State/Management

Item	Value	Unit
Animal Type	Lactating Cow	
Breed	Jersey	
Body Weight	439	kg
Mature Weight	450	kg
Age	42.0	months
Condition Score	3.00	(1-5)
Percent First Parity	0	(0-100)
Days in Milk	154	days
Age At First Calving	24	months
Days Pregnant	42	days
Temperature	20	deg C
In vitro NDF digest	Do not use	
Feeding Monensin	No	
Grazing	No	
Topography	Mild	Topography
Distance (Pasture to Parlor)	0.000	km
One-Way Trips	N/A	times/day

1.2 Entered Performance

Item	Value	Unit
Milk Production	29.5	kg
Milk Fat	5.25	%
Milk True Protein	3.41	%
Milk Lactose	4.71	%
Milk Fat	1.55	kg/d
Milk True Protein	1.01	kg/d
Milk Lactose	1.39	kg/d
Milk True Protein RHA	308	kg/305 d
Milk, Energy/Protein Corrected (ECM)	37.4	kg/d
Intake (Dry Matter)	18.20	kg/d
Estimated Intake Based on Animal (Dry Matter)	19.23	kg/d
Estimated Intake Based on Animal/Fiber (Dry Matter)	22.71	kg/d
Dry Matter Intake as Percent of Body Weight	4.15	% BW
ECM/DMI	2.06	kg/kg
Frame Gain	0.00	kg/d
Body Reserves Gain	-0.36	kg/d
Gravid Uterine Gain	0.00	kg/d
Total Body Gain	-0.36	kg/d

1.3 Predicted Production Variables

Item	Value	Unit
Milk, NEL Allowable	28.56	kg/d
Milk, MP Allowable	29.35	kg/d
Milk Production, Nutrient Predicted	25.6	kg
Milk True Protein, Nutrient Predicted	0.98	kg/day
Milk Fat, Nutrient Predicted	1.02	kg/day

Report 2. Diet Summary (DM Basis)

2.1 Macro-nutrients

Nutrient	Content
Dry Matter, %	53.1
Forage, % DM	57.1
CP, % DM	16.2
ME, Mcal/kg	2.73
MP, % DM	10.30
NEL, Mcal/kg	1.80
RDP, % DM	10.1
RUP, Base, % DM	6.1
Dig. RUP, % DM	5.0
ADF, % DM	18.3
NDF, % DM	29.1
ADF/NDF, Ratio	0.63
Forage NDF, % DM	22.1
Starch, % DM	28.4
WSC, % DM	3.2
Ash, % DM	9.0
Fatty Acids, % DM	5.05
Ca, % DM	0.85
P, % DM	0.40
Mg, % DM	0.34
K, % DM	1.46
Na, % DM	0.32
Cl, % DM	0.53
S, % DM	0.22
DCAD, mEq/kg	229
Cost, $/ton As Fed	0.00
Cost, $/day	0.00

2.2 Diet Ingredients

Ingredient	As Fed kg/d	% As Fed	DM kg/d	% of DM
01 *Corn silage (Updated After 2211DA Study)	22.201807	64.777045	7.371000	40.500000
02 *Alfalfa hay, (Updated After 2211DA Study)	3.362647	9.811018	3.023020	16.610000
03 Corn grain dry, fine grind	3.985249	11.627552	3.463460	19.030000
04 *DDGS, low fat (Dakota Gold) (Updated After 2211DA Study)	0.000000	0.000000	0.000000	0.000000
05 Soybean meal, solvent 48CP	1.557664	4.544714	1.390480	7.640000
06 *Soybean meal, expellers (soypass composition from NDS)	0.764580	2.230775	0.697060	3.830000
07 Soybean hulls	0.902226	2.632377	0.815360	4.480000
08 Calcium soaps	0.576747	1.682744	0.549640	3.020000
09 Blood meal, high dRUP	0.330494	0.964265	0.300300	1.650000
10 Sodium chloride (salt)	0.072800	0.212405	0.072800	0.400000
11 Sodium bicarbonate	0.091000	0.265506	0.091000	0.500000
12 Calcium carbonate	0.154700	0.451360	0.154700	0.850000
13 Calcium phosphate (di)	0.081900	0.238955	0.081900	0.450000
14 Magnesium oxide	0.054600	0.159304	0.054600	0.300000
15 Calcium sulfate (2H2O)	0.036400	0.106202	0.036400	0.200000
16 Rumen Protected Met	0.027857	0.081277	0.027300	0.150000
17 Rumen Protected Lys	0.055714	0.162554	0.054600	0.300000
18 VitTM Premix, generic	0.017804	0.051946	0.016380	0.090000
Totals	34.274	100.00	18.200	100.00

Report 3. Ingredient Macro-Nutrient Contributions (DM Basis)

Ingredient	Cost $/d	% of DM	BASE DE Mcal/d	CP kg/d	RDP g/d	RUP g/d	dRUP g/d	NDF kg/d	Starch kg/d	Fatty acids g/d
*Corn silage (Updated After 2211DA Study)	0.00	40.50	21.43	0.57	400	175	122	2.73	2.66	208
*Alfalfa hay, (Updated After 2211DA Study)	0.00	16.61	7.71	0.57	466	102	66	1.29	0.03	40
Corn grain dry, fine grind	0.00	19.03	12.27	0.29	175	120	88	0.34	2.44	133
*DDGS, low fat (Dakota Gold) (Updated After 2211DA Study)	0.00	0.00	0.00	0.00	0	0	0	0.00	0.00	0
Soybean meal, solvent 48CP	0.00	7.64	5.53	0.73	511	221	201	0.15	0.03	15
*Soybean meal, expellers (soypass composition from NDS)	0.00	3.83	2.65	0.33	129	199	185	0.22	0.00	43
Soybean hulls	0.00	4.48	2.24	0.10	63	33	23	0.54	0.01	13
Calcium soaps	0.00	3.02	2.98	0.00	0	0	0	0.00	0.00	464
Blood meal, high dRUP	0.00	1.65	1.36	0.29	80	205	175	0.00	0.00	4
Sodium chloride (salt)	0.00	0.40	0.00	0.00	0	0	0	0.00	0.00	0
Sodium bicarbonate	0.00	0.50	0.00	0.00	0	0	0	0.00	0.00	0
Calcium carbonate	0.00	0.85	0.00	0.00	0	0	0	0.00	0.00	0
Calcium phosphate (di)	0.00	0.45	0.00	0.00	0	0	0	0.00	0.00	0
Magnesium oxide	0.00	0.30	0.00	0.00	0	0	0	0.00	0.00	0
Calcium sulfate (2H2O)	0.00	0.20	0.00	0.00	0	0	0	0.00	0.00	0
Rumen Protected Met	0.00	0.15	0.00	0.02	5	15	14	0.00	0.00	0
Rumen Protected Lys	0.00	0.30	0.00	0.04	11	30	27	0.00	0.00	0
VitTM Premix, generic	0.00	0.09	0.00	0.00	2	1	1	0.00	0.00	0
Totals	0.00	100.00	56.17	2.94	1843	1102	901	5.29	5.17	920

Report 4. Energy

4.1 Energy Supply

Energy	Mcal/d	Mcal/kg	% of GE	% of DE	% of ME
GE	78.33	4.30	100.0		
DE	55.89	3.07	71.4	100.0	
Urinary E	1.98	0.11	2.5	3.5	
Gaseous E	4.21	0.23	5.4	7.5	
ME	49.70	2.73	63.4	88.9	100.0
NEL	32.80	1.80	41.9	58.7	66.0

4.2 NEL and ME Requirements

Requirement	ME Mcal/d	NEL Mcal/d	NE:DEIn Fraction	NE:ME Efficiency
Maintenance	14.52	9.59	0.17	0.66
Milk Production, User Entered	39.03	25.76	0.46	0.66
Pregnancy	0.03	0.02	0.00	0.14
Grazing Activity	0.00	0.00	0.00	0.66
Frame Gain	0.00	0.00	0.00	0.40
Reserves Gain	-2.65	-1.75	-0.04	0.89
Total Req, User Entered	50.94	33.62		
Balance (intake - required)	-1.24	-0.82		

4.3 Nutrient Contributions to DE

Nutrient	Intake kg/d	Base Digest %	Intake Adjusted Digest %	Truly Digested kg/d	Endog. Fecal kg/d	Apparently Digested kg/d	Apparently Digested %	Heat of combust Mcal/kg	DE Mcal/d
NDF	5.29	53.90	51.77	2.74	0.00	2.74	51.77	4.20	11.51
Starch	5.17	90.44	89.79	4.64	0.00	4.64	89.79	4.23	19.63
FA	0.92	73.00	73.00	0.67	0.00	0.67	73.00	9.40	6.31
rOM	2.27	96.00	96.00	2.18	0.62	1.55	68.46	4.00	6.21
CP	2.94	93.19	93.19	2.74	0.58	2.17	73.58	5.65	12.24
OM	16.56	79.19	78.31	12.97	1.20	11.77	71.05	4.75	55.89

Report 5. Fatty Acid Supply

Fatty Acid	Profile % of Total FA	Concentration % of DM	Intake g/d
C12:0	0.23	0.01	2.1
C14:0	1.33	0.07	12.2
C16:0	33.80	1.71	311.0
C16:1	0.21	0.01	1.9
C18:0	3.51	0.18	32.3
C18:1 trans	0.12	0.01	1.1
C18:1 cis	27.35	1.38	251.6
C18:2	27.26	1.38	250.8
C18:3	4.55	0.23	41.9
Others	1.59	0.08	14.6
Saturated Fatty Acids	40.47	2.05	372.3
Mono-Unsaturated Fatty Acids	27.68	1.40	254.7
Poly-Unsaturated Fatty Acids	31.81	1.61	292.7
Fatty Acids	100.00	5.05	920.0

Report 6. Protein and Amino Acid Supply and Requirements

6.1 Protein Supply

Item	Value	Unit
DE from Non-Protein Components	43.65	Mcal/d
Ruminal Digestion and Outflow		
Rumen Digested Starch	3.57	kg/d
Rumen Digested NDF	1.71	kg/d
Microbial Protein (MiCP)	1.48	kg/d
RDP - MiCP Balance	0.37	kg/d
Rumen Undegraded Protein	1.10	kg/d
Metabolized Protein Supply	1.87	kg/d
MP from Microbial CP	0.97	kg/d
MP from RUP	0.90	kg/d
MP from Body Weight Loss	0.03	kg/d
MP Supply / ME Supply	37.72	g/Mcal
MP Use / ME Use g/Mcal (a)	44.38	g/Mcal
CP Supply / ME Supply	60.84	g/Mcal
CP Use / ME Use g/Mcal (a)	71.57	g/Mcal

(a) MP and ME use are calculated using Target MP to NP efficiencies and predicted ME to NE efficiencies at user entered inputs and production.

6.2 NP, CP, and MP Supply and Use, g/d

Item	Net TP	CP	MP (a)
Scurf, g/d	7	8	10
Endogenous Urinary, g/d	145	N/A	145
Metabolic Fecal, g/d	206	282	298
Frame Growth, g/d	0	0	0
Body Reserves, g/d	-21	-44	-31
Pregnancy, g/d	1	1	2
Lactation, User Entered, g/d	1006	N/A	1458
Total Required at User Entered, g/d	1343	N/A	1882
Supply, g/d	N/A	2944	1875
Supply - Required at User Entered, g/d	N/A	N/A	-7
Required Eff at User Entered, g/g	N/A	N/A	0.69
Lactation, Nutrient Allowable, g/d	983	N/A	1447
Total Required at Nutrient Allowable, g/d	1320	1446	1875
Supply - Required at Nutrient Allowable	N/A	N/A	0
Predicted Eff at Nutr Allowable g/g (b)	N/A	N/A	0.68

(a) MP efficiency of 0.69 is used for all functions except for endogenous urinary and pregnancy, which are 1 and 0.33, respectively.
(b) Calculated using predicted MP efficiency for nutrient allowable milk protein production.

6.3 Predicted Milk Protein based on Supplied Amino Acids

Item	Independent Var	Regression Coeff (a)	Predicted Milk Protein, g/d
Intercept	N/A	N/A	-97.00
(BW - 612), kg (b)	-173.30	-0.42010	72.80
(Digested NDF - 17.06), % DM (c)	-2.01	-4.59500	9.22
Non-protein DEIn, Mcal/d	43.65	10.79000	471.03
Absorbed Arg, g/d	102.52	0.00000	0.00
Absorbed His, g/d	50.61	1.66663	84.34
Absorbed Ile, g/d	101.62	0.88058	89.48
Absorbed Leu, g/d	172.76	0.46367	80.10
Absorbed Lys, g/d	173.96	1.14724	199.58
Absorbed Met, g/d	52.63	1.82981	96.29
Absorbed Phe, g/d	108.32	0.00000	0.00
Absorbed Thr, g/d	96.88	0.00000	0.00
Absorbed Trp, g/d	24.77	0.00000	0.00
Absorbed Val, g/d	118.45	0.00000	0.00
Squared EAA, g^2/d (d)	75765.43	-0.00194	-146.61
Absorbed Other AA, g/d (e)	1604.45	0.07730	124.02
Nutr Allow Milk NP, g/d	N/A	N/A	983.26
Nutr Allow Milk NP / User Enter Max NP (305d RHA) (f)	N/A	N/A	0.59

(a) Regression coefficient from Eqn. 6.6 adjusted based on user entered Rolling Herd Average Protein,
(b) centered to 612 kg,
(c) centered to 17.06% of DM.
(d) the sum of the squared supplies of each EAA with non-zero coefficients,
(e) includes all AA other than the EAA with non-zero coefficients,
(f) nutrient allowable NP production as a proportion of the maximum calculated from the user entered 305d RHA milk protein. This ratio should not be greater than 0.80 under normal feeding conditions.

