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Effects of Feeding High-Moisture Corn Grain with Slow-Release Urea in Dairy Diets on Lactational Performance, Energy and Nitrogen Utilization, and Ruminal Fermentation Profiles by Lactating Cows

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EFFECTS OF FEEDING HIGH-MOISTURE CORN GRAIN WITH SLOW-RELEASE UREA IN DAIRY DIETS ON LACTATIONAL PERFORMANCE, ENERGY AND NITROGEN UTILIZATION, AND RUMINAL FERMENTATION PROFILES BY LACTATING COWS

by

Braden M. Tye

A thesis submitted in partial fulfillment
of requirements for the degree

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ABSTRACT

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by

Braden Tye, Master of Science

Utah State University, 2016

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Department: Animal, Dairy, and Veterinary Sciences

The objective of this experiment was to determine if nutrient utilization and energy partitioning by lactating dairy cows would differ in response to dietary corn grain (**CG**) types [steam-flaked corn (**SFC**) vs. high-moisture corn (**HMC**)] and to test if the types of CG would interact with slow-release urea (**SRU**) on lactational performance and energy utilization. Eight multiparous Holstein cows (32 ± 8.2 days-in-milk) were used in a duplicated 4 × 4 Latin square with one square consisting of ruminally cannulated cows. A 2 × 2 factorial arrangement was used to test 4 dietary treatments: SFC without SRU, SFC with SRU, HMC without SRU, and HMC with SRU. The experimental diets contained 60.5% dry matter (**DM**) of forages, whereas 12.9% or 14.4% DM of SFC or HMC was added in the diets, respectively. The SRU was supplemented at 0.46% DM, replacing a mixture of soybean meal and canola meal in a 50:50 ratio. Feeding HMC decreased intakes of DM, crude protein, and fiber compared with SFC. Supplementation of SRU did

not affect intakes of DM and nutrients, whereas it tended to increase intakes of DM or increased crude protein intake under SFC but no effect under HMC, leading to CG × SRU interactions on DM and crude protein intakes. Neither type of CG nor SRU supplementation affected milk production except that cows fed HMC-based diets tended to decrease energy-corrected milk yield compared to those fed SFC-based diets. Utilization of HMC in the diet had a tendency to increase dairy efficiency based on milk yield over SFC utilization. Cows fed HMC diets gained more body weight (**BW**) than those fed SFC diets, whereas supplementing SRU tended to reduce BW gain regardless of type of CG. Cows fed HMC diets shifted more net energy into BW compared with those fed SFC diets, whereas supplementing SRU tended to decrease a portion of net energy partitioned into BW gain under both SFC and HMC diets. Dietary treatments exerted minor impacts on ruminal fermentation profiles. Feeding HMC diets decreased fecal N excretion compared with SFC diets. In addition, supplementing SRU increased fecal N excretion under SFC, but it was decreased by SRU with HMC, leading to an interaction between CG and SRU. These collective results demonstrate that feeding HMC with SRU can be a practical option in high-forage lactation diets to maintain or improve nutrient and energy utilization efficiency and minimize negative environmental impacts.

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LIST OF ABBREVIATIONS

ADF = acid detergent fiber

AH = alfalfa hay

BCS = body condition score

BW = body weight

CG = corn grain

CP = crude protein

DE = digestible energy

DM = dry matter

DMI = dry matter intake

ECM = energy corrected milk

FCM = fat corrected milk

FE = feed efficiency

GE = gross energy

HI = heat increment

HMC = high-moisture corn

HMC+SRU = total mixed ration with high-moisture corn and slow-release urea

HMC–SRU = total mixed ration with high-moisture corn and no slow-release urea

HP = heat production

IOFC = income over feed cost

LRCpH = Lethbridge research centre ruminal pH measurement system

MCP = microbial crude protein

ME = metabolizable energy

MUN = milk urea nitrogen

N = nitrogen

NDF = neutral detergent fiber

NE = net energy

NE_G = net energy gain

NE_L = net energy lactation

NE_M = net energy metabolism

NEF = nitrogen efficiency

NEFA = non-esterified fatty acid

NH_3-N = ammonia nitrogen

NPN = non-protein nitrogen

NRC = national research council

OM = organic matter

RDP = ruminally degradable protein

RUP = ruminally undegradable protein

SARA = sub-acute ruminal acidosis

SFC = steam-flaked corn

SFC+SRU = total mixed ration with steam-flaked corn and slow-release urea

SFC–SRU = total mixed ration with steam-flaked corn and no slow-release urea

SBM = soybean meal

SBMCM = soybean meal canola meal 50:50 mix

SRU = slow release urea

TMR = total mixed ration

TTD = total-tract digestibility

TP = true protein

VFA = volatile fatty acid

INTRODUCTION

The major component of diets for lactating dairy cows is forage that provides energy and nutrients, and forage fiber is important for healthy cows, stimulating rumination and saliva production that aids in ruminal digestion and fermentation. In spite of their benefits to the cow, forages are not always efficiently utilized. For example, lactational performance may be limited by excessive ruminally degradable protein (**RDP**) from alfalfa and/or the availability of degradable starch from corn silage. In addition, too much physically effective neutral detergent fiber (**NDF**) can reduce DM intake (**DMI**) of dairy cows, as the concentration of physically effective NDF increases, DMI decreases due to rumen fill (Zebeli et al., 2007). To maximize nutrient utilization in high-forage lactation diets, the addition of highly digestible carbohydrates to the diets is a common method to increase the energy available to the cow. High-moisture corn (**HMC**) has consistently greater starch digestion in the rumen, the small intestine, and the total tract compared with dry-rolled and steam-flaked corn (**SFC**; Wilkerson et al., 1997; Knowlton et al., 1998; Firkins et al., 2001). Eun et al. (2014) found that feeding HMC in high-forage diets increased NDF and crude protein (**CP**) digestibilities, microbial protein synthesis, and feed and N utilization efficiencies with a decrease in DMI relative to SFC. Consequently, cows fed with HMC may have improved energetic efficiency compared with those fed with SFC. However, there is no information in literature in regard to how feeding HMC affects energy utilization and partitioning by lactating dairy cows fed high-forage diets.

In order to also maximize nutrient utilization in lactating diets, it is important to

synchronize nutrient availability in the rumen. Otherwise, excess feed N is deaminated and excreted as urea, a N waste compound, in urine and milk, while undigested rumen undegradable protein (**RUP**) and metabolic N (sloughed intestinal cells and hind gut fermentation products) are excreted in the feces (VandeHaar and St. Pierre, 2006). Thus, it is important to maintain microbial protein synthesis by meeting the protein requirement of the cow with the lowest dietary CP input, while still maintaining the best ratio between RDP and RUP to support milk production and optimize N utilization efficiency (Agle et al., 2010). A high-quality legume such as alfalfa hay (**AH**) alone is unable to meet these requirements and, therefore, must be supplemented with other protein sources. In addition to soybean meal and canola meal, very common protein supplements, slow-release urea (**SRU**), which is urea coated in vegetable oil, has been used in lactation rations by slowing its release of ammonia in the rumen. In a recent study, when SRU was added to high-forage dairy diets consisting of 24.5% AH and 30.4% corn silage of total dietary DM where SRU replaced SBM and canola meal, there were increased feed and N utilization efficiencies compared with a control diet (1.46 vs. 1.35 and 0.28 vs. 0.25, respectively; Neal et al., 2014). Hence, SRU has potential to improve nutrient utilization and lactational performance when supplemented in lactation diets consisting of a relatively great concentration of AH. The objective of the present study was to investigate the effects of nutrient utilization and energy partitioning by lactating dairy cows when fed diets that differed in dietary corn grain sources (SFC vs. HMC) and to test if the sources of corn grain would interact with SRU on lactational performance and energy utilization.

REVIEW OF LITERATURE

Improving the efficiency of conversion of feed energy and nutrients into milk by lactating dairy cows has been suggested as one of the most critical factors toward sustainable dairy production. A main focus of this review is to provide a recent development on energy metabolism and efficiency of dairy diets with a relatively high forage proportion, and how these metabolic events influence lactational performance of dairy cows.

Energy Partitioning

Energy is vital to the function of all cells, and thus physiologically, it is vital for tissue maintenance and growth, milk synthesis, and fetal development. Level of activity and environmental stress affect the energy required for body maintenance. In order to properly provide the energy needs to dairy cows, it is necessary to take into account the many facets of different metabolic functions, the energy content of tissue and milk, and the efficiency of energy utilization by different tissues. Equally important is determining the energy availability from different feeds. Composition of the feeds, physical and chemical forms of the feeds, and the effects of feed intake by the animals on digestibility affect the actual energy available from the feeds. Depending upon physiological status, the available energy will be partitioned between maintenance, tissue gain, and milk production by lactating dairy cows.