6.4 Duodenal AA Flows, g/d (a)

Item	Diet	Duodenal flow: RUP	Duodenal flow: MiCP	Duodenal flow: Endog	Duodenal flow: Total: True	Duodenal flow: Total: 24h Hydr	metabolizable EAA: From RUP	metabolizable EAA: From MiCP	metabolizable EAA: Total	Targ Supp at User Enter
Arg	154	58	67	11	136	128	49	53	103	N/A
His	82	35	27	7	69	64	29	22	51	49
Ile	119	41	85	10	135	121	34	68	102	103
Leu	261	102	112	18	232	218	83	90	173	172
Lys	202	96	115	15	225	211	82	92	174	145
Met	65	32	32	3	67	63	27	26	53	47
Phe	148	57	77	9	143	135	47	61	108	107
Thr	119	44	76	12	132	124	36	61	97	97
Trp	37	14	17	3	33	32	11	13	25	24
Val	161	63	84	12	159	144	11	67	118	114

(a)All flows include a correction to account for incomplete recovery of AA during a 24-h hydrolysis, except 'Total: 24h Hydr'; Target supply calculated using target efficiencies and net use detailed in Table 6.5.

6.5 Partition of net EAA utilization (g/d) and efficiency of utilization of EAA (a)

Item	Urine Endo.	Metab. Fecal	Scurf	Gest	Body Gain	Milk, Nutr. Allow	Milk, User Enter	Total, Nutr. Allow	Total, User Enter	Target Eff (b)	Nutr Allow Eff (b)	User Enter Eff (b)
Arg	2	12	1	0	-2	37	38	50	51	N/A	0.48	N/A
His	1	7	0	0	-1	29	29	37	37	0.75	0.72	0.73
Ile	1	11	0	0	-1	61	62	72	74	0.71	0.71	0.72
Leu	2	19	0	0	-2	104	106	124	126	0.73	0.71	0.73
Lys	2	16	0	0	-2	87	89	103	105	0.72	0.59	0.60
Met	1	4	0	0	-1	30	30	34	34	0.73	0.63	0.65
Phe	1	11	0	0	-1	52	53	63	64	0.60	0.58	0.59
Thr	1	15	0	0	-1	45	46	61	62	0.64	0.63	0.64
Trp	0	4	0	0	0	16	17	20	20	0.86	0.81	0.82
Val	1	14	0	0	-1	68	69	83	84	0.74	0.70	0.71

(a) Corrected for incomplete recovery of AA during a 24-h hydrolysis.
(b) Efficiencies for Urine endogenous and gestation are 1 and 0.33. Combined efficiencies calculated from MP supply for other functions. A target efficiency was not estimated for Arg due to semi-essentiality.

Report 7. Mineral and Vitamin Supply and Requirements

7.1 Minerals

Item	Diet Density	Req Density	AC	Absorb Req (TAR)	Diet Supply (TDS)	Absorb Supp (TAS)	**Diff TAS-TAR**	Metab Fecal	Urine	Preg	Milk	Growth
Macro Mineral	% DM	% DM	g/100g	g/d	g/d	g/d	**g/d**	g/d	g/d	g/d	g/d	g/d
Ca	0.85	0.51	48.7	46	155	75	**30**	16	N/A	0	33	-4
P	0.40	0.33	76.1	45	73	56	**10**	18	0	0	29	-2
Mg	0.34	0.19	25.6	9	62	16	**7**	5	0	0	3	0
Cl	0.53	0.29	92.0	49	96	88	**39**	20	N/A	0	30	0
K	1.46	0.97	100.0	177	265	265	**88**	46	88	0	44	-1
Na	0.32	0.21	100.0	38	58	58	**20**	26	N/A	0	12	-1
S	0.22	0.20	N/A	36	39	N/A	**3**	N/A	N/A	N/A	N/A	N/A
Micro Mineral (a)	mg/kg	mg/kg	g/100g	mg/d	mg/d	mg/d	**mg/d**	Maint. mg/d		mg/d	mg/d	mg/d
Co	0.00	0.20	0.0	4	0	0	**-4**	N/A		N/A	N/A	N/A
Cr	0.23	N/A	N/A	N/A	N/A	N/A	**N/A**	N/A		N/A	N/A	N/A
Cu	7.65	7.49	5.0	7	139	7	**0**	6		0	1	-1
Fe	209.66	9.47	10.0	17	3816	382	**364**	0		0	30	-12
I	0.07	0.46	N/A	8	1	N/A	**-7**	N/A		N/A	N/A	N/A
Mn	26.75	23.56	0.4	2	487	2	**0**	1		0	1	0
Se	0.15	0.30	N/A	5	3	N/A	**-3**	N/A		N/A	N/A	N/A
Zn	29.71	55.04	20.0	200	541	108	**-92**	91		0	118	-9

(a) For S, Co, I, and Se required is based on diet concentration and not absorbed amounts.

7.2 Vitamin Supply and Requirements

Item	Diet Density	Required Density	Diet Supply	Required	Supply - Required
Fat-soluble Vitamins	IU/kg	IU/kg	IU/d	IU/d	IU/d
A	504.0	2651.5	9173	48257	-39084
D	180.0	964.2	3276	17548	-14272
E	3.6	19.3	66	351	-285
Other Vitamins	mg/kg	mg/kg	mg/d	mg/d	mg/d
Beta Carotene	0.00	N/A	0	N/A	N/A
Biotin	0.00	N/A	0	N/A	N/A
Choline	0.00	N/A	0	N/A	N/A
Niacin	0.00	N/A	0	N/A	N/A

Report 8. Environmental Impact

8.1 Water, Volatile Solids, and Methane

Item	Value	Unit
Water Intake	81.8	kg/d
Wet Manure Output	55.3	kg/d
Manure Volatile Solids	6.12	kg/d
Enteric Methane Production	318	g/d
Enteric Methane Production	475	L/d
Water Intake	3.2	L H2O/kg Milk
Manure Water	1.9	L H2O/kg Milk
Manure Volatile Solids	1.9	kg/kg Milk
Enteric Methane Production	12	g CH4/kg Milk
Enteric Methane Production	19	L CH4/kg Milk

8.2 Nitrogen and Mineral Excretion

Item	Intake	Retained in Milk, Growth & Conceptus	Fecal & Urinary	Retained/Intake
Nitrogen and Macro-minerals	g/d	g/d	g/d	g/g
Nitrogen	471	159	264	0.34
Ca	155	29	126	0.19
P	73	27	46	0.37
Mg	62	3	59	0.05
Cl	96	29	66	0.30
K	265	43	222	0.16
Na	58	11	47	0.20
Micro-minerals	mg/d	mg/d	mg/d	g/g
Cu	139	0	139	0.00
Fe	3816	17	3799	0.00
Mn	487	1	486	0.00
Zn	541	109	431	0.20

Report 9. Ingredient Mineral Contributions

Ingredient	Ca g/d	P g/d	Mg g/d	Cl g/d	K g/d	Na g/d	S g/d	Co mg/d	Cu mg/d	I mg/d	Fe mg/d	Mn mg/d	Se mg/d	Zn mg/d
*Corn silage (Updated After	15	18	11	22	81	1	7	0	37	0	899	170	0	162
*Alfalfa hay, (Updated After	37	10	7	23	103	1	7	0	31	0	1300	129	1	80
Corn grain dry, fine grind	1	11	5	4	19	1	4	0	7	0	135	25	0	81
*DDGS, low fat (Dakota Gold)	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Soybean meal, solvent 48CP	6	10	5	1	34	0	6	0	22	0	260	58	0	74
*Soybean meal, expellers (so	3	6	2	0	15	0	3	0	8	0	146	22	0	30
Soybean hulls	5	1	2	0	11	0	1	0	6	0	378	17	0	39
Calcium soaps	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Blood meal, high dRUP	0	1	0	1	1	1	2	0	2	0	681	1	0	10
Sodium chloride (salt)	0	0	0	44	0	29	0	0	0	0	0	0	0	0
Sodium bicarbonate	0	0	0	0	0	25	0	0	0	0	0	0	0	0
Calcium carbonate	61	0	0	0	0	0	0	0	0	0	0	0	0	0
Calcium phosphate (di)	18	16	0	0	0	0	0	0	0	0	0	0	0	0
Magnesium oxide	0	0	31	0	0	0	0	0	0	0	0	0	0	0
Calcium sulfate (2H2O)	8	0	0	0	0	0	9	0	0	0	0	0	0	0
Rumen Protected Met	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Rumen Protected Lys	0	0	0	0	0	0	0	0	0	0	0	0	0	0
VitTM Premix, generic	0	0	0	0	0	0	0	0	26	1	16	66	1	66
Totals	155	73	62	96	265	58	39	0	139	1	3816	487	3	541

R-Alf

Report 1. Animal Inputs

1.1 Physiological State/Management

Item	Value	Unit
Animal Type	Lactating Cow	
Breed	Jersey	
Body Weight	446	kg
Mature Weight	450	kg
Age	42.0	months
Condition Score	2.90	(1-5)
Percent First Parity	0	(0-100)
Days in Milk	154	days
Age At First Calving	24	months
Days Pregnant	42	days
Temperature	20	deg C
In vitro NDF digest	Do not use	
Feeding Monensin	No	
Grazing	No	
Topography	Mild	Topography
Distance (Pasture to Parlor)	0.000	km
One-Way Trips	N/A	times/day

1.2 Entered Performance

Item	Value	Unit
Milk Production	31.5	kg
Milk Fat	5.15	%
Milk True Protein	3.59	%
Milk Lactose	4.73	%
Milk Fat	1.62	kg/d
Milk True Protein	1.13	kg/d
Milk Lactose	1.49	kg/d
Milk True Protein RHA	345	kg/305 d
Milk, Energy/Protein Corrected (ECM)	40.0	kg/d
Intake (Dry Matter)	19.40	kg/d
Estimated Intake Based on Animal (Dry Matter)	20.19	kg/d
Estimated Intake Based on Animal/Fiber (Dry Matter)	22.96	kg/d
Dry Matter Intake as Percent of Body Weight	4.35	% BW
ECM/DMI	2.06	kg/kg
Frame Gain	0.00	kg/d
Body Reserves Gain	-0.31	kg/d
Gravid Uterine Gain	0.00	kg/d
Total Body Gain	-0.31	kg/d

1.3 Predicted Production Variables

Item	Value	Unit
Milk, NEL Allowable	31.29	kg/d
Milk, MP Allowable	30.12	kg/d
Milk Production, Nutrient Predicted	27.2	kg
Milk True Protein, Nutrient Predicted	1.05	kg/day
Milk Fat, Nutrient Predicted	1.05	kg/day

Report 2. Diet Summary (DM Basis)

2.1 Macro-nutrients

Nutrient	Content
Dry Matter, %	52.6
Forage, % DM	49.9
CP, % DM	16.4
ME, Mcal/kg	2.78
MP, % DM	10.39
NEL, Mcal/kg	1.84
RDP, % DM	9.8
RUP, Base, % DM	6.6
Dig. RUP, % DM	5.2
ADF, % DM	15.5
NDF, % DM	28.2
ADF/NDF, Ratio	0.55
Forage NDF, % DM	19.0
Starch, % DM	29.5
WSC, % DM	2.5
Ash, % DM	8.9
Fatty Acids, % DM	5.79
Ca, % DM	0.89
P, % DM	0.39
Mg, % DM	0.32
K, % DM	1.43
Na, % DM	0.34
Cl, % DM	0.69
S, % DM	0.27
DCAD, mEq/kg	148
Cost, $/ton As Fed	0.00
Cost, $/day	0.00

2.2 Diet Ingredients

Ingredient	As Fed kg/d	% As Fed	DM kg/d	% of DM
01 *Corn silage (Updated After 2211DA Study)	24.257274	65.774563	8.053415	41.512446
02 *Alfalfa hay, (Updated After 2211DA Study	1.804590	4.893218	1.622326	8.362505
03 Corn grain dry, fine grind	4.242587	11.503943	3.687105	19.005695
04 *DDGS, low fat (Dakota Gold) (Updated After 2211DA Study)	2.787576	7.558623	2.522756	13.003896
05 Soybean meal, solvent 48CP	1.117388	3.029842	0.997459	5.141541
06 *Soybean meal, expellers (soypass composition from NDS)	0.495955	1.344802	0.452157	2.330706
07 Soybean hulls	0.465971	1.263499	0.421107	2.170655
08 Calcium soaps	0.614959	1.667486	0.586056	3.020907
09 Blood meal, high dRUP	0.352391	0.955522	0.320197	1.650500
10 Sodium chloride (salt)	0.077623	0.210478	0.077623	0.400119
11 Sodium bicarbonate	0.097029	0.263098	0.097029	0.500149
12 Calcium carbonate	0.302731	0.820867	0.302731	1.560469
13 Calcium phosphate (di)	0.029110	0.078933	0.029110	0.150052
14 Magnesium oxide	0.048515	0.131550	0.048515	0.250077
15 Calcium sulfate (2H2O)	0.000000	0.000000	0.000000	0.000000
16 Rumen Protected Met	0.029704	0.080544	0.029110	0.150052
17 Rumen Protected Lys	0.059405	0.161079	0.058217	0.300088
18 VitTM Premix, generic	0.018984	0.051476	0.017465	0.090026
19 Potassium chloride	0.077623	0.210478	0.077623	0.400119
Totals	36.879	100.00	19.400	100.00

Report 3. Ingredient Macro-Nutrient Contributions (DM Basis)

Ingredient	Cost $/d	% of DM	BASE DE Mcal/d	CP kg/d	RDP g/d	RUP g/d	dRUP g/d	NDF kg/d	Starch kg/d	Fatty acids g/d
*Corn silage (Updated After 2211DA Study)	0.00	41.51	23.41	0.63	437	191	134	2.99	2.91	227
*Alfalfa hay, (Updated After 2211DA Study	0.00	8.36	4.14	0.30	250	55	36	0.69	0.02	21
Corn grain dry, fine grind	0.00	19.01	13.07	0.31	186	128	93	0.36	2.59	142
*DDGS, low fat (Dakota Gold) (Updated After 2211DA Study)	0.00	13.00	8.81	0.77	439	331	248	0.88	0.18	188
Soybean meal, solvent 48CP	0.00	5.14	3.96	0.53	367	159	144	0.11	0.02	11
*Soybean meal, expellers (soypass composition from NDS)	0.00	2.33	1.72	0.21	84	129	120	0.14	0.00	28
Soybean hulls	0.00	2.17	1.16	0.05	33	17	12	0.28	0.00	7
Calcium soaps	0.00	3.02	3.17	0.00	0	0	0	0.00	0.00	495
Blood meal, high dRUP	0.00	1.65	1.45	0.30	85	219	186	0.00	0.00	4
Sodium chloride (salt)	0.00	0.40	0.00	0.00	0	0	0	0.00	0.00	0
Sodium bicarbonate	0.00	0.50	0.00	0.00	0	0	0	0.00	0.00	0
Calcium carbonate	0.00	1.56	0.00	0.00	0	0	0	0.00	0.00	0
Calcium phosphate (di)	0.00	0.15	0.00	0.00	0	0	0	0.00	0.00	0
Magnesium oxide	0.00	0.25	0.00	0.00	0	0	0	0.00	0.00	0
Calcium sulfate (2H2O)	0.00	0.00	0.00	0.00	0	0	0	0.00	0.00	0
Rumen Protected Met	0.00	0.15	0.00	0.02	6	16	15	0.00	0.00	0
Rumen Protected Lys	0.00	0.30	0.00	0.04	11	32	29	0.00	0.00	0
VitTM Premix, generic	0.00	0.09	0.00	0.00	2	1	1	0.00	0.00	0
Potassium chloride	0.00	0.40	0.00	0.00	0	0	0	0.00	0.00	0
Totals	0.00	100.00	60.90	3.18	1900	1277	1017	5.47	5.73	1123

Report 4. Energy

4.1 Energy Supply

Energy	Mcal/d	Mcal/kg	% of GE	% of DE	% of ME
GE	84.38	4.35	100.0		
DE	60.41	3.11	71.6	100.0	
Urinary E	2.12	0.11	2.5	3.5	
Gaseous E	4.30	0.22	5.1	7.1	
ME	53.98	2.78	64.0	89.4	100.0
NEL	35.63	1.84	42.2	59.0	66.0

4.2 NEL and ME Requirements

Requirement	ME Mcal/d	NEL Mcal/d	NE:DEIn Fraction	NE:ME Efficiency
Maintenance	14.70	9.70	0.16	0.66
Milk Production, User Entered	41.77	27.57	0.46	0.66
Pregnancy	0.03	0.02	0.00	0.14
Grazing Activity	0.00	0.00	0.00	0.66
Frame Gain	0.00	0.00	0.00	0.40
Reserves Gain	-2.25	-1.48	-0.03	0.89
Total Req, User Entered	54.26	35.81		
Balance (intake - required)	-0.28	-0.18		

4.3 Nutrient Contributions to DE

Nutrient	Intake kg/d	Base Digest %	Intake Adjusted Digest %	Truly Digested kg/d	Endog. Fecal kg/d	Apparently Digested kg/d	Apparently Digested %	Heat of combust Mcal/kg	DE Mcal/d
NDF	5.47	55.69	52.67	2.88	0.00	2.88	52.67	4.20	12.09
Starch	5.73	90.44	89.59	5.13	0.00	5.13	89.59	4.23	21.70
FA	1.12	73.00	73.00	0.82	0.00	0.82	73.00	9.40	7.70
rOM	2.21	96.00	96.00	2.12	0.67	1.46	65.90	4.00	5.83
CP	3.18	91.81	91.81	2.92	0.60	2.32	72.88	5.65	13.08
OM	17.67	79.70	78.50	13.87	1.27	12.60	71.32	4.79	60.41

Report 5. Fatty Acid Supply

Fatty Acid	Profile % of Total FA	Concentration % of DM	Intake g/d
C12:0	0.20	0.01	2.2
C14:0	1.16	0.07	13.0
C16:0	31.13	1.80	349.5
C16:1	0.16	0.01	1.8
C18:0	3.28	0.19	36.8
C18:1 trans	0.07	0.00	0.8
C18:1 cis	27.60	1.60	309.8
C18:2	31.68	1.83	355.7
C18:3	3.33	0.19	37.4
Others	1.35	0.08	15.2
Saturated Fatty Acids	37.12	2.15	416.7
Mono-Unsaturated Fatty Acids	27.83	1.61	312.5
Poly-Unsaturated Fatty Acids	35.02	2.03	393.1
Fatty Acids	100.00	5.79	1122.6

Report 6. Protein and Amino Acid Supply and Requirements

6.1 Protein Supply

Item	Value	Unit
DE from Non-Protein Components	47.33	Mcal/d
Ruminal Digestion and Outflow		
Rumen Digested Starch	3.78	kg/d
Rumen Digested NDF	1.97	kg/d
Microbial Protein (MiCP)	1.52	kg/d
RDP - MiCP Balance	0.38	kg/d
Rumen Undegraded Protein	1.28	kg/d
Metabolized Protein Supply	2.02	kg/d
MP from Microbial CP	1.00	kg/d
MP from RUP	1.02	kg/d
MP from Body Weight Loss	0.03	kg/d
MP Supply / ME Supply	37.34	g/Mcal
MP Use / ME Use g/Mcal (a)	45.55	g/Mcal
CP Supply / ME Supply	60.23	g/Mcal
CP Use / ME Use g/Mcal (a)	73.47	g/Mcal

(a) MP and ME use are calculated using Target MP to NP efficiencies and predicted ME to NE efficiencies at user entered inputs and production.

6.2 NP, CP, and MP Supply and Use, g/d

Item	Net TP	CP	MP (a)
Scurf, g/d	7	8	10
Endogenous Urinary, g/d	148	N/A	148
Metabolic Fecal, g/d	218	298	316
Frame Growth, g/d	0	0	0
Body Reserves, g/d	-18	-37	-26
Pregnancy, g/d	1	1	2
Lactation, User Entered, g/d	1131	N/A	1639
Total Required at User Entered, g/d	1486	N/A	2087
Supply, g/d	N/A	3177	2016
Supply - Required at User Entered, g/d	N/A	N/A	-72
Required Eff at User Entered, g/g	N/A	N/A	0.72
Lactation, Nutrient Allowable, g/d	1046	N/A	1559
Total Required at Nutrient Allowable, g/d	1401	1535	2017
Supply - Required at Nutrient Allowable	N/A	N/A	-1
Predicted Eff at Nutr Allowable g/g (b)	N/A	N/A	0.67

(a) MP efficiency of 0.69 is used for all functions except for endogenous urinary and pregnancy, which are 1 and 0.33, respectively.
(b) Calculated using predicted MP efficiency for nutrient allowable milk protein production.

6.3 Predicted Milk Protein based on Supplied Amino Acids

Item	Independent Var	Regression Coeff (a)	Predicted Milk Protein, g/d
Intercept	N/A	N/A	-97.00
(BW - 612), kg (b)	-166.30	-0.42010	69.86
(Digested NDF - 17.06), % DM (c)	-2.22	-4.59500	10.20
Non-protein DEIn, Mcal/d	47.33	10.79000	510.64
Absorbed Arg, g/d	96.95	0.00000	0.00
Absorbed His, g/d	49.98	1.65784	82.86
Absorbed Ile, g/d	99.06	0.87593	86.77
Absorbed Leu, g/d	175.32	0.46122	80.86
Absorbed Lys, g/d	171.94	1.14118	196.22
Absorbed Met, g/d	54.03	1.82015	98.35
Absorbed Phe, g/d	107.17	0.00000	0.00
Absorbed Thr, g/d	95.86	0.00000	0.00
Absorbed Trp, g/d	24.00	0.00000	0.00
Absorbed Val, g/d	117.18	0.00000	0.00
Squared EAA, g^2/d (d)	75530.19	-0.00171	-129.11
Absorbed Other AA, g/d (e)	1767.87	0.07730	136.66
Nutr Allow Milk NP, g/d	N/A	N/A	1046.31
Nutr Allow Milk NP / User Enter Max NP (305d RHA) (f)	N/A	N/A	0.57

(a) Regression coefficient from Eqn. 6.6 adjusted based on user entered Rolling Herd Average Protein,
(b) centered to 612 kg,
(c) centered to 17.06% of DM.
(d) the sum of the squared supplies of each EAA with non-zero coefficients,
(e) includes all AA other than the EAA with non-zero coefficients,
(f) nutrient allowable NP production as a proportion of the maximum calculated from the user entered 305d RHA milk protein. This ratio should not be greater than 0.80 under normal feeding conditions.

6.4 Duodenal AA Flows, g/d (a)

		Duodenal flow					metabolizable EAA			
Item	Diet	RUP	MiCP	Endog	Total: True	Total: 24h Hydr	From RUP	From MiCP	Total	Targ Supp at User Enter
Arg	129	51	68	11	130	123	42	55	97	N/A
His	76	34	28	7	69	64	28	22	50	55
Ile	101	36	87	10	134	119	29	70	99	115
Leu	253	104	115	19	238	223	83	92	175	192
Lys	178	91	118	15	224	210	78	94	172	162
Met	64	33	33	3	69	65	28	26	54	52
Phe	133	55	79	10	143	135	44	63	107	119
Thr	105	42	78	13	132	124	34	62	96	107
Trp	31	13	17	3	33	31	10	14	24	26
Val	144	60	86	13	159	144	10	69	117	127

(a)All flows include a correction to account for incomplete recovery of AA during a 24-h hydrolysis, except 'Total: 24h Hydr'; Target supply calculated using target efficiencies and net use detailed in Table 6.5.

6.5 Partition of net EAA utilization (g/d) and efficiency of utilization of EAA (a)

Item	Urine Endo.	Metab. Fecal	Scurf	Gest	Body Gain	Milk, Nutr. Allow	Milk, User Enter	Total, Nutr. Allow	Total, User Enter	Target Eff (b)	Nutr Allow Eff (b)	User Enter Eff (b)
Arg	2	13	1	0	-1	39	42	53	57	N/A	0.54	N/A
His	1	8	0	0	-1	31	33	39	41	0.75	0.77	0.83
Ile	1	12	0	0	-1	65	70	77	82	0.71	0.78	0.83
Leu	2	20	0	0	-1	110	119	132	141	0.73	0.75	0.80
Lys	2	17	0	0	-1	92	100	110	118	0.72	0.64	0.68
Met	1	4	0	0	0	32	34	36	38	0.73	0.66	0.71
Phe	1	12	0	0	-1	55	59	67	72	0.60	0.62	0.67
Thr	1	16	0	0	-1	48	52	65	69	0.64	0.68	0.72
Trp	0	4	0	0	0	17	19	21	23	0.86	0.89	0.95
Val	1	15	0	0	-1	72	78	88	94	0.74	0.75	0.80

(a) Corrected for incomplete recovery of AA during a 24-h hydrolysis.

(b) Efficiencies for Urine endogenous and gestation are 1 and 0.33. Combined efficiencies calculated from MP supply for other functions. A target efficiency was not estimated for Arg due to semi-essentiality.

Report 7. Mineral and Vitamin Supply and Requirements

7.1 Minerals

Item	Diet Density	Req Density	AC	Absorb Req (TAR)	Diet Supply (TDS)	Absorb Supp (TAS)	**Diff TAS-TAR**	Metab Fecal	Urine	Preg	Milk	Growth
Macro Mineral	% DM	% DM	g/100g	g/d	g/d	g/d	**g/d**	g/d	g/d	g/d	g/d	g/d
Ca	0.89	0.54	48.3	51	174	84	**33**	17	N/A	0	36	-3
P	0.39	0.33	75.8	49	76	58	**9**	19	0	0	31	-2
Mg	0.32	0.19	26.2	9	63	16	**7**	6	0	0	3	0
Cl	0.69	0.30	92.0	53	134	123	**71**	22	N/A	0	32	0
K	1.43	0.95	100.0	184	277	277	**93**	49	89	0	47	-1
Na	0.34	0.21	100.0	40	66	66	**26**	28	N/A	0	13	0
S	0.27	0.20	N/A	39	53	N/A	**14**	N/A	N/A	N/A	N/A	N/A
Micro Mineral (a)	mg/kg	mg/kg	g/100g	mg/d	mg/d	mg/d	**mg/d**	Maint. mg/d		mg/d	mg/d	mg/d
Co	0.00	0.20	0.0	4	0	0	**-4**	N/A		N/A	N/A	N/A
Cr	0.19	N/A	N/A	N/A	N/A	N/A	**N/A**	N/A		N/A	N/A	N/A
Cu	6.87	7.33	5.0	7	133	7	**0**	6		0	1	-1
Fe	167.25	10.86	10.0	21	3245	324	**303**	0		0	32	-10
I	0.07	0.44	N/A	9	1	N/A	**-7**	N/A		N/A	N/A	N/A
Mn	23.34	23.44	0.4	2	453	2	**0**	1		0	1	0
Se	0.17	0.30	N/A	6	3	N/A	**-2**	N/A		N/A	N/A	N/A
Zn	32.89	55.58	20.0	216	638	128	**-88**	97		0	126	-7

(a) For S, Co, I, and Se required is based on diet concentration and not absorbed amounts.