Energy Distribution in Dairy Cattle

Gross energy (**GE**) intake is the amount of energy that is in the feed an animal consumes. This energy amount is often determined using the heat of combustion for each individual ingredient which is measured by using a bomb calorimetry device. Digestible energy (**DE**) is the GE minus the energy excreted in the feces. Metabolizable energy (**ME**) is DE minus the energy used in gas (methane) production and through urine excretion. Therefore, ME is the energy available for metabolism by the animal. However, heat generated from digestion and fermentation is lost and does not account for energy available for metabolic processes in the animal and should be deducted from the ME value. The heat loss from fermentation of feed in the digestive tract (primarily in the rumen) and from metabolism of cells is referred to as heat increment (**HI**). The ME minus HI provides the amount of energy actually available for cell function and is thus referred to as net energy (**NE**). The NE unit is subdivided into the energy needed for maintenance, growth, and lactation. The reason for this is that energy used for different processes is used with different efficiencies. For mature dairy cattle, only NE for lactation ($\mathbf{NE_L}$) is used, because the efficiency of energy utilization for maintenance (0.62) is similar to that for lactation (0.64; Moe et al., 1971). Therefore, the NE_L for dry cows includes the energy needed for maintenance and fetal growth, and the NE_L for lactating cows includes the energy needed for maintenance and lactation. Because energy use for growth is only 50 to 70% as efficient as the energy for maintenance, NE for maintenance ($\mathbf{NE_M}$) and NE for growth ($\mathbf{NE_G}$) are used separately for growing dairy animals. Therefore, in the computer model used in the current NRC (2001), NE_L feed

values express the requirement for maintenance, pregnancy, milk production, and changes in body reserves of adult dairy cows (NRC, 2001). Defining these terms and their respective energy values is critical to the dairy industry, so there has been strong needs to better understand the energy utilization by dairy cows and to improve the system to optimize ration formulation.

The amount and type of nutrient supplied can have a large impact on nutrient and energy available for partitioning. Van Knegsel et al. (2007) found that lipogenic nutrients when compared with glucogenic nutrients can shift energy partitioning from milk fat synthesis to adipose tissue deposition in the body. In ruminants, lipogenic nutrients originate from one of three sources: 1) dietary fiber consumption that stimulates the ruminal production of acetate and butyrate, 2) dietary fat, and 3) mobilized body reserves (Van Knegsel et al., 2007). Van Soest (1963) suggested that diets that depress milk fat lower the priority for milk fat synthesis relative to fat deposition in body reserves. This suggests that lipogenic nutrients increase the partitioning of ME into milk and decrease the ME partitioned into body reserves (Van Knegsel et al., 2007). On the other hand, glucogenic nutrients are derived mainly from starch and fiber fermentation in the rumen. The three main glucogenic precursors for gluconeogenesis by the liver are propionate, lactate, and glucogenic amino acids like alanine (Aschenbach et al., 2010). These glucogenic substrates increase blood glucose and insulin concentrations and can decrease milk fat concentration. This implies that glucogenic nutrients may cause the cow to partition ME toward body tissue.

Not only the type of nutrients in the diet affects energy partitioning, but also the

amount of certain nutrients has been found to have an effect on the way energy is used by lactating dairy cows. McCarthy et al. (2015) showed this concept in a study where 70 Holstein dairy cows were fed diets with a low- (21.5% DM) or a high-starch (26.2% DM) diet. Cows fed the high-starch diet had less negative energy balance and decreased body condition score (**BCS**) change; while both diets resulted in the loss of BCS, cows fed the high-starch diet lost less than those fed the low-starch diet (–2.05 vs. –4.50 Mcal/d, respectively; McCarthy et al., 2015). These results suggest that the cows fed the high-starch diet partitioned more energy into body reserves, and consequently retained greater BCS than those fed the low-starch ration.

Although energy utilization can be investigated by its partitioning, intermediary metabolism must be studied in relation to overall body processes of the cow as a whole. This approach will result in comprehensive understanding on energy metabolism and its impacts on animal performance. Thus, study of feed composition, energy balance, and productive measurements need to be supplemented with knowledge gained through the study of intermediary metabolism.

Energy Balance

The term energy balance generally refers to the body energy state of dairy cows and can be defined as the difference between dietary energy intake and energy utilization required by production, body maintenance, and gestation (Butler and Smith, 1989). Negative energy balance corresponds with loss of body weight (**BW**) while positive energy balance with gain in BW. High producing dairy cows usually cause a negative

energy balance in early lactation due to the extreme energy demand for milk production with energy demands around 3-fold greater for lactation than for late gestation (Stocks and Allen, 2014). This is often coupled with lower feed intake after parturition which can exacerbate the situation further by lowering the amount of nutrients entering the body for digestion. The energy demand required by a cow to produce milk can vary and is often dependent on the amount of energy that can be supplied to the mammary gland (Coffey et al., 2002). Decreased DMI often causes the animal to mobilize their body fat and in some cases protein reserves in order to maintain the energy demand for milk production. The resultant adipose tissue mobilization and lipolysis cause elevated concentration of non-esterified fatty acids (**NEFA**) circulating in blood serum and, if their complete oxidation fails in the liver, ketone bodies in the serum will also rise (Mahrt et al., 2014). Elevated ketone bodies in the blood serum (subclinical/clinical ketosis) have been linked to many negative health effects even after positive energy balance has been restored, which usually happens around 40 to 80 d post-partum (Coffey et al., 2002; Mann et al., 2015). These events of subclinical ketosis not only cause economic loss but also increase the risk of displaced abomasum, metritis, lameness, reproductive performance, and milk yield (Mahrt et al., 2014).

Energy Requirements for Lactation

The NRC (2001) describes the NE_L as the energy contained in the milk produced or, in other words, the amount of energy in the individual milk components such as fat, protein, and lactose. The energy for these individual components are found by using the

energy value of the heat of combustion for these components and their reported values are 9.29, 5.71, and 3.95 Mcal/kg for milk fat, protein, and lactose, respectively. Fat and protein concentrations in the milk vary significantly based upon DMI, health status, and environmental factors. However, lactose concentration in the milk is fairly constant at approximately 4.85% of milk (NRC, 2001). When calculating the NE_L requirements from milk production, the NRC has outlined formulas from milk production data. Equation 1 uses all three components:

1) NE_L (MCAL/KG) = 0.0929 × FAT, % + 0.0547 × CRUDE PROTEIN, % + 0.0395 × LACTOSE, %.

Equation 2 uses fat and protein components:

2) NE_L (MCAL/KG) = 0.0929 × FAT, % + 0.0547 × CRUDE PROTEIN, % + 0.192.

Equation 3 uses milk fat content only:

3) NE_L (MCAL/KG) = 0.0969 × FAT, % + 0.360.

The NE_L value can also be calculated as the proportion of ME that is used for milk production. Equation 4 calculates the difference between ME and heat production (**HP**):

4) NE_L (MCAL/KG) = ME (MCAL/KG) – HP (MCAL/KG).

Wilkerson et al. (1997) showed that high-moisture corn yielded greater values of NE_L when fed in a lactation dairy diet when compared with dry corn (1.84 vs. 1.63 Mcal/kg, respectively). Boerman et al. (2015) showed that high-fiber, high-fat diet containing a 50:50 of forage:concentrate containing a C16:0-enriched fatty acid supplement at 2.5% DM increased the proportion of energy partitioned toward milk (72.8 vs. 67.9%) and

reduced that of energy partitioned toward body tissue gain (4.03 vs. 10.1%) when compared with a high-starch diet containing a 40:60 of forage:concentrate containing a mixture of dry ground and high-moisture corn. The two previous mentioned studies show the importance in different ration ingredients in the value of NE_L. The current system of NE_L calculations outlined above takes a broad consideration at general requirements for total milk energy output. The NRC (2001) has suggested that future NE requirements identify more detailed substrate requirements for the individual components of milk. This approach may yield greater information on how to utilize feed ingredients to partition energy to higher NE_L values.

Efficiency of Dairy Cattle

In the dairy industry, efficiency has come under increasing attention from not only the public but from government agencies and dairy producers themselves, as efficiency has been shown to affect farm profitability and the environment (Phuong et al., 2013). There are several measures to use when determining efficiency in dairy cattle; however, some of the more critical could be as follows.

Feed Efficiency

In lactating dairy cattle feed efficiency (**FE**) is often stated as the relative ratio of output to input, with a common criteria of measurement being milk produced per unit of feed consumed, which can be seen in Equation 5:

5) FE = MILK YIELD (KG/D)/DMI (KG/D).

This FE is often calculated ignoring the milk component composition. In order to calculate for milk composition, fat-corrected (**FCM**) or energy-corrected milk (**ECM**) can be used in calculations with the following equations (6 and 7):

6) FE = FCM YIELD (KG/D)/DMI (KG/D),

6a) FCM = [0.4324 × milk yield (kg/d)] + [16.216 × fat (kg/d)],

7) FE = ECM YIELD (KG/D)/DMI (KG/D),

7a) ECM = [0.327 × milk yield (kg/d)] + [12.95 × fat (kg/d)] + [7.65 × protein (kg/d)].

These FE measurements can be used as a benchmark when evaluating herd efficiency performance of DM into salable milk (Britt et al., 2003).

Nitrogen Efficiency

Nitrogen efficiency (**NEF**) in dairy cows can be described as the conversion of dietary nitrogen (**N**) into milk N and can often be calculated using the 2 equations as follows (8 and 9):

8) NEF = CP IN MILK/CP INTAKE,

9) NEF = MILK N/N INTAKE.

Economic Efficiency

Economic efficiency can be an important goal for dairy production enterprises, as feed prices are the single largest milk expense in milk production (Wolf, 2010). Therefore, increase of main incomes to the farm while reducing the main variable cost

(feed) can potentially improve profitability of dairy cows. Income over feed cost (**IOFC**) is defined as the income from milk sales minus the feed cost to produce it, which can be seen in equation 10:

10) IOFC ($/D/COW) = GROSS INCOME ($/D/COW) – FEED COSTS ($/D/COW).