7.2 Vitamin Supply and Requirements

Item	Diet Density	Required Density	Diet Supply	Required	Supply - Required
Fat-soluble Vitamins	IU/kg	IU/kg	IU/d	IU/d	IU/d
A	504.1	2527.2	9780	49027	-39247
D	180.1	919.0	3493	17828	-14335
E	3.6	18.4	70	357	-287
Other Vitamins	mg/kg	mg/kg	mg/d	mg/d	mg/d
Beta Carotene	0.00	N/A	0	N/A	N/A
Biotin	0.00	N/A	0	N/A	N/A
Choline	0.00	N/A	0	N/A	N/A
Niacin	0.00	N/A	0	N/A	N/A

Report 8. Environmental Impact

8.1 Water, Volatile Solids, and Methane

Item	Value	Unit
Water Intake	85.6	kg/d
Wet Manure Output	59.2	kg/d
Manure Volatile Solids	6.52	kg/d
Enteric Methane Production	324	g/d
Enteric Methane Production	486	L/d
Water Intake	3.1	L H2O/kg Milk
Manure Water	2.0	L H2O/kg Milk
Manure Volatile Solids	2.0	kg/kg Milk
Enteric Methane Production	12	g CH4/kg Milk
Enteric Methane Production	18	L CH4/kg Milk

8.2 Nitrogen and Mineral Excretion

Item	Intake	Retained in Milk, Growth & Conceptus	Fecal & Urinary	Retained/Intake
Nitrogen and Macro-minerals	g/d	g/d	g/d	g/g
Nitrogen	508	170	288	0.34
Ca	174	33	140	0.19
P	76	29	47	0.38
Mg	63	3	59	0.05
Cl	134	31	103	0.23
K	277	46	230	0.17
Na	66	12	54	0.18
Micro-minerals	mg/d	mg/d	mg/d	g/g
Cu	133	1	133	0.00
Fe	3245	21	3224	0.01
Mn	453	1	452	0.00
Zn	638	119	519	0.19

Report 9. Ingredient Mineral Contributions

Ingredient	Ca g/d	P g/d	Mg g/d	Cl g/d	K g/d	Na g/d	S g/d	Co mg/d	Cu mg/d	I mg/d	Fe mg/d	Mn mg/d	Se mg/d	Zn mg/d
*Corn silage (Updated After	17	20	12	24	89	2	8	0	40	0	983	185	0	177
*Alfalfa hay, (Updated After	20	6	4	12	55	0	4	0	17	0	698	69	0	43
Corn grain dry, fine grind	1	11	5	4	21	1	4	0	8	0	144	26	0	86
*DDGS, low fat (Dakota Gold)	1	21	9	6	32	5	28	0	15	0	201	36	1	159
Soybean meal, solvent 48CP	4	7	3	1	24	0	4	0	16	0	187	41	0	53
*Soybean meal, expellers (so	2	4	1	0	9	0	2	0	5	0	95	14	0	19
Soybean hulls	3	1	1	0	6	0	0	0	3	0	195	9	0	20
Calcium soaps	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Blood meal, high dRUP	0	1	0	1	1	1	2	0	2	0	726	1	0	11
Sodium chloride (salt)	0	0	0	47	0	31	0	0	0	0	0	0	0	0
Sodium bicarbonate	0	0	0	0	0	26	0	0	0	0	0	0	0	0
Calcium carbonate	119	0	0	0	0	0	0	0	0	0	0	0	0	0
Calcium phosphate (di)	6	6	0	0	0	0	0	0	0	0	0	0	0	0
Magnesium oxide	0	0	27	0	0	0	0	0	0	0	0	0	0	0
Calcium sulfate (2H2O)	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Rumen Protected Met	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Rumen Protected Lys	0	0	0	0	0	0	0	0	0	0	0	0	0	0
VitTM Premix, generic	0	0	0	0	0	0	0	0	28	1	17	70	1	70
Potassium chloride	0	0	0	39	39	0	0	0	0	0	0	0	0	0
Totals	174	76	63	134	277	66	53	0	133	1	3245	453	3	638

R-Gc

Report 1. Animal Inputs

1.1 Physiological State/Management

Item	Value	Unit
Animal Type	Lactating Cow	
Breed	Jersey	
Body Weight	440	kg
Mature Weight	450	kg
Age	42.0	months
Condition Score	3.00	(1-5)
Percent First Parity	0	(0-100)
Days in Milk	154	days
Age At First Calving	24	months
Days Pregnant	42	days
Temperature	20	deg C
In vitro NDF digest	Do not use	
Feeding Monensin	No	
Grazing	No	
Topography	Mild	Topography
Distance (Pasture to Parlor)	0.000	km
One-Way Trips	N/A	times/day

1.2 Entered Performance

Item	Value	Unit
Milk Production	30.0	kg
Milk Fat	5.09	%
Milk True Protein	3.46	%
Milk Lactose	4.71	%
Milk Fat	1.53	kg/d
Milk True Protein	1.04	kg/d
Milk Lactose	1.41	kg/d
Milk True Protein RHA	317	kg/305 d
Milk, Energy/Protein Corrected (ECM)	37.6	kg/d
Intake (Dry Matter)	18.90	kg/d
Estimated Intake Based on Animal (Dry Matter)	19.28	kg/d
Estimated Intake Based on Animal/Fiber (Dry Matter)	22.57	kg/d
Dry Matter Intake as Percent of Body Weight	4.30	% BW
ECM/DMI	1.99	kg/kg
Frame Gain	0.00	kg/d
Body Reserves Gain	-0.25	kg/d
Gravid Uterine Gain	0.00	kg/d
Total Body Gain	-0.25	kg/d

1.3 Predicted Production Variables

Item	Value	Unit
Milk, NEL Allowable	29.57	kg/d
Milk, MP Allowable	29.78	kg/d
Milk Production, Nutrient Predicted	26.1	kg
Milk True Protein, Nutrient Predicted	0.98	kg/day
Milk Fat, Nutrient Predicted	1.04	kg/day

Report 2. Diet Summary (DM Basis)

2.1 Macro-nutrients

Nutrient	Content
Dry Matter, %	53.2
Forage, % DM	57.1
CP, % DM	16.6
ME, Mcal/kg	2.72
MP, % DM	10.31
NEL, Mcal/kg	1.79
RDP, % DM	10.4
RUP, Base, % DM	6.3
Dig. RUP, % DM	4.9
ADF, % DM	19.9
NDF, % DM	33.3
ADF/NDF, Ratio	0.60
Forage NDF, % DM	22.1
Starch, % DM	22.6
WSC, % DM	2.5
Ash, % DM	9.0
Fatty Acids, % DM	5.51
Ca, % DM	0.88
P, % DM	0.39
Mg, % DM	0.34
K, % DM	1.47
Na, % DM	0.34
Cl, % DM	0.54
S, % DM	0.28
DCAD, mEq/kg	198
Cost, $/ton As Fed	0.00
Cost, $/day	0.00

2.2 Diet Ingredients

Ingredient	As Fed kg/d	% As Fed	DM kg/d	% of DM
01 *Corn silage (Updated After 2211DA Study)	23.055723	64.931247	7.654500	40.500000
02 *Alfalfa hay, (Updated After 2211DA Study	3.491980	9.834375	3.139290	16.610000
03 Corn grain dry, fine grind	2.072526	5.836802	1.801170	9.530000
04 *DDGS, low fat (Dakota Gold) (Updated After 2211DA Study)	2.714917	7.645952	2.457000	13.000000
05 Soybean meal, solvent 48CP	0.876539	2.468575	0.782460	4.140000
06 *Soybean meal, expellers (soypass composition from NDS)	0.275719	0.776500	0.251370	1.330000
07 Soybean hulls	1.491137	4.199451	1.347570	7.130000
08 Calcium soaps	0.598930	1.686751	0.570780	3.020000
09 Blood meal, high dRUP	0.343205	0.966560	0.311850	1.650000
10 Sodium chloride (salt)	0.075600	0.212910	0.075600	0.400000
11 Sodium bicarbonate	0.094500	0.266138	0.094500	0.500000
12 Calcium carbonate	0.226800	0.638731	0.226800	1.200000
13 Calcium phosphate (di)	0.037800	0.106455	0.037800	0.200000
14 Magnesium oxide	0.047250	0.133069	0.047250	0.250000
15 Calcium sulfate (2H2O)	0.000000	0.000000	0.000000	0.000000
16 Rumen Protected Met	0.028929	0.081472	0.028350	0.150000
17 Rumen Protected Lys	0.057857	0.162941	0.056700	0.300000
18 VitTM Premix, generic	0.018489	0.052070	0.017010	0.090000
19 Potassium chloride	0.000000	0.000000	0.000000	0.000000
Totals	35.508	100.00	18.900	100.00

Report 3. Ingredient Macro-Nutrient Contributions (DM Basis)

Ingredient	Cost $/d	% of DM	BASE DE Mcal/d	CP kg/d	RDP g/d	RUP g/d	dRUP g/d	NDF kg/d	Starch kg/d	Fatty acids g/d
*Corn silage (Updated After 2211DA Study)	0.00	40.50	22.25	0.60	416	181	127	2.84	2.76	216
*Alfalfa hay, (Updated After 2211DA Study	0.00	16.61	8.01	0.59	484	106	69	1.34	0.03	41
Corn grain dry, fine grind	0.00	9.53	6.38	0.15	91	62	46	0.18	1.27	69
*DDGS, low fat (Dakota Gold) (Updated After 2211DA Study)	0.00	13.00	8.58	0.75	427	322	242	0.86	0.18	183
Soybean meal, solvent 48CP	0.00	4.14	3.11	0.41	288	124	113	0.09	0.01	8
*Soybean meal, expellers (soypass composition from NDS)	0.00	1.33	0.96	0.12	47	72	67	0.08	0.00	15
Soybean hulls	0.00	7.13	3.70	0.16	105	55	38	0.90	0.01	22
Calcium soaps	0.00	3.02	3.09	0.00	0	0	0	0.00	0.00	482
Blood meal, high dRUP	0.00	1.65	1.42	0.30	83	213	181	0.00	0.00	4
Sodium chloride (salt)	0.00	0.40	0.00	0.00	0	0	0	0.00	0.00	0
Sodium bicarbonate	0.00	0.50	0.00	0.00	0	0	0	0.00	0.00	0
Calcium carbonate	0.00	1.20	0.00	0.00	0	0	0	0.00	0.00	0
Calcium phosphate (di)	0.00	0.20	0.00	0.00	0	0	0	0.00	0.00	0
Magnesium oxide	0.00	0.25	0.00	0.00	0	0	0	0.00	0.00	0
Calcium sulfate (2H2O)	0.00	0.00	0.00	0.00	0	0	0	0.00	0.00	0
Rumen Protected Met	0.00	0.15	0.00	0.02	6	16	14	0.00	0.00	0
Rumen Protected Lys	0.00	0.30	0.00	0.04	11	31	28	0.00	0.00	0
VitTM Premix, generic	0.00	0.09	0.00	0.00	2	1	1	0.00	0.00	0
Potassium chloride	0.00	0.00	0.00	0.00	0	0	0	0.00	0.00	0
Totals	0.00	100.00	57.50	3.14	1958	1185	925	6.29	4.27	1041

Report 4. Energy

4.1 Energy Supply

Energy	Mcal/d	Mcal/kg	% of GE	% of DE	% of ME
GE	81.83	4.33	100.0		
DE	57.94	3.07	70.8	100.0	
Urinary E	2.15	0.11	2.6	3.7	
Gaseous E	4.41	0.23	5.4	7.6	
ME	51.37	2.72	62.8	88.7	100.0
NEL	33.91	1.79	41.4	58.5	66.0

4.2 NEL and ME Requirements

Requirement	ME Mcal/d	NEL Mcal/d	NE:DEIn Fraction	NE:ME Efficiency
Maintenance	14.55	9.60	0.17	0.66
Milk Production, User Entered	39.15	25.84	0.45	0.66
Pregnancy	0.03	0.02	0.00	0.14
Grazing Activity	0.00	0.00	0.00	0.66
Frame Gain	0.00	0.00	0.00	0.40
Reserves Gain	-1.80	-1.19	-0.03	0.89
Total Req, User Entered	51.93	34.27		
Balance (intake - required)	-0.56	-0.37		

4.3 Nutrient Contributions to DE

Nutrient	Intake kg/d	Base Digest %	Intake Adjusted Digest %	Truly Digested kg/d	Endog. Fecal kg/d	Apparently Digested kg/d	Apparently Digested %	Heat of combust Mcal/kg	DE Mcal/d
NDF	6.29	55.32	56.45	3.55	0.00	3.55	56.45	4.20	14.91
Starch	4.27	90.00	89.21	3.81	0.00	3.81	89.21	4.23	16.11
FA	1.04	73.00	73.00	0.76	0.00	0.76	73.00	9.40	7.14
rOM	2.48	96.00	96.00	2.38	0.65	1.73	69.89	4.00	6.94
CP	3.14	91.74	91.74	2.88	0.61	2.27	72.24	5.65	12.83
OM	17.20	77.63	77.85	13.39	1.26	12.13	70.52	4.78	57.94

Report 5. Fatty Acid Supply

Fatty Acid	Profile % of Total FA	Concentration % of DM	Intake g/d
C12:0	0.23	0.01	2.4
C14:0	1.09	0.06	11.4
C16:0	32.29	1.78	336.2
C16:1	0.21	0.01	2.1
C18:0	3.43	0.19	35.7
C18:1 trans	0.08	0.00	0.8
C18:1 cis	27.35	1.51	284.7
C18:2	29.62	1.63	308.4
C18:3	4.14	0.23	43.1
Others	1.53	0.08	16.0
Saturated Fatty Acids	38.58	2.13	401.7
Mono-Unsaturated Fatty Acids	27.63	1.52	287.7
Poly-Unsaturated Fatty Acids	33.76	1.86	351.5
Fatty Acids	100.00	5.51	1041.2

Report 6. Protein and Amino Acid Supply and Requirements

6.1 Protein Supply

Item	Value	Unit
DE from Non-Protein Components	45.11	Mcal/d
Ruminal Digestion and Outflow		
Rumen Digested Starch	2.84	kg/d
Rumen Digested NDF	2.45	kg/d
Microbial Protein (MiCP)	1.55	kg/d
RDP - MiCP Balance	0.40	kg/d
Rumen Undegraded Protein	1.18	kg/d
Metabolized Protein Supply	1.95	kg/d
MP from Microbial CP	1.02	kg/d
MP from RUP	0.93	kg/d
MP from Body Weight Loss	0.02	kg/d
MP Supply / ME Supply	37.94	g/Mcal
MP Use / ME Use g/Mcal (a)	44.83	g/Mcal
CP Supply / ME Supply	61.20	g/Mcal
CP Use / ME Use g/Mcal (a)	72.30	g/Mcal

(a) MP and ME use are calculated using Target MP to NP efficiencies and predicted ME to NE efficiencies at user entered inputs and production.

6.2 NP, CP, and MP Supply and Use, g/d

Item	Net TP	CP	MP (a)
Scurf, g/d	7	8	10
Endogenous Urinary, g/d	146	N/A	146
Metabolic Fecal, g/d	221	302	320
Frame Growth, g/d	0	0	0
Body Reserves, g/d	-14	-30	-21
Pregnancy, g/d	1	1	2
Lactation, User Entered, g/d	1038	N/A	1504
Total Required at User Entered, g/d	1397	N/A	1960
Supply, g/d	N/A	3143	1949
Supply - Required at User Entered, g/d	N/A	N/A	-11
Required Eff at User Entered, g/g	N/A	N/A	0.69
Lactation, Nutrient Allowable, g/d	985	N/A	1482
Total Required at Nutrient Allowable, g/d	1344	1476	1950
Supply - Required at Nutrient Allowable	N/A	N/A	-1
Predicted Eff at Nutr Allowable g/g (b)	N/A	N/A	0.66

(a) MP efficiency of 0.69 is used for all functions except for endogenous urinary and pregnancy, which are 1 and 0.33, respectively.
(b) Calculated using predicted MP efficiency for nutrient allowable milk protein production.

6.3 Predicted Milk Protein based on Supplied Amino Acids

Item	Independent Var	Regression Coeff (a)	Predicted Milk Protein, g/d
Intercept	N/A	N/A	-97.00
(BW - 612), kg (b)	-172.20	-0.42010	72.34
(Digested NDF - 17.06), % DM (c)	1.73	-4.59500	-7.94
Non-protein DEIn, Mcal/d	45.11	10.79000	486.74
Absorbed Arg, g/d	91.86	0.00000	0.00
Absorbed His, g/d	47.62	1.66428	79.25
Absorbed Ile, g/d	96.89	0.87934	85.20
Absorbed Leu, g/d	167.34	0.46302	77.48
Absorbed Lys, g/d	169.22	1.14562	193.87
Absorbed Met, g/d	52.65	1.82723	96.20
Absorbed Phe, g/d	103.74	0.00000	0.00
Absorbed Thr, g/d	93.95	0.00000	0.00
Absorbed Trp, g/d	23.46	0.00000	0.00
Absorbed Val, g/d	114.28	0.00000	0.00
Squared EAA, g^2/d (d)	71066.72	-0.00187	-133.24
Absorbed Other AA, g/d (e)	1707.89	0.07730	132.02
Nutr Allow Milk NP, g/d	N/A	N/A	984.93
Nutr Allow Milk NP / User Enter Max NP (305d RHA) (f)	N/A	N/A	0.58

(a) Regression coefficient from Eqn. 6.6 adjusted based on user entered Rolling Herd Average Protein,
(b) centered to 612 kg,
(c) centered to 17.06% of DM.
(d) the sum of the squared supplies of each EAA with non-zero coefficients,
(e) includes all AA other than the EAA with non-zero coefficients,
(f) nutrient allowable NP production as a proportion of the maximum calculated from the user entered 305d RHA milk protein. This ratio should not be greater than 0.80 under normal feeding conditions.

6.4 Duodenal AA Flows, g/d (a)

		Duodenal flow					metabolizable EAA			
Item	Diet	RUP	MiCP	Endog	Total: True	Total: 24h Hydr	From RUP	From MiCP	Total	Targ Supp at User Enter
Arg	122	44	70	11	125	118	36	56	92	N/A
His	73	31	28	7	66	62	25	23	48	51
Ile	100	33	89	10	132	118	25	72	97	108
Leu	238	92	118	18	229	215	73	95	167	179
Lys	179	86	121	15	222	208	73	97	169	152
Met	62	30	34	3	67	64	26	27	53	49
Phe	130	49	81	9	140	132	39	65	104	111
Thr	105	38	80	12	130	122	30	64	94	101
Trp	32	12	18	3	32	31	9	14	23	25
Val	144	55	88	13	156	142	9	70	114	119

(a)All flows include a correction to account for incomplete recovery of AA during a 24-h hydrolysis, except 'Total: 24h Hydr'; Target supply calculated using target efficiencies and net use detailed in Table 6.5.

6.5 Partition of net EAA utilization (g/d) and efficiency of utilization of EAA (a)

Item	Urine Endo.	Metab. Fecal	Scurf	Gest	Body Gain	Milk, Nutr. Allow	Milk, User Enter	Total, Nutr. Allow	Total, User Enter	Target Eff (b)	Nutr Allow Eff (b)	User Enter Eff (b)
Arg	2	13	1	0	-1	37	39	52	54	N/A	0.55	N/A
His	1	8	0	0	0	29	30	37	39	0.75	0.78	0.81
Ile	1	12	0	0	-1	61	64	73	77	0.71	0.76	0.79
Leu	2	20	0	0	-1	104	110	126	131	0.73	0.75	0.78
Lys	2	17	0	0	-1	87	92	105	110	0.72	0.62	0.64
Met	1	4	0	0	0	30	31	34	36	0.73	0.64	0.67
Phe	1	12	0	0	-1	52	55	64	67	0.60	0.62	0.64
Thr	1	16	0	0	-1	46	48	63	65	0.64	0.66	0.69
Trp	0	4	0	0	0	16	17	20	21	0.86	0.87	0.91
Val	1	15	0	0	-1	68	72	84	88	0.74	0.74	0.77

(a) Corrected for incomplete recovery of AA during a 24-h hydrolysis.
(b) Efficiencies for Urine endogenous and gestation are 1 and 0.33. Combined efficiencies calculated from MP supply for other functions. A target efficiency was not estimated for Arg due to semi-essentiality.

Report 7. Mineral and Vitamin Supply and Requirements

7.1 Minerals

Item	Diet Density	Req Density	AC	Absorb Req (TAR)	Diet Supply (TDS)	Absorb Supp (TAS)	**Diff TAS-TAR**	Metab Fecal	Urine	Preg	Milk	Growth
Macro Mineral	% DM	% DM	g/100g	g/d	g/d	g/d	**g/d**	g/d	g/d	g/d	g/d	g/d
Ca	0.88	0.55	46.6	48	167	78	**29**	17	N/A	0	34	-2
P	0.39	0.32	76.6	47	74	56	**10**	19	0	0	29	-1
Mg	0.34	0.19	26.2	9	63	17	**7**	6	0	0	3	0
Cl	0.54	0.29	92.0	51	102	94	**43**	21	N/A	0	30	0
K	1.47	0.95	100.0	180	278	278	**98**	47	88	0	45	-1
Na	0.34	0.21	100.0	39	64	64	**25**	27	N/A	0	12	0
S	0.28	0.20	N/A	38	53	N/A	**15**	N/A	N/A	N/A	N/A	N/A
Micro Mineral (a)	mg/kg	mg/kg	g/100g	mg/d	mg/d	mg/d	**mg/d**	Maint. mg/d		mg/d	mg/d	mg/d
Co	0.00	0.20	0.0	4	0	0	**-4**	N/A		N/A	N/A	N/A
Cr	0.24	N/A	N/A	N/A	N/A	N/A	**N/A**	N/A		N/A	N/A	N/A
Cu	7.58	7.50	5.0	7	143	7	**0**	6		0	1	0
Fe	216.82	11.45	10.0	22	4098	410	**388**	0		0	30	-8
I	0.07	0.44	N/A	8	1	N/A	**-7**	N/A		N/A	N/A	N/A
Mn	26.23	23.93	0.4	2	496	2	**0**	1		0	1	0
Se	0.19	0.30	N/A	6	4	N/A	**-2**	N/A		N/A	N/A	N/A
Zn	34.01	55.18	20.0	209	643	129	**-80**	95		0	120	-6

(a) For S, Co, I, and Se required is based on diet concentration and not absorbed amounts.

7.2 Vitamin Supply and Requirements

Item	Diet Density	Required Density	Diet Supply	Required	Supply - Required
Fat-soluble Vitamins	IU/kg	IU/kg	IU/d	IU/d	IU/d
A	504.0	2559.7	9526	48378	-38852
D	180.0	930.8	3402	17592	-14190
E	3.6	18.6	68	352	-284
Other Vitamins	mg/kg	mg/kg	mg/d	mg/d	mg/d
Beta Carotene	0.00	N/A	0	N/A	N/A
Biotin	0.00	N/A	0	N/A	N/A
Choline	0.00	N/A	0	N/A	N/A
Niacin	0.00	N/A	0	N/A	N/A

Report 8. Environmental Impact

8.1 Water, Volatile Solids, and Methane

Item	Value	Unit
Water Intake	85.9	kg/d
Wet Manure Output	58.0	kg/d
Manure Volatile Solids	6.45	kg/d
Enteric Methane Production	333	g/d
Enteric Methane Production	498	L/d
Water Intake	3.3	L H2O/kg Milk
Manure Water	2.0	L H2O/kg Milk
Manure Volatile Solids	2.0	kg/kg Milk
Enteric Methane Production	13	g CH4/kg Milk
Enteric Methane Production	19	L CH4/kg Milk

8.2 Nitrogen and Mineral Excretion

Item	Intake	Retained in Milk, Growth & Conceptus	Fecal & Urinary	Retained/Intake
Nitrogen and Macro-minerals	g/d	g/d	g/d	g/g
Nitrogen	503	161	291	0.32
Ca	167	31	135	0.19
P	74	28	46	0.38
Mg	63	3	60	0.05
Cl	102	30	73	0.29
K	278	44	233	0.16
Na	64	12	53	0.18
Micro-minerals	mg/d	mg/d	mg/d	g/g
Cu	143	1	142	0.00
Fe	4098	22	4076	0.01
Mn	496	1	495	0.00
Zn	643	114	529	0.18

Report 9. Ingredient Mineral Contributions

Ingredient	Ca g/d	P g/d	Mg g/d	Cl g/d	K g/d	Na g/d	S g/d	Co mg/d	Cu mg/d	I mg/d	Fe mg/d	Mn mg/d	Se mg/d	Zn mg/d
*Corn silage (Updated After	16	19	11	23	84	2	8	0	38	0	934	176	0	168
*Alfalfa hay, (Updated After	38	11	7	24	107	1	8	0	32	0	1350	134	1	83
Corn grain dry, fine grind	1	6	2	2	10	0	2	0	4	0	70	13	0	42
*DDGS, low fat (Dakota Gold)	1	20	9	5	31	5	27	0	14	0	196	35	1	155
Soybean meal, solvent 48CP	3	6	3	0	19	0	3	0	13	0	146	32	0	41
*Soybean meal, expellers (so	1	2	1	0	5	0	1	0	3	0	53	8	0	11
Soybean hulls	9	2	4	1	19	0	2	0	10	0	625	28	0	64
Calcium soaps	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Blood meal, high dRUP	0	1	0	1	1	1	2	0	2	0	707	1	0	10
Sodium chloride (salt)	0	0	0	46	0	30	0	0	0	0	0	0	0	0
Sodium bicarbonate	0	0	0	0	0	26	0	0	0	0	0	0	0	0
Calcium carbonate	89	0	0	0	0	0	0	0	0	0	0	0	0	0
Calcium phosphate (di)	8	7	0	0	0	0	0	0	0	0	0	0	0	0
Magnesium oxide	0	0	27	0	0	0	0	0	0	0	0	0	0	0
Calcium sulfate (2H2O)	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Rumen Protected Met	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Rumen Protected Lys	0	0	0	0	0	0	0	0	0	0	0	0	0	0
VitTM Premix, generic	0	0	0	0	0	0	0	0	27	1	17	68	1	68
Potassium chloride	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Totals	167	74	63	102	278	64	53	0	143	1	4098	496	4	643

R-GcAlf

Report 1. Animal Inputs

1.1 Physiological State/Management

Item	Value	Unit
Animal Type	Lactating Cow	
Breed	Jersey	
Body Weight	440	kg
Mature Weight	450	kg
Age	42.0	months
Condition Score	2.90	(1-5)
Percent First Parity	0	(0-100)
Days in Milk	154	days
Age At First Calving	24	months
Days Pregnant	42	days
Temperature	20	deg C
In vitro NDF digest	Do not use	
Feeding Monensin	No	
Grazing	No	
Topography	Mild	Topography
Distance (Pasture to Parlor)	0.000	km
One-Way Trips	N/A	times/day