Factors Affecting Efficiency

There are many factors to consider when evaluating efficiency in dairy cattle. Level of milk production, type of diet, body size, changes in body tissue mass, environmental conditions, exercise, and age at first calving can all have an effect on dairy cow efficiency (VandeHaar, 1998). Among these, level of production is one of the most important factors, with greater milk production being associated with more efficient partitioning of feed nutrients (VandeHaar, 1998). Arndt et al. (2015) reported this concept when the authors assessed the differences between high- and low-FE cows. The authors concluded that high-FE cows had 103% greater milk production (43.1 vs. 21.2 kg/d) with just a 15% increase in DMI (23.7 vs. 20.6 kg/d). This resulted in 78% greater (1.82 vs. 1.03 milk kg/kg of DMI) FE in high-FE cows when compared to low-FE cows.

Energy balance often follows level of milk production with negative energy balance starting in early lactation and continuing past peak lactation. When calculating FE it is necessary to take into account adjustments for energy balance. Under negative energy balance, the cow typically retrieves nutrients from its body reserves to put toward milk production. This may make the cow seem more efficient than it really is by not

calculating for the nutrients that are being provided from endogenous sources. The same deception can happen with late lactation when the cow is in positive energy balance and partitioning dietary nutrients back into its body reserves (Arndt et al., 2015). However, this loss of body tissue during early lactation and replenishment in later lactation are efficient processes (VandeHaar, 1998). Moe et al. (1971) calculated the net efficiency of converting feed to body tissue and then to milk and concluded that it was as efficient as converting feed directly to milk. Using the idea of utilizing body reserves in order to efficiently produce milk, VandeHaar (1998) reported that loss of 1 BCS unit during the first 60 d of lactation would result in 8 kg of milk/d more from the energy provided. The extra 8 kg/d of milk would result in 2,000 kg more milk over a 305-d lactation period. This was calculated by assuming that 1 unit of BCS (five-point scale, from 1 = thin to 5 = obese) has a tissue energy of 400 Mcal of ME (Ferguson, 1991), and that tissue energy is converted to milk with 82% efficiency (Moe et al., 1981). While this concept of using body reserve energy as an efficient way to produce milk is intriguing, the negative effects of losing that much BCS during the lactation period would have to be weighed when considering the usefulness of this strategy.

A more rational way to approach improving efficiency in dairy cattle is to address nutritional and dietary factors, with dietary level of energy being one of the most critical (Smith, 1988). Whether calculating on a GE, DE, or ME basis, the net efficiency of converting fiber to milk is less than that for starch and protein (VandaHaar, 1998). Therefore, focusing on getting the best efficiency out of starch sources in the diet of lactating dairy cows has become increasingly important. In the US, corn grain is the

principal source of dietary starch for lactating dairy cows (Oba and Allen, 2003). How that corn grain in the diet is processed can affect efficiency in dairy cows. Ferraretto et al. (2014) reported that when HMC was used in the diet of lactating Holstein cows, it increased FE over the use of dry ground corn. Values of kg of milk/kg of DMI increased from 1.50 to 1.58 when HMC replaced dry ground corn in the diet. The HMC also had a significant effect on the total-tract digestibility as a percentage of intake when compared to dry corn with values of 94.2 and 92.0%, respectively. These results from Ferraretto et al. (2014) suggest that the increased digestibility of HMC can increase the FE of a diet. Ferraretto et al. (2014) also concluded that there was no effect in FE when HMC was ensiled at < 2,000 μm or > 2,000 μm particle size (1.67 vs. 1.65 milk/DMI). The same results of no difference were found for dry corn when it was processed at 500-1,000 μm, 1,000-1,500 μm, 1,500-2,000 μm, 3,000-3,500 μm with values of 1.55, 1.56, 1.50, 1.53 milk/DMI, respectively. Collectively, these data further suggest that it is the difference in processing that affects FE. Eun et al. (2014) reported in a study where corn was fed with low-quality or high-quality alfalfa hay, HMC improved FE over SFC in lactating dairy cows. The authors reported values of milk/DMI increased from 1.11 to 1.25 when HMC was used with fair-quality alfalfa hay and 1.10 to 1.18 when HMC was used with high-quality alfalfa hay. Results like these from Eun et al. (2014) show when corn is utilized in a diet as an energy source the processing method of the corn can help improve FE. This can have important benefits in dairy nutrition, because energy is the most limiting nutrient to the modern US dairy cow. Energy is also the nutrient most closely connected to level of milk production (VandeHaar, 1998). However, in order to ensure proper

function of ruminal microorganisms, appropriate ratios of structural and non-structural carbohydrates, fat, and protein must be included in the diet (Fox et al, 1992).

In addition to dietary energy, dietary CP can also affect FE. Levels of CP in the diet are important to reach maximal milk production and can often be fed in excess to make sure that there is a sufficient supply of metabolizable protein. However, research has continually shown that lowering protein concentration of the diet is the most effective strategy to improve the efficiency of dietary N utilization for milk protein synthesis (Giallongo et al., 2015). Excess levels of dietary protein or high degradability of dietary protein can increase ammonia production in the rumen. As a result, ammonia concentrations in the blood increases, causing increased rate of ammonia conversion to urea in the liver. This rise in ammonia conversion requires extra energy and thus reduces energetic efficiency (Martin and Blaxter, 1965).

Research has shown that feeding diets with excessively high CP concentration or even excess ruminally degradable protein decreases protein efficiency in dairy cows (Olmos Colmenero and Broderick, 2006; Wang et al., 2007). Lower NEF coupled with the relative high price for protein supplements also impacts economic efficiencies (Broderick, 2003). Feeding 1 kg of excess CP is equivalent to 0.72 Mcal of NE_L. If a lactating dairy cow is producing 45 kg of milk/d and consuming 25 kg of DMI in a diet that requires 17% CP DM, then feeding an extra 2 percentage units and increasing the DM CP to 19%, would decrease milk yield by 0.5 kg/d and decrease gross efficiency by 0.3 percentage unit (VandeHaar, 1998), which may seem minimal; however, the decrease in percentage units of gross efficiency may be worth noting in this situation.

One challenge when dealing with FE is that when feed intake is increased, feed digestibility does down. So even though the cow consumes more nutrients, the cow may not be receiving more energy as a result. This loss of digestible energy associated with high rate of passage in cows with high DMI and milk production and the fact that marginal increases in FE decreases with increasing milk production, may affect future selection for higher milk production alone in dairy cows (NRC, 2001). Therefore, there needs to be further exploration on ways to improve FE. Theoretically this can be achieved by altering the amount of GE that is available for distribution to metabolic and productive uses. This can then in turn reduce the amount of energy lost in any of the following: feces, urine, gas production, maintenance, body gain, or heat. Consideration of the way energy is utilized can then help determine where the sensitivities are into improving energy and N efficiencies in high producing dairy cows (Arndt et al., 2015).

Forage versus Concentrate as a Source of Energy

Utilization of Forage

Evaluating dairy rations and their component parts can help in understanding how these nutrients participate in energy partitioning and efficiencies in dairy cattle. When evaluating dairy rations one major component is fiber, which provides energy and nutrients. Forage fiber also plays a critical role in ruminal digestion and fermentation by stimulating rumination and saliva production (Zebeli et al., 2007). Highly digestible forage in the ration can also increase nutrient utilization and increase rumen function.

Because of this, it is common in the dairy industry to feed high producing cows a ration that is high in forage concentration (Nocek and Tamminga, 1991). Two common sources of forage in high producing dairy cows is corn silage and legume or grass hay (Lopes et al., 2015). It is common for lactation dairy diets in the western US to utilize high amounts of forage and feed a ration with a 60:40 of forage:concentrate (Holt et al., 2010). A benefit of greater forage-to-concentrate ratio in the diet is that it can help increase the amount of milk fat precursors that are available for milk synthesis. Cellulolytic bacteria in the rumen digest the fiber from forages and ferment it to mainly acetate and butyrate. These two VFA, especially acetate, are known to be used as precursors in the synthesis of milk fat in the mammary gland. Moe (1981) showed this concept when he reported that as the forage-to-concentrate ratio in the diet increased, the acetate-to-propionate ratio also increased. Moe (1981) reported acetate-to-propionate ratios of 2.00, 2.57, and 3.32 with forage-to-concentrate ratios of 20:80, 40:60, and 60:40, respectively. These increases in acetate from Moe (1981) also correlated with greater milk fat concentrations, with 2.7, 3.0, and 3.5% from 20:80, 40:60, and 60:40 of forage:concentrate in the diet. Likewise, Yang and Beauchemin (2007) showed that when diet was changed from 35:65 to 60:40 of forage:concentrate, milk fat concentration increased from 3.45 to 3.84%.