1.2 Entered Performance

Item	Value	Unit
Milk Production	30.1	kg
Milk Fat	5.05	%
Milk True Protein	3.53	%
Milk Lactose	4.74	%
Milk Fat	1.52	kg/d
Milk True Protein	1.06	kg/d
Milk Lactose	1.43	kg/d
Milk True Protein RHA	323	kg/305 d
Milk, Energy/Protein Corrected (ECM)	37.7	kg/d
Intake (Dry Matter)	18.30	kg/d
Estimated Intake Based on Animal (Dry Matter)	19.58	kg/d
Estimated Intake Based on Animal/Fiber (Dry Matter)	22.76	kg/d
Dry Matter Intake as Percent of Body Weight	4.16	% BW
ECM/DMI	2.06	kg/kg
Frame Gain	0.00	kg/d
Body Reserves Gain	-0.28	kg/d
Gravid Uterine Gain	0.00	kg/d
Total Body Gain	-0.27	kg/d

1.3 Predicted Production Variables

Item	Value	Unit
Milk, NEL Allowable	28.87	kg/d
Milk, MP Allowable	29.52	kg/d
Milk Production, Nutrient Predicted	25.8	kg
Milk True Protein, Nutrient Predicted	0.99	kg/day
Milk Fat, Nutrient Predicted	1.03	kg/day

Report 2. Diet Summary (DM Basis)

2.1 Macro-nutrients

Nutrient	Content
Dry Matter, %	53.2
Forage, % DM	54.4
CP, % DM	16.7
ME, Mcal/kg	2.75
MP, % DM	10.63
NEL, Mcal/kg	1.81
RDP, % DM	10.2
RUP, Base, % DM	6.5
Dig. RUP, % DM	5.3
ADF, % DM	18.0
NDF, % DM	30.0
ADF/NDF, Ratio	0.60
Forage NDF, % DM	21.0
Starch, % DM	26.9
WSC, % DM	2.9
Ash, % DM	8.9
Fatty Acids, % DM	5.39
Ca, % DM	0.88
P, % DM	0.40
Mg, % DM	0.32
K, % DM	1.43
Na, % DM	0.33
Cl, % DM	0.54
S, % DM	0.25
DCAD, mEq/kg	202
Cost, $/ton As Fed	0.00
Cost, $/day	0.00

2.2 Diet Ingredients

Ingredient	As Fed kg/d	% As Fed	DM kg/d	% of DM
01 *Corn silage (Updated After 2211DA Study)	22.310407	64.813464	7.407055	40.475708
02 *Alfalfa hay, (Updated After 2211DA Study	2.827781	8.214923	2.542175	13.891666
03 Corn grain dry, fine grind	3.430231	9.965087	2.981111	16.290223
04 *DDGS, low fat (Dakota Gold) (Updated After 2211DA Study)	1.313577	3.816043	1.188787	6.496103
05 Soybean meal, solvent 48CP	1.257963	3.654480	1.122946	6.136317
06 *Soybean meal, expellers (soypass composition from NDS)	0.768320	2.232029	0.700470	3.827705
07 Soybean hulls	0.997708	2.898419	0.901649	4.927043
08 Calcium soaps	0.579569	1.683693	0.552329	3.018191
09 Blood meal, high dRUP	0.332111	0.964808	0.301769	1.649011
10 Sodium chloride (salt)	0.073156	0.212524	0.073156	0.399760
11 Sodium bicarbonate	0.091445	0.265655	0.091445	0.499699
12 Calcium carbonate	0.212153	0.616321	0.212153	1.159306
13 Calcium phosphate (di)	0.054867	0.159393	0.054867	0.299820
14 Magnesium oxide	0.045723	0.132829	0.045723	0.249852
15 Calcium sulfate (2H2O)	0.016460	0.047818	0.016460	0.089945
16 Rumen Protected Met	0.027994	0.081325	0.027434	0.149913
17 Rumen Protected Lys	0.055987	0.162647	0.054867	0.299820
18 VitTM Premix, generic	0.017891	0.051975	0.016460	0.089945
19 Potassium chloride	0.009145	0.026567	0.009145	0.049973
Totals	34.422	100.00	18.300	100.00

Report 3. Ingredient Macro-Nutrient Contributions (DM Basis)

Ingredient	Cost $/d	% of DM	BASE DE Mcal/d	CP kg/d	RDP g/d	RUP g/d	dRUP g/d	NDF kg/d	Starch kg/d	Fatty acids g/d
*Corn silage (Updated After 2211DA Study)	0.00	40.48	21.53	0.58	402	176	123	2.75	2.67	209
*Alfalfa hay, (Updated After 2211DA Study	0.00	13.89	6.49	0.48	392	86	56	1.09	0.02	33
Corn grain dry, fine grind	0.00	16.29	10.57	0.25	151	103	75	0.29	2.10	114
*DDGS, low fat (Dakota Gold) (Updated After 2211DA Study)	0.00	6.50	4.15	0.36	207	156	117	0.42	0.09	88
Soybean meal, solvent 48CP	0.00	6.14	4.46	0.59	413	179	162	0.12	0.02	12
*Soybean meal, expellers (soypass composition from NDS)	0.00	3.83	2.67	0.33	130	200	186	0.22	0.00	43
Soybean hulls	0.00	4.93	2.47	0.11	70	37	25	0.60	0.01	15
Calcium soaps	0.00	3.02	2.99	0.00	0	0	0	0.00	0.00	467
Blood meal, high dRUP	0.00	1.65	1.37	0.29	80	206	175	0.00	0.00	4
Sodium chloride (salt)	0.00	0.40	0.00	0.00	0	0	0	0.00	0.00	0
Sodium bicarbonate	0.00	0.50	0.00	0.00	0	0	0	0.00	0.00	0
Calcium carbonate	0.00	1.16	0.00	0.00	0	0	0	0.00	0.00	0
Calcium phosphate (di)	0.00	0.30	0.00	0.00	0	0	0	0.00	0.00	0
Magnesium oxide	0.00	0.25	0.00	0.00	0	0	0	0.00	0.00	0
Calcium sulfate (2H2O)	0.00	0.09	0.00	0.00	0	0	0	0.00	0.00	0
Rumen Protected Met	0.00	0.15	0.00	0.02	5	15	14	0.00	0.00	0
Rumen Protected Lys	0.00	0.30	0.00	0.04	11	30	27	0.00	0.00	0
VitTM Premix, generic	0.00	0.09	0.00	0.00	2	1	1	0.00	0.00	0
Potassium chloride	0.00	0.05	0.00	0.00	0	0	0	0.00	0.00	0
Totals	0.00	100.00	56.70	3.05	1862	1189	962	5.50	4.92	986

Report 4. Energy

4.1 Energy Supply

Energy	Mcal/d	Mcal/kg	% of GE	% of DE	% of ME
GE	79.25	4.33	100.0		
DE	56.59	3.09	71.4	100.0	
Urinary E	2.10	0.12	2.7	3.7	
Gaseous E	4.17	0.23	5.3	7.4	
ME	50.32	2.75	63.5	88.9	100.0
NEL	33.21	1.81	41.9	58.7	66.0

4.2 NEL and ME Requirements

Requirement	ME Mcal/d	NEL Mcal/d	NE:DEIn Fraction	NE:ME Efficiency
Maintenance	14.56	9.61	0.17	0.66
Milk Production, User Entered	39.35	25.97	0.46	0.66
Pregnancy	0.03	0.02	0.00	0.14
Grazing Activity	0.00	0.00	0.00	0.66
Frame Gain	0.00	0.00	0.00	0.40
Reserves Gain	-2.02	-1.33	-0.03	0.89
Total Req, User Entered	51.93	34.27		
Balance (intake - required)	-1.61	-1.06		

4.3 Nutrient Contributions to DE

Nutrient	Intake kg/d	Base Digest %	Intake Adjusted Digest %	Truly Digested kg/d	Endog. Fecal kg/d	Apparently Digested kg/d	Apparently Digested %	Heat of combust Mcal/kg	DE Mcal/d
NDF	5.50	55.00	53.76	2.96	0.00	2.96	53.76	4.20	12.41
Starch	4.92	90.34	89.68	4.41	0.00	4.41	89.68	4.23	18.66
FA	0.99	73.00	73.00	0.72	0.00	0.72	73.00	9.40	6.76
rOM	2.24	96.00	96.00	2.15	0.63	1.53	68.02	4.00	6.11
CP	3.05	92.56	92.56	2.82	0.58	2.24	73.41	5.65	12.65
OM	16.67	78.99	78.38	13.06	1.21	11.85	71.11	4.78	56.59

Report 5. Fatty Acid Supply

Fatty Acid	Profile % of Total FA	Concentration % of DM	Intake g/d
C12:0	0.22	0.01	2.1
C14:0	1.21	0.07	11.9
C16:0	32.51	1.75	320.5
C16:1	0.19	0.01	1.9
C18:0	3.44	0.19	33.9
C18:1 trans	0.11	0.01	1.1
C18:1 cis	27.36	1.47	269.6
C18:2	29.30	1.58	288.7
C18:3	4.14	0.22	40.8
Others	1.49	0.08	14.7
Saturated Fatty Acids	38.87	2.09	383.1
Mono-Unsaturated Fatty Acids	27.66	1.49	272.6
Poly-Unsaturated Fatty Acids	33.43	1.80	329.5
Fatty Acids	100.00	5.39	985.6

Report 6. Protein and Amino Acid Supply and Requirements

6.1 Protein Supply

Item	Value	Unit
DE from Non-Protein Components	43.94	Mcal/d
Ruminal Digestion and Outflow		
Rumen Digested Starch	3.36	kg/d
Rumen Digested NDF	1.90	kg/d
Microbial Protein (MiCP)	1.49	kg/d
RDP - MiCP Balance	0.37	kg/d
Rumen Undegraded Protein	1.19	kg/d
Metabolized Protein Supply	1.95	kg/d
MP from Microbial CP	0.98	kg/d
MP from RUP	0.96	kg/d
MP from Body Weight Loss	0.02	kg/d
MP Supply / ME Supply	38.67	g/Mcal
MP Use / ME Use g/Mcal (a)	45.13	g/Mcal
CP Supply / ME Supply	62.38	g/Mcal
CP Use / ME Use g/Mcal (a)	72.79	g/Mcal

(a) MP and ME use are calculated using Target MP to NP efficiencies and predicted ME to NE efficiencies at user entered inputs and production.

6.2 NP, CP, and MP Supply and Use, g/d

Item	Net TP	CP	MP (a)
Scurf, g/d	7	8	10
Endogenous Urinary, g/d	146	N/A	146
Metabolic Fecal, g/d	208	286	302
Frame Growth, g/d	0	0	0
Body Reserves, g/d	-16	-33	-23
Pregnancy, g/d	1	1	2
Lactation, User Entered, g/d	1063	N/A	1540
Total Required at User Entered, g/d	1408	N/A	1976
Supply, g/d	N/A	3051	1946
Supply - Required at User Entered, g/d	N/A	N/A	-30
Required Eff at User Entered, g/g	N/A	N/A	0.70
Lactation, Nutrient Allowable, g/d	992	N/A	1498
Total Required at Nutrient Allowable, g/d	1338	1466	1947
Supply - Required at Nutrient Allowable	N/A	N/A	-1
Predicted Eff at Nutr Allowable g/g (b)	N/A	N/A	0.66

(a) MP efficiency of 0.69 is used for all functions except for endogenous urinary and pregnancy, which are 1 and 0.33, respectively.
(b) Calculated using predicted MP efficiency for nutrient allowable milk protein production.

6.3 Predicted Milk Protein based on Supplied Amino Acids

Item	Independent Var	Regression Coeff (a)	Predicted Milk Protein, g/d
Intercept	N/A	N/A	-97.00
(BW - 612), kg (b)	-171.90	-0.42010	72.22
(Digested NDF - 17.06), % DM (c)	-0.91	-4.59500	4.18
Non-protein DEIn, Mcal/d	43.94	10.79000	474.10
Absorbed Arg, g/d	100.88	0.00000	0.00
Absorbed His, g/d	50.29	1.66280	83.62
Absorbed Ile, g/d	101.02	0.87855	88.75
Absorbed Leu, g/d	173.29	0.46260	80.17
Absorbed Lys, g/d	173.08	1.14460	198.11
Absorbed Met, g/d	52.85	1.82560	96.48
Absorbed Phe, g/d	107.97	0.00000	0.00
Absorbed Thr, g/d	96.63	0.00000	0.00
Absorbed Trp, g/d	24.60	0.00000	0.00
Absorbed Val, g/d	117.96	0.00000	0.00
Squared EAA, g^2/d (d)	75515.30	-0.00184	-138.70
Absorbed Other AA, g/d (e)	1687.33	0.07730	130.43
Nutr Allow Milk NP, g/d	N/A	N/A	992.36
Nutr Allow Milk NP / User Enter Max NP (305d RHA) (f)	N/A	N/A	0.57

(a) Regression coefficient from Eqn. 6.6 adjusted based on user entered Rolling Herd Average Protein,
(b) centered to 612 kg,
(c) centered to 17.06% of DM.
(d) the sum of the squared supplies of each EAA with non-zero coefficients,
(e) includes all AA other than the EAA with non-zero coefficients,
(f) nutrient allowable NP production as a proportion of the maximum calculated from the user entered 305d RHA milk protein. This ratio should not be greater than 0.80 under normal feeding conditions.