Along with fiber, high producing dairy cows are often fed an increased amount of concentrates and energy supplements to meet the demand of energy toward milk production. While this practice is beneficial to reach maximum milk production potential, it can cause health and functionality issues in the rumen (Beauchemin et al., 2003). When greater amounts of concentrates are fed in the diet, it can lower ruminal pH from

increased fermentation acid production (NRC, 2001). This is detrimental, because the rumen is designed to function optimally between pH range of 6.2 and 7.2 (Yang and Beauchemin, 2007). A drop in ruminal pH puts the cow at risk for subclinical ruminal acidosis (**SARA**). When a cow is experiencing SARA conditions, fiber digestion and microbial protein synthesis and AA supply to the small intestine are typically reduced (Yang and Beauchemin, 2007). Formulating diets to insure adequate fiber particle length has become a means of reducing SARA in the rumen. This concept, known as physically effective fiber, was introduced originally by Mertens (1997), and has the ability to promote chewing and increase salivary secretions that can then buffer the ruminal fermentation acids and raise the pH. The use of high-quality alfalfa hay has been shown by Eun et al. (2014) to keep the mean pH of the rumen at an optimal functioning range of 6.31, even with the supplementation of a highly digestible energy supplement such as HMC in the diet. The use of high-quality alfalfa hay in Eun et al. (2014) also showed an increase in milk yield (30.4 vs. 28.1 kg/d) when compared to fair-quality alfalfa hay used with HMC in the diet, thus showing the improved benefits of utilizing high-quality forages in lactation dairy rations.

Even with the reported benefits to the cow, forages are not always utilized efficiently. Lactational performance, for example, may be infringed by excessive RDP from high-quality alfalfa. Also, having too much physically effective fiber in the diet may reduce DMI due to increased rumen fill (Zebeli et al., 2007). Mertens (1997) described that when too much fiber is included in the ration, this can cause energy density, DMI, and productivity to all be lowered. This makes the use of physically effective fiber essential,

and there needs to be adequate amounts of dietary forage in the ration.

Utilization of Corn Grain

Along with fiber carbohydrates, corn grain is also extensively used in dairy cattle rations. Corn grain is the most common energy source used in dairy cattle rations and is the major source of dietary starch for lactating dairy cattle in the US (Oba and Allen 2003). Huntington (1997) describes how the biological function of the grain itself reflects its structure. The corn grain structure is set up that the embryo, or germ, and the endosperm are housed inside the pericarp. Inside the endosperm is the aleurone layer. Beneath the aleurone are the peripheral and corneous endosperm. These 2 layers contain starch granules that are embedded in a protein rich matrix. Beneath those layers is the floury endosperm. The floury endosperm contains the highest starch granule concentration. These starch granules in the floury endosperm are not housed in a protein matrix and are most susceptible to grain processing (Huntington, 1997). The structure of the starch granules constitutes mainly amylopectin and amylose, which are composed of α1-4 and α1-6 bonds. Along with the starch granules, there are small amounts of pectin and sugars, which collectively make up the non-structural carbohydrates in the grain (Nocek and Tamminga, 1991).

Starch is the most important part of the corn grain when considering its energetic value and houses most of the energy content that is released when the corn is fermented in the rumen. Corn grains contain as much as 70-80% starch inside their endosperm layers (Nocek and Tamminga, 1991). The starch in corn grain is very important to the

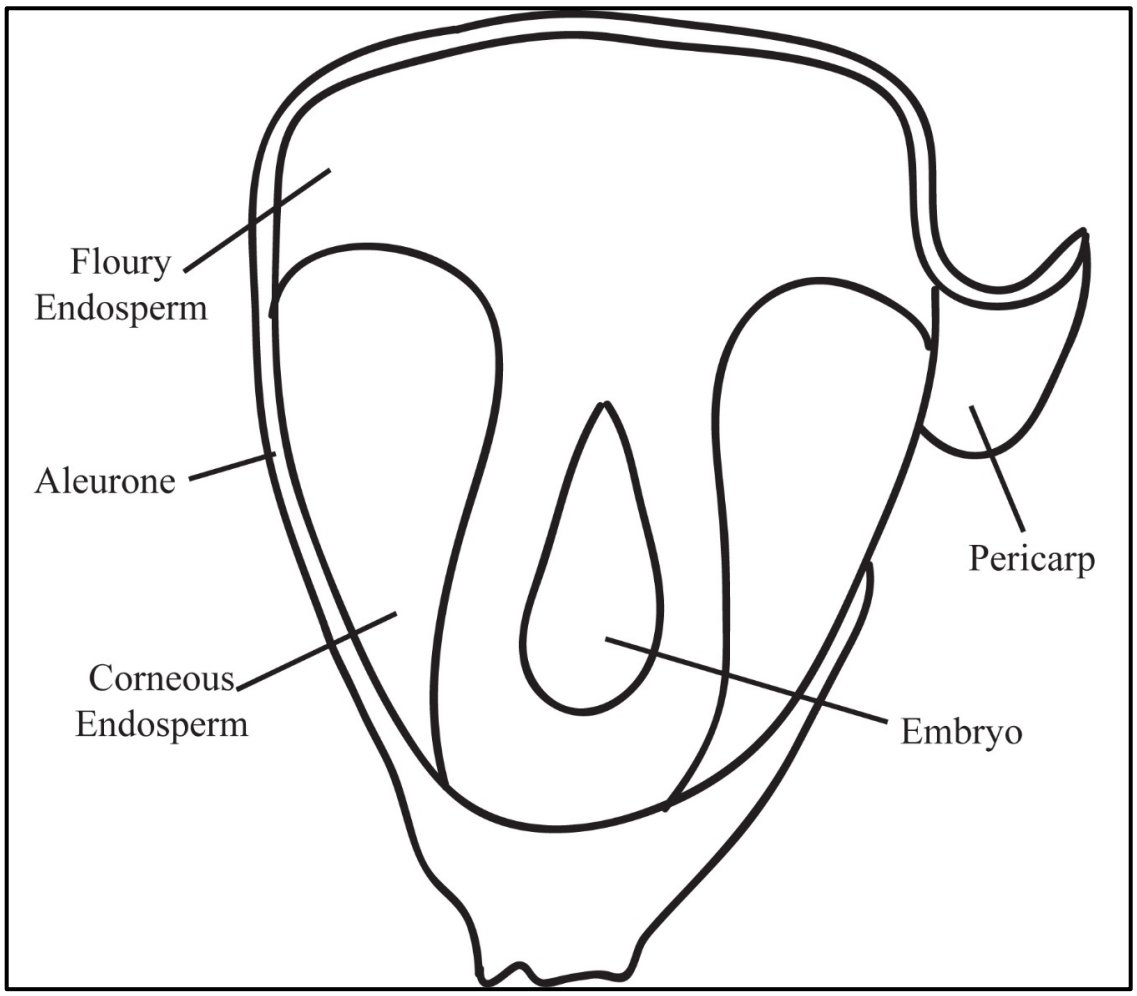

Figure 1. Corn grain diagram

dairy industry, because it provides energy to the ruminal microbes that are trying to undergo microbial synthesis. Carbohydrates are the main sources of energy used by ruminal microorganisms for maintenance and growth, and they have been shown to grow faster when using readily fermentable carbohydrates (Nocek and Tamminga, 1991). The majority of starch fermentation in the rumen is performed by ruminal bacteria which attach to the starch particles and have a high amylase activity (McAllister et al., 1994).

This allows the ruminal bacteria to release the amylolytic enzymes which hydrolyze the α1-4 and α 1-6 chemical bonds. This fermentation process of attachment by the major starch fermenting ruminal bacteria shows the importance of corn grain processing in ruminant feeds. Whole grain with an intact pericarp is almost entirely resistant to digestion by ruminal bacteria, because they are unable to attach (Huntington, 1997). That is why the practice of processing corn grains fed to ruminants is widely used today. Processing corn grains for cattle consumption has been used for many years and prior to 1960 there had not been much more to processing corn than simple grinding or dry rolling (Hale, 1973). Processing grain is performed by applying one or more of the applications of mechanical action, heat, and moisture (Theurer et al., 1999). Processing corn grains allows access to starch in the inner endosperm layers for attachment and access by bacterial enzymes in the rumen. If there is no mechanical action of breaking the grain open, then the animal relies on chewing to break open the pericarp layer.

There is a body of evidence to indicate clearly that there is an advantage of processing the corn grain on its digestibility in the animal. Research has shown that simple rolling or cracking whole corn increased its digestibility by up to 25% (Moe et al., 1973; Clark et al., 1975), and the authors contributed the increase in digestibility to an increase in the availability of the starch to the rumen microbes. Firkins et al. (2001) showed different ways in which the total-tract digestibility (**TTD**) of starch increased due to altering the corn grain in diets fed to lactating dairy cows. The authors reported that SFC had a higher TTD than steam-rolled corn (94.2 vs. 88.8%), and steam-rolled corn had a higher TTD than dry-rolled corn (88.8 vs. 85.0%). High-moisture corn was

reported to have greater ruminal starch digestion than SFC with values reported as 86.8% of intake for HMC and 56.9% of intake for SFC (Firkins et al., 2001). Similarly, Huntington (1997) reported that the amount of ruminal starch degraded was influenced by the type of processing done to the corn grain. The author reported HMC had the greatest ruminal starch digestibility with 89.9%, while SFC had 84.8% and dry-ground corn was the least with 76.2%. Firkins et al. (2001) also found that particle size had an effect on starch digestibility with finely ground corn having a greater TTD of starch than rolled or cracked corn. In a review of 17 different lactation studies, Theurer et al. (1999) reported that the TTD of starch in these 17 studies increased from 83.7 to 97.1% when SFC was compared with dry-rolled corn. This same review also showed greater ruminal starch digestibility for SFC when compared with dry-rolled corn (52 vs. 35 %; Theurer et al., 1999). Not all research has found the increased digestibility of starch when improved processing has been performed on the corn grain. Joy et al. (1997) reported no difference in ruminal or post-ruminal starch digestion in lactating Holstein cows when SFC was compared with dry-rolled corn. However, research has continued to show that SFC and HMC have a greater starch digestibility than whole corn or even dry-cracked or dry-rolled corn.

Lopes et al. (2009) found both in vitro and in situ experiments that differing amounts of endosperm contained in the corn affected nutrient digestibility in lactating dairy cows. The authors reported that using floury and opaque endosperm corns increased in vitro ruminal starch digestibility when compared with vitreous endosperm corn with values reported at 91, 85, and 62% starch digested, respectively, over 7 h. Floury and opaque

endosperm was also shown to increase TTD when fed to dairy cows. The reported values of TTD in Lopes et al. (2009) increased from 89.6 to 95.1 and 96.6% for vitreous, floury, and opaque endosperm treatments, respectively. Taylor and Allen (2005) also found that using a floury, 3% vitreousness, over a 67% vitreousness endosperm in dry corn fed to lactating dairy cows, increased ruminal and total-tract starch digestibility.

It is important to point out that if there is too much starch digestion in the rumen it can lead to a decrease in DMI. In a review by Allen (2000), the author reported that greater starch digestion in the rumen was associated with reductions in DMI in 3 out of 10 comparisons. This is of importance, because if the use of greatly fermentable starch in early lactation diets causes reduced DMI, it can reduce energy intake and further exacerbate the negative energy balance of that animal. Oba and Allen (2003) reported that when HMC was compared with dry-ground corn, HMC decreased DMI by 1.7 kg/d (20.8 vs. 22.5 kg/d). The authors reported that difference was due to increased ruminal digestion of starch. In a recent study, Eun et al. (2014) reported that when HMC was compared with SFC in a lactation dairy diet fed with high-quality alfalfa hay, DMI decreased with HMC from 27.4 to 25.7 kg/d. However, not all findings report a decrease in DMI due to increased starch digestion or fermentability. Alvarez et al. (2001) performed a study in which HMC replaced dry-cracked corn in a lactation grazing diet of Holstein cows. High-moisture corn replaced dry-cracked corn as an added energy source from greater starch digestion. In this study, total DMI was not different for either corn grain (21.0 vs. 20.5 kg/d). In a study performed using 24 lactating Holstein cows fed SFC compared with steam-rolled corn, Chen et al. (1994) found that with a similar starch

concentration (30.0 vs 29.6%) no difference in DMI was seen between the 2 corn grain treatments. Allen (2000) reported that there are many factors that can influence DMI and energy intake in dairy cows and stated that some of those factors are fiber concentration, ease of hydrolysis of starch and fiber, particle size, particle fragility, silage fermentation products, concentration and characteristics of fat, and the amount and ruminal degradation of protein. Corn grain processing is intimately intertwined with many of these factors and should be evaluated when determining best practices for DMI of lactating dairy cows.

Whether through increased energy available or increased efficiencies, processed corn grains like SFC and HMC have been shown to increase milk production in lactating dairy cows. Theurer et al. (1999) performed a review of 19 different lactation studies in which they assessed the effects of steam flaking influenced the performance of lactating dairy cows. The authors found that when SFC was compared with steam-rolled corn, the milk yield was greater (38.0 vs.35.8 kg/d). It was also shown that SFC had greater milk protein yield compared with steam-rolled corn (1.16 vs. 1.07 kg/d). Chen et al. (1994) also found that SFC yielded greater milk production compared with steam-rolled corn (34.6 vs. 32.1 kg/d) with increased milk protein yield (1.01 vs. 0.92 kg/d). Similarly, Firkins et al. (2001) showed that milk yield increased from 35.8 to 38.0 kg/d when feeding HMC compared with steam-rolled corn. The difference seen in milk production in these 3 previously mentioned studies could be related to findings of Preston et al. (1993) who reported that starch digestibility was positively related to the degree of starch that was gelatinized in processing, and the authors reported that steam-flaking increased

gelatinization even over steam-rolling. The results of greater milk protein yields could have been due to the digestibility of energy and N in the rumen to increase microbial protein outflow.

Utilization of Protein and Nitrogen

An overview of protein metabolism can show that it is multifaceted and requires examination of protein quality, protein segments, and protein interaction with other nutrients in the diet and rumen microorganisms. Dietary CP content includes both protein and non-protein nitrogen (**NPN**), which can be in the form of rumen degradable protein (**RDP**) and rumen undegradable protein (**RUP**; NRC, 2001). Dietary RDP is made up of protein segments that are degraded in the rumen and consist of peptides, AA, and ammonia-N which can be used to support microbial protein (**MCP**) synthesis. In contrast, RUP consists of protein segments that escape ruminal fermentation and pass on to the lower gastrointestinal tract where it is available to be absorbed. Along with dietary CP sources, there are N fractions metabolized from endogenous sources as well. Endogenous sources of protein can include sloughed-off cells of the gastrointestinal tract and metabolic enzymes secreted in the abomasum (Tamminga, 1992). Protein requirements in lactating dairy cows are met from the supply and absorption of AA reaching the small intestine that are needed to carry out the synthesis of proteins required for maintenance, growth, reproduction, and milk production of dairy cattle (NRC, 2001). These AA come from MCP, RUP, and endogenous secretions, which constitutes metabolizable protein

(**MP**). Nitrogen output from the rumen mainly consists of microbial protein, RUP, and ammonia-N; with up to 50-80% being comprised of microbial protein (Bach et al., 2005). The varying range in microbial supply to the small intestine depends on the nutrient availability and efficiency by the ruminal bacteria (Bach et al., 2005).

Proper feeding of RDP can reduce the risk of depressed MCP, ruminal digestion, and energy and protein availability to the cow (Clark et al., 1992). This makes feeding the proper requirements of RDP for MCP synthesis critical in any feeding regime. Klusmeyer et al. (1990) observed no differences in microbial N flow to the small intestine when feeding diets containing 5.7% RDP (11% CP) compared with an 8.7% RDP (14.5% CP) diet. However, reducing dietary CP from 14.5 to 11.0% decreased milk production (29.3 vs. 26.9 kg/d). Similarly, Olmos Colmenero and Broderick (2006) reported that when CP was raised from 15.6 to 16.6% in the diet, milk production increased from 38.8 to 40.0 kg/d. Thus, dietary CP should be balanced to supply adequate amounts of RDP for maximal MCP yield, which can then provide quality AA required for milk and protein production (Olmos Colmenero and Broderick, 2006). On the other hand, there have been many attempts to substitute high RUP sources in the diet to increase MCP flow to the small intestine; such attempts have brought varying results. After reviewing 15 in vivo studies, Santos et al. (1998) concluded that when soybean meal was replaced with high RUP, it did not increase the duodenal flows of MCP or essential AA. In fact, supplementing high RUP in the diet decreased MCP flow to the small intestine in 76% of the studies (Santos et al., 1998). Olmos Colmenero and Broderick (2006) concluded in their study that greater RUP (5.4 vs. 4.8% DM) resulted in similar production and

efficiencies; however, greater RUP resulted in increases in blood urea N and milk urea N concentrations (15.6 vs. 13.7 mg/100 mL and 10.4 vs. 9.8 mg/100 mL, respectively).

When examining protein metabolism in dairy cows, it comprises 2 processes: protein degradation and MCP synthesis. In order to start microbial degradation of protein, there must first be an attachment of rumen microbe to feed particles (Bach et al., 2005). Many strains and species of bacteria, protozoa, and anaerobic fungi are used in microbial protein degradation by supplying a variety of proteases, peptidases, and deaminases (Wallace et al., 1997). About 80% of microbial organisms in the rumen attach to undigested feed particles (Craig et al., 1987). Outside of the microbial cell, degradable protein will be converted into peptides and AA by microbial proteases (Brock et al., 1982). These peptides and AA will be taken into the cell where proteolytic enzymes will further breakdown the peptides into AA which can be used to make MCP or further deaminated to ammonia and VFA. The determination of the fate of the AA once inside the cell often depends on the energy available from accessible carbohydrate sources (Tamminga et al., 1979). Ruminal pH plays a vital role in N metabolism and its degradation. The optimal pH range of rumen proteolytic enzymes is from 5.5-7.0, with a reduced rate of protein degradation at the lower end of this spectrum (Bach et al. 2005).

In the formation of MCP, ammonia is the main N source for growth and essential for several of the rumen microbial species (Brito et al., 2007). Rumen microbial cells derive about 80% of their N from ammonia whereas protozoa cannot use ammonia (Bach et al., 2005). With microbial protein accounting for the majority of the total AA flow to the small intestine, it is critical to maintain ammonia concentrations in the rumen at a level

that will allow maximal MCP synthesis. Satter and Slyter (1974) reported a minimal concentration of 5 mg/100 mL ruminal ammonia for maximal MCP. This concentration of 5 mg/100 mL corresponds to a 13% dietary CP level. However, Reynal and Broderick (2005) suggest a concentration of almost double (9.2 mg/100 mL) for ruminal ammonia required for maximal MCP synthesis in lactating dairy cows.