6.4 Duodenal AA Flows, g/d (a)

		Duodenal flow					metabolizable EAA			
Item	Diet	RUP	MiCP	Endog	Total: True	Total: 24h Hydr	From RUP	From MiCP	Total	Targ Supp at User Enter
Arg	143	56	67	11	134	126	47	54	101	N/A
His	79	34	27	7	68	64	29	22	50	52
Ile	112	39	86	10	135	121	32	69	101	109
Leu	253	102	114	18	233	219	82	91	173	181
Lys	192	93	116	15	224	210	80	93	173	153
Met	63	32	32	3	67	64	27	26	53	49
Phe	141	56	78	9	143	135	46	62	108	112
Thr	113	43	77	12	132	124	35	61	97	101
Trp	34	13	17	3	33	31	11	13	25	25
Val	153	61	85	12	159	144	11	68	118	120

(a)All flows include a correction to account for incomplete recovery of AA during a 24-h hydrolysis, except 'Total: 24h Hydr'; Target supply calculated using target efficiencies and net use detailed in Table 6.5.

6.5 Partition of net EAA utilization (g/d) and efficiency of utilization of EAA (a)

Item	Urine Endo.	Metab. Fecal	Scurf	Gest	Body Gain	Milk, Nutr. Allow	Milk, User Enter	Total, Nutr. Allow	Total, User Enter	Target Eff (b)	Nutr Allow Eff (b)	User Enter Eff (b)
Arg	2	12	1	0	-1	37	40	51	54	N/A	0.49	N/A
His	1	7	0	0	0	29	31	37	39	0.75	0.73	0.77
Ile	1	11	0	0	-1	61	66	73	78	0.71	0.72	0.77
Leu	2	19	0	0	-1	105	112	125	133	0.73	0.72	0.76
Lys	2	16	0	0	-1	88	94	105	111	0.72	0.60	0.64
Met	1	4	0	0	0	30	32	34	36	0.73	0.64	0.68
Phe	1	11	0	0	-1	52	56	64	68	0.60	0.59	0.62
Thr	1	15	0	0	-1	46	49	62	65	0.64	0.64	0.67
Trp	0	4	0	0	0	16	18	20	21	0.86	0.82	0.87
Val	1	15	0	0	-1	68	73	84	89	0.74	0.71	0.75

(a) Corrected for incomplete recovery of AA during a 24-h hydrolysis.
(b) Efficiencies for Urine endogenous and gestation are 1 and 0.33. Combined efficiencies calculated from MP supply for other functions. A target efficiency was not estimated for Arg due to semi-essentiality.

Report 7. Mineral and Vitamin Supply and Requirements

7.1 Minerals

Item	Diet Density	Req Density	AC	Absorb Req (TAR)	Diet Supply (TDS)	Absorb Supp (TAS)	**Diff TAS-TAR**	Metab Fecal	Urine	Preg	Milk	Growth
Macro Mineral	% DM	% DM	g/100g	g/d	g/d	g/d	**g/d**	g/d	g/d	g/d	g/d	g/d
Ca	0.88	0.54	48.3	48	161	78	**30**	16	N/A	0	34	-3
P	0.40	0.33	76.0	46	73	55	**9**	18	0	0	29	-2
Mg	0.32	0.19	26.2	9	59	16	**7**	5	0	0	3	0
Cl	0.54	0.30	92.0	50	99	91	**41**	20	N/A	0	30	0
K	1.43	0.97	100.0	178	261	261	**83**	46	88	0	45	-1
Na	0.33	0.21	100.0	38	60	60	**22**	27	N/A	0	12	0
S	0.25	0.20	N/A	37	45	N/A	**9**	N/A	N/A	N/A	N/A	N/A
Micro Mineral (a)	mg/kg	mg/kg	g/100g	mg/d	mg/d	mg/d	**mg/d**	Maint. mg/d		mg/d	mg/d	mg/d
Co	0.00	0.20	0.0	4	0	0	**-4**	N/A		N/A	N/A	N/A
Cr	0.22	N/A	N/A	N/A	N/A	N/A	**N/A**	N/A		N/A	N/A	N/A
Cu	7.48	7.69	5.0	7	137	7	**0**	6		0	1	-1
Fe	201.27	11.34	10.0	21	3683	368	**348**	0		0	30	-9
I	0.07	0.46	N/A	8	1	N/A	**-7**	N/A		N/A	N/A	N/A
Mn	25.79	24.48	0.4	2	472	2	**0**	1		0	1	0
Se	0.17	0.30	N/A	5	3	N/A	**-2**	N/A		N/A	N/A	N/A
Zn	31.85	56.09	20.0	205	583	117	**-89**	92		0	120	-7

(a) For S, Co, I, and Se required is based on diet concentration and not absorbed amounts.

7.2 Vitamin Supply and Requirements

Item	Diet Density	Required Density	Diet Supply	Required	Supply - Required
Fat-soluble Vitamins	IU/kg	IU/kg	IU/d	IU/d	IU/d
A	503.7	2645.4	9218	48411	-39193
D	179.9	962.0	3292	17604	-14312
E	3.6	19.2	66	352	-286
Other Vitamins	mg/kg	mg/kg	mg/d	mg/d	mg/d
Beta Carotene	0.00	N/A	0	N/A	N/A
Biotin	0.00	N/A	0	N/A	N/A
Choline	0.00	N/A	0	N/A	N/A
Niacin	0.00	N/A	0	N/A	N/A

Report 8. Environmental Impact

8.1 Water, Volatile Solids, and Methane

Item	Value	Unit
Water Intake	83.1	kg/d
Wet Manure Output	55.3	kg/d
Manure Volatile Solids	6.14	kg/d
Enteric Methane Production	315	g/d
Enteric Methane Production	471	L/d
Water Intake	3.2	L H2O/kg Milk
Manure Water	1.9	L H2O/kg Milk
Manure Volatile Solids	1.9	kg/kg Milk
Enteric Methane Production	12	g CH4/kg Milk
Enteric Methane Production	18	L CH4/kg Milk

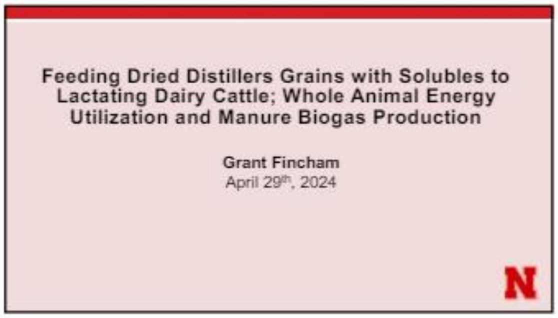
8.2 Nitrogen and Mineral Excretion

Item	Intake	Retained in Milk, Growth & Conceptus	Fecal & Urinary	Retained/Intake
Nitrogen and Macro-minerals	g/d	g/d	g/d	g/g
Nitrogen	488	162	278	0.33
Ca	161	32	129	0.20
P	73	28	45	0.38
Mg	59	3	56	0.05
Cl	99	30	69	0.30
K	261	44	216	0.17
Na	60	12	48	0.19
Micro-minerals	mg/d	mg/d	mg/d	g/g
Cu	137	1	136	0.00
Fe	3683	21	3662	0.01
Mn	472	1	471	0.00
Zn	583	114	469	0.20

Report 9. Ingredient Mineral Contributions

Ingredient	Ca g/d	P g/d	Mg g/d	Cl g/d	K g/d	Na g/d	S g/d	Co mg/d	Cu mg/d	I mg/d	Fe mg/d	Mn mg/d	Se mg/d	Zn mg/d
*Corn silage (Updated After	16	19	11	22	81	1	7	0	37	0	904	170	0	163
*Alfalfa hay, (Updated After	31	9	6	19	87	1	6	0	26	0	1093	109	1	67
Corn grain dry, fine grind	1	9	4	3	17	1	3	0	6	0	116	21	0	70
*DDGS, low fat (Dakota Gold)	0	10	4	3	15	2	13	0	7	0	95	17	0	75
Soybean meal, solvent 48CP	4	8	4	1	27	0	5	0	18	0	210	46	0	59
*Soybean meal, expellers (so	3	6	2	0	15	0	4	0	8	0	146	22	0	30
Soybean hulls	6	1	2	0	13	0	1	0	7	0	418	18	0	43
Calcium soaps	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Blood meal, high dRUP	0	1	0	1	1	1	2	0	2	0	684	1	0	10
Sodium chloride (salt)	0	0	0	44	0	29	0	0	0	0	0	0	0	0
Sodium bicarbonate	0	0	0	0	0	25	0	0	0	0	0	0	0	0
Calcium carbonate	84	0	0	0	0	0	0	0	0	0	0	0	0	0
Calcium phosphate (di)	12	11	0	0	0	0	0	0	0	0	0	0	0	0
Magnesium oxide	0	0	26	0	0	0	0	0	0	0	0	0	0	0
Calcium sulfate (2H2O)	4	0	0	0	0	0	4	0	0	0	0	0	0	0
Rumen Protected Met	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Rumen Protected Lys	0	0	0	0	0	0	0	0	0	0	0	0	0	0
VitTM Premix, generic	0	0	0	0	0	0	0	0	26	1	16	66	1	66
Potassium chloride	0	0	0	5	5	0	0	0	0	0	0	0	0	0
Totals	161	73	59	99	261	60	45	0	137	1	3683	472	3	583

APPENDIX D: FINAL DEFENSE PRESENTATION



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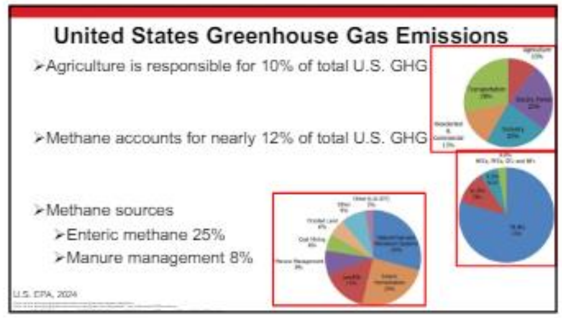

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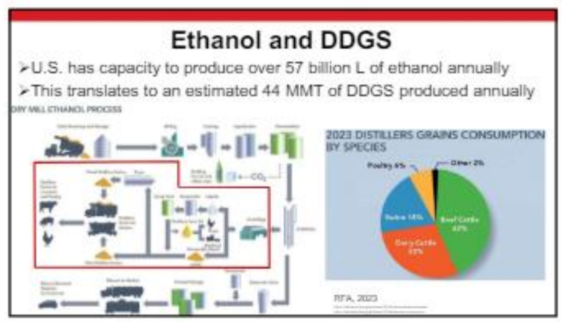

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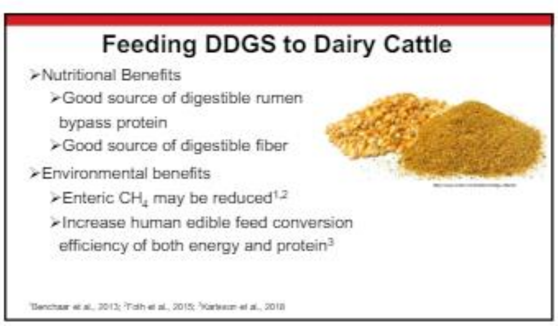

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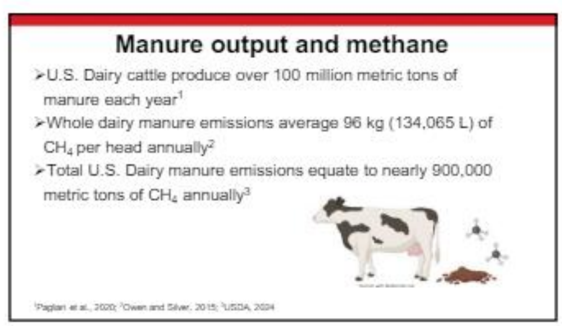
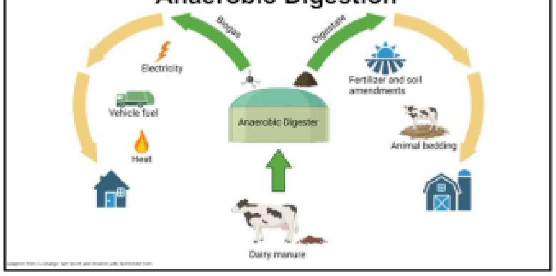


6

Anaerobic Digestion



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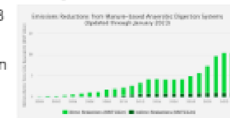
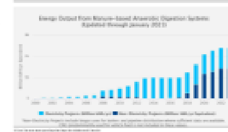
Anaerobic Manure Digesters

- As of January 2023, there were 343 U.S. manure-based anaerobic digesters, and 86 under construction
- In 2022, manure-based digestors:
  - reduced GHG emissions by 10.4 million metric tons of CO_2 equivalent
  - generated 2.42 million megawatt-hour equivalents of energy





U.S. EPA, 2023

8

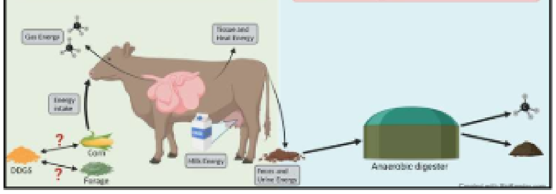
Replacing dietary ingredients with DDGS in diets fed to lactating dairy cattle

Experiment 1: Energy utilization, milk production, methane production, and manure output

The Problem: Productive responses to feeding DDGS are inconsistent among studies

Experiment 2: Manure composition and resulting biogas production in an anaerobic digester

The Problem: Little is known about how diet changes affect manure methane production in an anaerobic digester



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Replacing dietary ingredients with DDGS in diets fed to lactating dairy cattle. I. Energy utilization, milk production, methane production, and manure output

G.M. Fincham, A.L. Carroll, K.J. Herrick, and P.J. Kononoff

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Introduction

- When DDGS replace a source of fiber which is lower in digestibility, energy supply and milk production may increase[1,3,4]
- When DDGS replace a low fiber ingredient such as ground corn, feed efficiency may be increased through a reduction in DMI[2]