Even though not used as much on a percentage basis, the other protein degradation products such as peptides and AA still contribute to MCP. The usefulness of these protein degradation products was reported by Argyle and Baldwin (1989) when the authors reported that adding only 1 mg/L of differing protein AA and 1 mg/L of peptides to rumen microorganisms in vitro increased the microbial yield by more than two-fold. The authors also concluded that ruminal microorganisms used the peptide and AA substrates very efficiently at the low levels of concentration normally found in the rumen (Argyle and Baldwin, 1989). It has also been reported that the amount of peptide and AA N used for cell N depends on the bacteria's fermentation substrate preference (Bach et al., 2005). It was proposed that microbes that degrade structural carbohydrates, known as cellulolytic bacteria, grow slowly and tend to use ammonia-N as their main N source (Russel et al., 1992). Microorganisms that degrade non-structural carbohydrates, known as amylolytic bacteria, grow rapidly and generally use more peptides and AA as a N source than cellulolytic microbes (Russel et al., 1992). In certain situations, the bacteria themselves can be used as an N source. Protozoa account for approximately 40% of rumen biomass, utilize carbohydrates as their energy substrate, and use bacteria as their source of AA (Russel et al., 1992). This is important in regard to ruminal protein

synthesis because of the large percentage of biomass protozoa represent; however, protozoa only contribute around 11% of the total protein delivered to the small intestine (Shabi et al., 2000). For those bacteria that do not utilize whole peptides or AA, carbohydrates can be used as carbon skeletons along with ammonia for protein synthesis. This emphasizes the importance of nutrient interaction and adequate supply of carbohydrates for optimal MCP synthesis (Bach et al., 2005).

Another important source of N for MCP comes from recycled N from the blood to the rumen. Recycled urea from the blood comes back to the rumen where it is converted to ammonia. This is beneficial, because if the recycled urea can be used in MCP, that potentially leaves less to be excreted in the urine (Lapierre and Lobley, 2001). This utilization of recycled urea can be helpful in increasing N efficiency. Remond et al. (2002) reported net recycling of blood urea into the rumen when ammonia concentrations were below 9.5 mg/100 mL improved N efficiency and reduced microbial reliance on RDP.

Use of Urea as a N supplement

Urea use as an NPN source in diets fed to cattle has been recognized for over a century. As early as 1891, Zuntz (1891) proposed a theory that bacteria in the rumen could utilize NPN compounds to produce bacterial protein that could then be accessed by the animal with digestion in the small intestine. After the Zuntz' theory not much research was done on NPN until 1937 when Krebs (1937), after reviewing earlier research, concluded that there was conflicting evidence and doubt to whether the theory

of using NPN compounds and converting them to significant amounts of protein was a solid theory (Holder, 2012). However, Hart et al. (1939) reported in long-term trials with cattle when plant protein was replaced with NPN compounds it resulted in normal growth in growing cattle. Further, Reid (1953) showed that when urea was fed to ruminants, the tissues of those growing animals were of normal protein composition. Bryant and Robinson (1962) reported that when rumen bacteria were grown on defined media containing branched chain VFA, 56% of the isolates grew when all of their N was provided as either ammonia or enzymatic casein hydrolysate and another 25% required ammonia only. Eventually, Virtanen (1966) reported that when dairy cows were fed NPN compounds as their sole protein source, the cows were able to live, reproduce, and make a moderate supply of milk. However, Oltjen et al. (1968) reported that use of only NPN as the source of dietary CP reduced growth rate, feed efficiency, and N retention in ruminants when he summarized a number of trials that compared CP from solely urea or isolated soy protein. The author reported that the N retention for urea was about 65% that of the soy protein treatments, and consequently ascribed enhanced microbial yield to the soy peptides and AA supply. After performing an experiment where continuous culture fermenters were used, Satter and Slyter (1974) concluded that MCP yield did not increase with urea addition above an average dietary CP of 13.4%. This study was performed by feeding continuous culture fermenters diets in which CP was increased above 4% on a DM basis with the addition of urea only. After about 13% of the DM being dietary CP the MCP yield did not increase. However, up to that point it increased linearly with the increase in dietary CP form urea. The results from Oltjen et al. (1968) and Satter and

Slyter (1974) showed that use of NPN in diets of ruminants as a supplementation of RDP and not the sole source showed positive benefits. Thus, for the next 40 years considerable research has focused on utilizing NPN compounds in the rumen of cattle. This has eventually lead to the practice today of feeding urea in lactation dairy diets to help increase the RDP portion of N available in the rumen.

Although urea has been found to be beneficial as an N source to rumen microbes, the use of urea in lactation dairy diets has its limitations and disadvantages. Reid (1953) reported that urea was less effective in diets that already contained 12% or more CP, and that urea became unpalatable or reduced feed intake when dietary addition exceeded 1% of DM. Brito et al. (2007) reported that DMI was reduced from 24.7 to 22.4 kg/d when urea replaced SBM in the diet. Nitrogen intake was also lower with intakes of 590 vs. 653 g/d; however, there was no difference reported in RDP supplied in g/d between urea and SBM, showing that urea can be used as an acceptable RDP supplementation. However, there was greater microbial efficiency with the use of SBM over urea (29.0 vs 26.3 g of non-ammonia N/kg of organic matter truly digested in the rumen), with a corresponding tendency ($P = 0.08$) for more microbial N flow to the small intestine with the use of SBM. In a companion study, Brito and Broderick (2007) reported lower milk yield (32.9 vs. 40.0 kg/d) and milk components (milk fat, 1.01 vs. 1.22 kg/d; protein, 0.92 vs. 1.23 kg/d) for urea treatments. The authors concluded that this lower milk yield, milk fat, and milk protein could be from lower flows of MCP and AA into the small intestine. Although urea did not decrease RDP, it showed lower RUP and MCP leaving the rumen. Broderick et al. (1993) reported in a study with lactating dairy cows, that supplementing

urea in the diet for soybean meal (**SBM**) did not decrease DMI (25.4 vs. 25.3 kg/d) when fed with a diet of 55:45 of forage:concentrate with alfalfa hay and corn silage as forage sources. There was also no difference in milk yield with either urea or SBM (32.9 and 32.6 kg/d, respectively; Broderick et al., 1993). However, when dietary concentration of alfalfa silage was lowered from 59.7 to 39.0%, there was a difference in DMI and milk yield when urea was compared with SBM. Intake of DM was reduced to 24.2 from 26.2 kg/d, and milk yield was decreased from 38.5 to 35.4 kg/d (Broderick et al., 1993).

On the other hand, Wohlt et al. (1991) and Santos et al. (1998) showed no difference in DMI when urea was used. Santos et al. (1998) found that when feeding urea with steam-flaked sorghum it increased DMI by 2.6 kg/d over SBM. This could be due to the fact that the highly degradable starch may have improved synchronization of energy and RDP fermentation in the rumen (Bartley et al., 1976). These results suggest that supplementation of urea can be beneficial in some conditions but can have a negative effect in others, making evaluating urea use a priority in any feeding regime.

Broderick and Reynal (2009) performed a study in which they investigated the effects of feeding differing proportions of RDP supplied from SBM and urea on production and ruminal metabolism. Concentration of RDP in the diet remained at 10.5%, while urea concentration that made up the RDP increased. The authors found no difference in milk yield when the urea was increased from 0 to 1.2 to 2.4% (39.3 vs. 38.6 vs. 38.5 kg/d, respectively). However, when urea supplementation reached 3.7% of RDP, then milk yield dropped to 36.0 kg/d. This greater concentration of urea at 3.7% also corresponded to increased urinary urea-N and fecal N excretions. Therefore, not only did overfeeding

urea reduce production and risk lower profit, it excreted more N into the environment.

Modern feeding systems account for the fact that dietary N can be used to feed the ruminal microorganisms as well as the animal directly (NRC, 2001). However, these feeding systems have to account for the fact that urea may only be used as a ruminal supply of N. If it is to be used after it passes through the rumen, it must be absorbed as urea or ammonia and recycled back to the rumen. However, NRC (2001) assumes that the degradation rate of NPN in the rumen is equal to infinity. Therefore, passage rate of NPN would be zero, and there would be no post-ruminal absorption. Thus, innovations have been made to reduce the rate of degradation of urea in the rumen.

Slow-Release Urea

Urea in the rumen is rapidly hydrolyzed to ammonia by microbial enzymes which often occurs at a greater rate than urea utilization by the rumen bacteria (Highstreet et al., 2010). Because of this, urea is used somewhat inefficiently for production of protein products (Broderick and Reynal, 2009). This has led to many endeavors to produce a form of urea that would have a slower degradation rate in the rumen (Holder, 2012). Early attempts at this have led to products such as biuret, which is formed with 2 molecules of urea and has been studied since the 1970's (Fonnesbeck et al., 1975). There has also been the use of starea, a product composed of cooking grains and urea together (Deyoe, 1968), also urea phosphate (Oltjen et al., 1968). Urea has been bound to lignin (Castro et al., 1999) or calcium chloride (Huntington et al., 2006) to slow degradation. There has also been encapsulating urea particles with polymers (Galo et al., 2003) or

lipids (Owens et al., 1980; Garret et al., 2005) in expectations of slowing degradation rate by the rumen microbial enzymes (Holder, 2012).

Some authors have reported using a slow-release urea (**SRU**) product that increased DMI and/or digestibility when compared with urea, leading to increased efficiency of N for milk production. Cherdthong et al. (2011) reported that when SRU was used compared with non-treated urea, OM intake increased along with OM digestibility (9.8 vs. 8.3 kg/d and 73.2 vs. 68.5%, respectively). The use of SRU also improved milk production (13.4 vs. 10.1 kg/d) when compared to non-treated urea. Xin et al. (2010) found similar results with regards to DMI (22.8 vs. 20.2 kg/d) when a polyurethane-coated SRU was compared with urea. This increase in DMI did correlate with an increase in milk production (34.53 vs. 32.48 kg/d; Xin et al., 2010). However, not all reports show an increase in DMI with SRU. Galo et al. (2003) fed a polymer-coated SRU in a corn silage-based dairy diet and did not find any difference in DMI. It has been shown that SRU may improve efficiencies when fed to ruminants. Cherdthong et al. (2011) reported that supplementation of SRU resulted in increased efficiency of MCP compared with non-treated urea (18.9 vs. 13.5 g/kg OM digested in the rumen, respectively). Xin et al. (2010) reported that in vitro microbial efficiency was greater when SRU was used compared with feed-grade urea in the diet. Golombeski (2006) also reported increased FE coupled with reduced DMI due to addition of SRU, but it did not affect milk production. Neal et al. (2014) also showed increased FE when using SRU over a combination of SBM and canola meal at 50:50 in DM. Galo et al. (2003) fed a polymer-coated SRU in a corn silage-based diet and did not find any difference in DMI. The authors also reported

that there was no difference in milk yield between SRU and control diet (35.6 and 34.8 kg/d, respectively).

Slow-release urea has been shown to improve ruminal fermentation characteristics. The very idea of SRU is that it will reduce the rate of release of ammonia in the rumen. This can help reduce the risk of ammonia toxicity over the use of urea (Owens et al., 1980). Cherdthong et al. (2011) reported that SRU resulted in increased counts of total and cellulolytic bacteria in diets based on cassava chips fed to lactating dairy cows. When SRU was used, total bacteria count numbered 8.6×10^{11} (real-time PCR technique, copies/ml of rumen content) compared with urea with a count of 3.1×10^{11}. The authors contribute this increase to the fact that readily fermentable carbohydrates, such as cassava chips, are more effective in promoting microbial growth. However, Galo et al. (2003) reported no difference in MCP yield (1706.1 vs. 1784.6 g/d) with use of SRU in a typical TMR diet fed to lactating dairy cattle. It has been reported that ruminal MCP synthesis depends on the supply of adequate amounts and type of carbohydrates used as an energy source for the synthesis of peptide bonds (Bach et al., 2005). Perhaps an even better advantage of SRU is that it can lead to better synchronization of energy and N substrate fermentation in the rumen (Bach et al., 2005).

Synchronization of Ruminal Fermentation between Protein and Energy

The concept of synchronizing protein and energy fermentation in the rumen is of sound theoretical basis but has a very complex and uncertain reality (Bach et al., 2005).

The first concept in this theory is the availability of nutrients to the rumen microorganisms for utilization. If there is a deficient utilization of protein, digestibility of carbohydrates can be reduced. Likewise, if there is insufficient carbohydrate or energy to match available N, excess N may be lost as ruminal ammonia (Nocek and Russell, 1988). The availability of these nutrients depend on the amount available in the feed and the mechanisms and exposure to the rumen microbes that degrade them. Rumen environmental factors such as pH, major population of bacteria, substrate availability, and passage rate of digesta all play a role in the amount and efficiency of nutrient degradation and synthesis in the rumen (Bach et al., 2005). As was discussed previously, many attempts have been made to alter feed ingredients to supply adequate amounts and in suitable timing to the rumen. Better synchronization of nutrients in ruminal fermentation has been shown to increase efficiency and yield of MCP and productive performance (Theurer et al., 1999; Cherdthong et al., 2011; Neal et al., 2014).

The use of high-quality forages in dairy diets has been shown to maintain proper pH range and VFA concentrations in the rumen. However, there is a need to choose a right source of readily fermentable carbohydrate to capture degradable N fraction from the forages such as alfalfa hay; 80% of alfalfa protein can be degraded in the rumen or during ensiling process, and up to one-third of alfalfa protein is ultimately excreted in urine as urea. Corn grain processing has been shown to increase its digestibility in the rumen and throughout the total gastrointestinal tract. Corn grain processing has also been shown to increase energy availability to the cow and rumen microorganisms. Because of this property, SFC and HMC were selected in this trial to use in a lactation dairy diet.

Therefore, this trail was designed to use high-quality alfalfa hay along with corn silage to facilitate proper rumen function and adequate amounts of VFA for metabolism. In order to more fully utilize the energy available from the processed corn grains, a SRU product was selected to be used in replacement of 40% of conventional protein supplement, a combined SBM and canola meal.

Therefore, the objective of this experiment was to determine if nutrient utilization and energy partitioning by lactating dairy cows would differ in response to dietary corn grain types [steam-flaked corn vs. high-moisture corn] and to test if the types of CG would interact with slow-release urea on lactational performance and energy utilization.

MATERIALS AND METHODS

The dairy cows used in the present study were cared for according to the Live Animal Use in Research Guidelines of the Institutional Animal Care and Use Committee at Utah State University. The study was conducted at the Caine Dairy Research Center (Wellsville, UT), Utah State University from August to October, 2014.

Cows, Experimental Design, and Diets

Eight multiparious lactating Holstein cows were used during this trial. Four of the cows were surgically fitted with rumen cannulas. Cows began the experiment averaging 32 ± 8.2 DIM, and average BW was 682 ± 68.2 and 709 ± 66.6 kg at the beginning and the end of the experiment, respectively.

The experiment was performed in a double 4 × 4 Latin square design. Within each square, cows were randomly assigned to a sequence of 4 diets during each of the four 21-d periods (14 d of treatment adaptation and 7 d of data collection and sampling). Within each square, cows were randomly assigned to a sequence of 4 dietary treatments with a 2 × 2 factorial arrangement: SFC without SRU diet (**SFC−SRU**); SFC with SRU diet (**SFC+SRU**); HMC without SRU diet (**HMC−SRU**), and HMC with SRU diet (**HMC+SRU**; Table 1).

Whole corn (Pioneer 3730; Pioneer Hi-bred International, Inc., Johnston, IA) was processed with a mobile roller mill (model number ATG3600B, Automatic Equipment Manufacturing Co., Pender, NE) which resulted in a mean particle size of 1017 μm. The

ground HMC was ensiled in a 2.4- × 9.0-m bag (Ag-Bag International, Blair, NE). The SFC grain used in this study was supplied by Intermountain Farmers Association (Logan, UT). Average thickness of the SFC was 2.0 mm, and its bulk density was averaged at 0.35 kg/L. Alfalfa was preserved as sun-cured hay and processed for approximately 15 min in a TMR wagon (model 455, Roto-Mix, Dodge City, KS). A commercial SRU product (Optigen®, Alltech Inc., Nicholasville, KY) was supplemented at 0.46 and 0.45% DM in the SFC+SRU and the HMC+SRU, respectively, in order for cows to consume approximately 127 g/d. The dietary concentration of the SRU was chosen based on a previous lactation study (Neal et al., 2014). Slow-release urea has a CP concentration of 256%, which is slightly lower than urea due to the vegetable oil coating of SRU.

The alfalfa hay used in our study had a chemical composition of 21.2, 37.7, and 27.4% DM for CP, NDF, and ADF, respectively, whereas corn silage contained 8.40, 36.8, and 20.1% DM for CP, NDF, and ADF, respectively. While SFC comprised 9.01, 9.08, and 61.6% DM for CP, NDF, and starch, respectively, HMC consisted of 8.80, 9.11, and 64.8% DM for CP, NDF, and starch, respectively. A similar CP concentration (17.4 % DM on average) across treatments was maintained by replacing mixture of SBM and canola meal (**SBMCM**) in 50:50 with the SRU (Table 1), and the SBMCM had 74 and 26% of RDP and RUP, respectively, as a % CP. In addition, diets had similar RDP and RUP fractions. Diets were formulated based on the NRC (2001) recommendations to provide sufficient NE_L, MP, vitamins, and minerals to produce 40 kg/d of milk with 3.5% fat and 3.0% true protein (**TP**).

Cows were housed individually in tie stalls fitted with rubber mattresses covered with

straw, and had free access to water. Cows were individually fed twice daily for ad libitum intake at a level of 110% expected daily intake with 70% of allotted feed fed at 0600 h and 30% fed at 1500 h. Feed offered and refused was recorded daily, and samples taken during the sampling week to determine DMI.

Cows were milked twice daily at 0400 and 1600 h, and milk production was recorded throughout the entire experiment. Milk was sampled for 2 consecutive days (d 16 and 17) during the a.m. and p.m. milkings each period. Individual milk samples were analyzed by the Rocky Mountain DHIA Laboratory (Wellsville, UT) for fat, TP, lactose, and MUN. Milk composition was expressed on weighted milk yield of a.m. and p.m. samples. Milk fat and protein yields were calculated by multiplying milk yield from the respective day by fat and TP concentration of the milk from an individual cow. To convert milk TP to milk N, 6.38 was used as the conversion factor (DePeters and Cant, 1992), and total milk N (kg/d) was calculated as milk TP/6.38 + MUN, where milk TP and MUN were expressed as g/d.