[1]Clark and Armentano, 1993; Ranathunga et al., [2]2010, [3]2018, [4]2019

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Objectives

- Examine the effects of feeding DDGS in place of ground corn, alfalfa hay, or a combination of the two on enteric methane production, feed intake, energy and N utilization, manure output, and milk production in lactating Jersey cows

Hypothesis

- Replacing alfalfa would increase DMI, energy supply, and milk production
- Replacing ground corn would decrease DMI without changing energy supply or milk production
- Replacing ground corn would increase fecal NDF content
- Replacing alfalfa would increase both manure and manure volatile solids (VS) output

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Material and Methods

Experimental Design and Animals

- 4x4 Latin Square
- 4 periods of 28 d
- 12 multiparous Jersey cows
- Housed in tie stalls at the UNL Dairy Metabolism Facility
- Milked twice daily at 0700 and 1800 h
- Fed once daily at 0930 h

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Material and Methods

Diets

	Treatment			
Item (%DM)	CON	R-Alf	R-Gc	R-GcAlf
Corn silage	40.5	41.5	40.5	40.5
Alfalfa hay	16.6	8.36	16.6	13.9
Ground corn	19.0	19.0	9.53	16.3
DDGS	-	13.0	13.0	6.50
Soybean meal	7.64	5.14	4.14	6.14
Non-enzymatically browned soybean meal (NEBSBM)	3.83	2.33	1.33	3.83
Soybean hulls	4.48	2.17	7.13	4.93
Calcium soaps	3.02	3.02	3.02	3.02
Blood meal	1.65	1.65	1.65	1.65
Rumen protected Met	0.15	0.15	0.15	0.15
Rumen protected Lys	0.30	0.30	0.30	0.30
Mineral and Vitamin	2.79	3.35	2.64	2.84

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Material and Methods

Collection period

Each individual cow's feed intake measured

Feces and Urine Collection

Headbox-type indirect calorimeter

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Results

Chemical Composition of Diets

	Treatment			
Item (% DM)	CON	R-Alf	R-Gc	R-GcAlf
CP	16.4 (0.53)	16.6 (0.10)	16.7 (0.27)	16.8 (0.39)
Total fatty acids	5.36 (0.167)	6.08 (0.142)	5.93 (0.225)	5.96 (0.253)
16C fatty acids	1.71 (0.077)	1.73 (0.077)	1.77 (0.093)	1.82 (0.108)
18C fatty acids	3.50 (0.089)	4.21 (0.084)	4.00 (0.125)	3.99 (0.134)
Starch	28.2 (0.91)	29.7 (0.78)	22.8 (0.75)	27.2 (0.67)
NDF	29.3 (1.26)	27.6 (0.76)	32.6 (0.84)	29.9 (1.18)
aNDFom[1]	28.6 (1.34)	26.9 (0.80)	31.9 (0.86)	29.2 (1.16)
fNDF	22.1 (0.63)	18.9 (0.54)	22.1 (0.63)	20.9 (0.59)
ADF	18.5 (0.77)	15.9 (0.11)	20.6 (0.79)	18.0 (0.42)
Lignin	2.91 (0.163)	2.51 (0.210)	3.15 (0.192)	2.95 (0.422)
Ash	8.73 (1.625)	9.26 (1.437)	8.74 (1.328)	8.73 (1.421)
K	1.47 (0.087)	1.44 (0.079)	1.50 (0.088)	1.44 (0.080)
NEL, Mcal/kg[2]	1.80	1.84	1.79	1.81

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Results

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Results

Energy Intake

	Treatments				SEM	P-value
Item (Mcal/d)	CON	R-Alf	R-Gc	R-GcAlf		
GE	79.0^{c}	84.4^{a}	83.3^{ab}	80.6^{bc}	2.67	0.02
DE	52.8	56.1	54.6	53.6	1.75	0.15
ME	46.9	50.1	48.5	47.7	1.60	0.15
NEL	33.4	35.4	34.0	34.0	1.20	0.41

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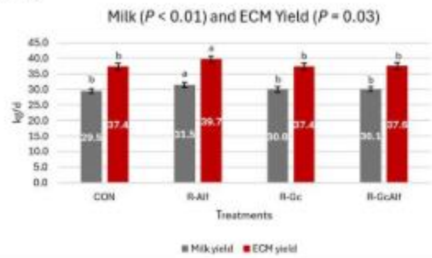
Results



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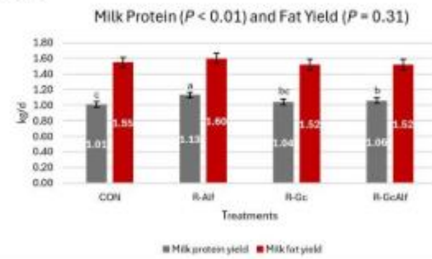
Results



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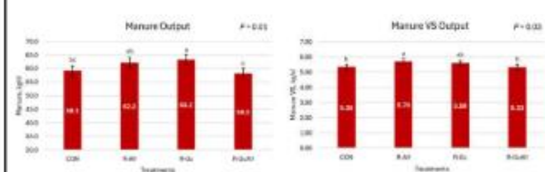
Results



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Conclusions

- Replacing a portion of dietary alfalfa with DDGS increased:
  - DMI and energy intake
  - ECM and both milk protein yield and concentration
  - Manure VS output
- Milk fat yield and concentration were not affected by dietary treatments
- Manure output increased when DDGS replaced ground corn

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Replacing dietary ingredients with DDGS in diets fed to lactating dairy cattle. II. Manure composition and resulting biogas and methane production in an anaerobic digester

G.M. Fincham, A.L. Carroll, K.J. Herrick, and P.J. Kononoff

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Introduction

- Manure digesters capture the energy in methane before it can negatively impact the environment[1]
- How do dietary changes affect dairy cattle manure composition and its subsequent methane production in an anaerobic manure digester?

[1]U.S. EPA, 2023; [2]Hao et al., 2009; [3]Genke et al., 2010; [4]Lee et al., 2020

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Objectives

- Test how differences in diet composition can affect the chemical composition of manure and resulting methane production in an anerobic digester

Hypothesis

- As diets resulted in an increase in manure NDF content, methane production in the anaerobic digester would also increase

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Materials and Methods

Terminology

Term	Abbreviation	Units	Definition
Total Solids	TS	%	The material residue left after evaporation and subsequent drying of a sample
Volatile Solids	VS	%	The portion of solids lost after ignition of dry solids at 550° C
Biochemical Methane Potential	BMP	ml/g VS	The maximum amount of methane that can be produced by anaerobic digestion of a specific substrate
Substrate	-	-	The main source of energy and carbon for the anaerobic microorganisms
Inoculum	-	-	The source of microorganisms that start the substrate degradation process

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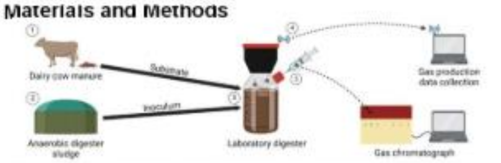
Materials and Methods



1. Manure samples were collected from Experiment 1 to be used as the substrate for a BMP test
2. Inoculum was obtained from two different anaerobic manure digesters on two different commercial dairy farms
3. Substrate and inoculum were loaded into the laboratory digester on a VS basis
4. Cumulative headspace pressure was wirelessly transmitted to the gas production software. Pressure was then used to calculate the volume of gas produced
5. Throughout the experiment, headspace gas samples were taken and analyzed to determine methane concentration

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Materials and Methods

Manure Collection

- Manure samples were collected from the previously described feeding study
- Feces and urine were weighed into a single composite according to the proportions by which they were produced by each cow in each treatment

Manure substrate treatment composite

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Materials and Methods

Inoculum Collection

- Inoculum was obtained from two different anaerobic manure digesters
  - Inoculum A was from a Jersey dairy
  - Inoculum B was from a Holstein dairy
- Digester sludge was collected from the effluent pit of the digester

Anaerobic manure digester

Digester effluent pit

Inside digester effluent pit

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Materials and Methods

Test Setup

Digesters loaded with 100 g of combined manure and inoculum (1:1 VS basis) and DDW

Digesters sealed with ANKOM RF gas production modules and maintained in an agitating water bath

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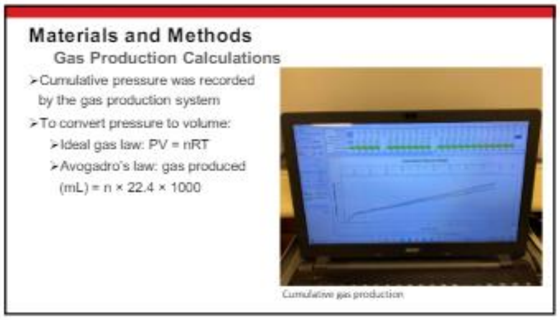

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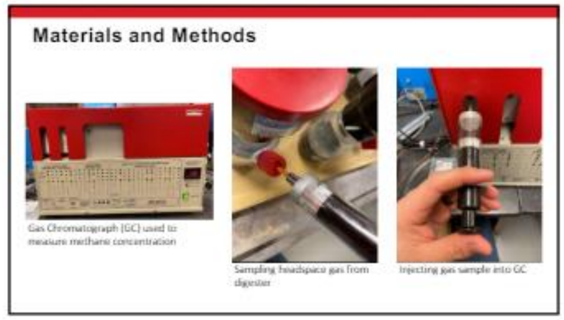

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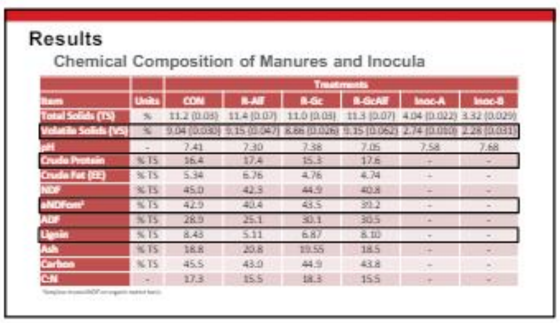

		Treatments					
Item	Units	CON	R-Alf	R-Gc	R-GcAlf	Inoc-A	Inoc-B
Total Solids (TS)	%	11.2 (0.03)	11.4 (0.07)	11.0 (0.03)	11.3 (0.07)	4.04 (0.022)	3.32 (0.029)
Volatile Solids (VS)	%	9.04 (0.030)	9.15 (0.047)	8.86 (0.026)	9.15 (0.062)	2.74 (0.010)	2.28 (0.031)
pH	-	7.41	7.30	7.38	7.05	7.58	7.68
Crude Protein	% TS	16.4	17.4	15.3	17.6	-	-
Crude Fat (EE)	% TS	5.34	6.76	4.76	4.74	-	-
NDF	% TS	45.0	42.3	44.9	40.8	-	-
aNDFom[1]	% TS	42.9	40.4	43.5	39.2	-	-
ADF	% TS	28.9	25.1	30.1	30.5	-	-
Lignin	% TS	8.43	5.11	6.87	8.10	-	-
Ash	% TS	18.8	20.8	19.55	18.5	-	-
Carbon	% TS	45.5	43.0	44.9	43.8	-	-
C:N	-	17.3	15.5	18.3	15.5	-	-

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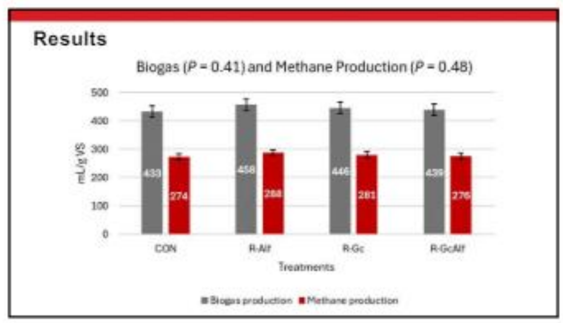

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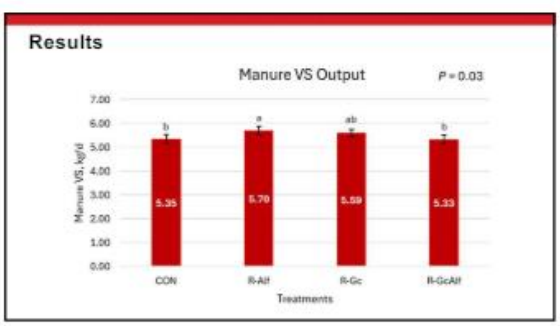

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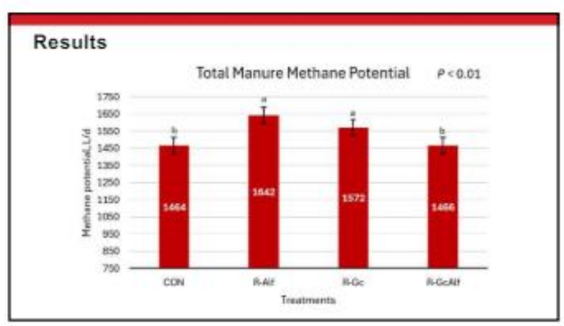

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Conclusions

- Dietary treatments did not affect manure methane and biogas production in laboratory anaerobic digesters
- Manure VS output increased when DDGS replaced alfalfa
- Total manure methane potential increase when DDGS replaced alfalfa or ground corn

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Overall Conclusions

- Productive responses to feeding DDGS are largely influenced by what ingredient in the diet is replaced
- Total manure methane potential may be influenced by diet composition
- Future research should explore how to manipulate the digestible VS portion of manure and influence its methane production in an anaerobic digester

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