Energy Partitioning Calculations

Energy partitioning was determined during treatment periods using data of milk yield, milk composition, and BW of experimental animals. Cows were weighed for 2 consecutive d after the a.m. milking and before the a.m. feeding at the beginning and end of each period. These weights were used to calculate the mean BW of cows for each experimental period. Energy utilization was determined by calculating energy for maintenance as $BW^{0.75} \times 0.08$ (NRC, 2001). Energy of BW change was assumed to be

5.114 Mcal/kg of gain or 4.924 Mcal/kg of loss (NRC, 2001). Milk energy was calculated as (0.0929 × milk fat concentration) + (0.0563 × milk TP concentration) + (0.0395 × milk lactose concentration) (NRC, 2001). Estimated NE_L/kg was calculated by total net energy utilization (maintenance, BW gain, and milk) divided by DMI (Neal et al., 2014).

Feed Sampling and Analysis

Samples of alfalfa hay and corn silage were taken weekly to determine DM, and diets were adjusted accordingly for change in DM concentration. Samples were composited by month, ground to pass a 1-mm screen (standard model 4; Arthur H. Thomas Co., Swedesboro, NJ), and stored for chemical analysis. Samples of TMR and orts were collected from individual cows on d 15 to d 21, composited, dried at 60°C for 48 h, and ground as previously described. The DM concentrations of samples were used to calculate intakes of DM and nutrients.

Analytical DM concentration of samples was determined by oven drying overnight at 105°C, and OM was determined by ashing at 550°C for 5 h (AOAC, 2000; method 942.05). Concentration of CP was determined using an automated N combustion analyzer (Elementar, Analysensysteme GmbH, Hanau, Germany; AOAC, 2000; method 968.06). Concentrations of NDF and ADF were sequentially determined using a fiber analyzer (200/220, ANKOM Technology, Macedon, NY) according to the methodology supplied by the company, which is based on the methods described by Van Soest et al. (1991). Sodium sulfite was used in the procedure for NDF determination and pre-treated with heat-stable amylase (Type XI-A from *Bacillus subtilis*; Sigma-Aldrich Corporation, St.

Louis, MO). Ether extract was measured using a fat analyzer (XT20, ANKOM Technology; AOAC, 2000; method 2003.05). In addition, samples of corn grain (SFC and HMC) and TMR were analyzed for starch by the Dairyland Laboratories, Inc. (Arcadia, WI) according to Knudsen (1997).

Urine Samplings and Analyses

On d 15 to 17, spot urine samples were collected from each cow at 0600 and 1800 h for a total of 6 samples per cow. Using 4 *M* HCl, urine samples were acidified to pH < 4.0 and composited by cow per period. Samples were frozen and stored at −40°C. The samples were thawed at a later date, and urinary-urea N was assayed using a kit (Stanbio Urea Nitrogen Kit 580, Stanbio Laboratory, Inc., San Antonio, TX) according to its instructions.

Ruminal Fermentation Characteristics

Ruminal pH was measured continuously starting on d 18 for 2 consecutive days using indwelling pH meters in the cannulated cows. The Lethbridge Research Centre Ruminal pH Measurement System (**LRCpH**; Dascor, Escondido, CA) as described by Penner et al. (2006) was used. Prior to placing the LRCpH system in the rumen, readings in pH buffers 4 and 7 were recorded. Meters were placed in the rumen taking a pH measurement every 30 s, which was stored by the data logger. The LRCpH was removed from the rumen after 48 h of continuous pH measurements and washed in 39°C water. The daily ruminal pH data were averaged for each minute and summarized as minimum,

mean, and maximum pH. Also, when ruminal pH was less than 5.8, daily episodes, duration (h/d), and area (pH × min) were calculated. The threshold of 5.8 was chosen because it has been previously described by others (Beauchemin and Yang, 2005) to cause ruminal acidosis.

Ruminal contents were sampled from cannulated cows at 0, 3, and 6 h after the a.m. feeding on d 18 and 19. Approximately 1 L of ruminal contents was obtained from different locations within the rumen (anterior dorsal, anterior ventral, medial ventral, posterior dorsal, and posterior ventral) and strained through a polyester screen (pore size 355 μm; B & S H Thompson, Ville Mont-Royal, QC, Canada). Five mL of the filtered ruminal fluid was added to 1 mL of 1% sulfuric acid, and samples were retained for ammonia-N (**NH_3-N**) determination. Concentration of NH_3-N in the ruminal contents was measured as described by Rhine et al. (1998). Another 5 mL of filtered ruminal fluid was added to 1 mL of 25% meta-phosphoric acid, and the samples were retained for VFA determination. The VFA were quantified using a gas chromatograph (model 5890, Hewlett-Packard Lab, Palo Alto, CA) with a capillary column (30 m × 0.32 mm i.d., 1 μm phase thickness, Zebron ZB-FAAP, Phenomenex, Torrance, CA) and flame-ionization detection. The oven temperature was 170°C held for 4 min, which was then increased by 5°C/min to 185°C, and then by 3°C/min to 220°C, and held at this temperature for 1 min. The injector temperature was 225°C, the detector temperature was 250°C, and the carrier gas was helium (Eun and Beauchemin, 2007).

Statistical Analysis

Data were summarized for each cow by measurement period. All data were statistically analyzed using the mixed-model procedure in SAS (SAS Institute, 2013). Data for intake, milk production, energy partitioning, pH and VFA profiles, and N utilization were analyzed with a model that included the effects of type of CG (SFC vs. HMC), supplementation of SRU (−SRU vs. +SRU), and their interaction. Cow and period were the terms of the random statement. Data for NH_3-N concentration were analyzed using the model described above, except that the fixed effect of time after feeding was included using the repeated option. For each variable analyzed, cow nested within treatment was subjected to 3 covariance structures: compound symmetry, autoregressive order 1, and unstructured covariance. The covariance structure that resulted in the lowest values for the Akaike information criterion and the Schwartz Bayesian criterion was used (Littell et al., 1998). Residual errors were used to test main effects and interactions. Differences were considered significant at $P \leq 0.05$, and trends were discussed at $P > 0.05$ to $P < 0.10$.

RESULTS AND DISCUSSION

Diet Composition and Dietary Treatments

Chemical composition and ingredients of treatment diets are listed in Table 1. High-forage experimental diets (60.5% DM of forage on average) consisted of 35.0% DM of alfalfa hay and 25.5% DM of corn silage on average across diets. To the SFC+SRU and the HMC+SRU, SBMCM concentration was reduced by 44% due to SRU supplementation at 0.46 and 0.45% DM, respectively. Diets were formulated to have similar CP concentration and NE_L value (17.4% DM and 1.66 Mcal/kg on average, respectively), while starch and NFC concentrations were slightly greater in HMC diets compared with SFC diets due to greater starch concentration of HMC than SFC (64.8 vs. 61.6% DM) and greater dietary addition of HMC fed than SFC.

Intake, Milk Production, and Feed efficiency

Feeding HMC decreased intakes of DM, organic matter (**OM**), CP, NDF, and acid-detergent fiber (**ADF**) compared with SFC (Table 2). Supplementation of SRU did not affect intakes of DM and nutrients, whereas it tended to increase intakes of DM and OM ($P < 0.08$) or increased CP intake under SFC but no effect under HMC, leading to tendencies of CG × SRU interaction on DM and OM intakes ($P < 0.07$) and CG × SRU interaction on CP intake. In a previous study done by our group (Eun et al., 2014), we reported decreases in intake of DM and nutrients when cows were fed with HMC added at 17.0% DM in similar high-forage diets (58.0% DM of forage) to the ones tested in the

Table 1. Ingredient and chemical composition (% of DM, unless otherwise noted) of the experimental diets fed to lactating Holstein dairy cows (n = 4)

	Experimental diet[1]			
	SFC		HMC	
Item	−SRU	+SRU	−SRU	+SRU
Ingredient				
Alfalfa hay	35.5	35.1	34.9	34.5
Corn silage	25.8	25.6	25.4	25.1
Corn grain (steam-flaked)	12.9	12.8	-	-
Corn grain (high moisture)	-	-	14.4	14.3
Corn distillers grains	6.07	6.76	5.97	6.64
SBMCM[2]	7.17	4.01	7.05	3.94
Soybean hull (pellet)	4.55	6.00	4.48	5.90
Beet pulp (shreds)	4.55	6.00	4.48	5.90
Slow-release urea[3]	-	0.46	-	0.45
Fat supplement[4]	0.65	0.65	0.64	0.64
Yeast culture[5]	0.19	0.19	0.18	0.18
Vitamins and minerals[6]	1.88	1.86	1.85	1.83
Sodium bicarbonate	0.66	0.66	0.65	0.65
Chemical composition				
DM, %	59.9 ± 3.69	57.6 ± 1.75	56.4 ± 3.57	55.2 ± 2.18
OM	89.2 ± 0.61	90.0 ± 0.95	89.2 ± 0.92	90.5 ± 0.92
CP	17.5 ± 1.21	17.8 ± 1.43	17.3 ± 1.44	17.1 ± 0.41
RDP[7]	10.7	11.0	10.7	10.6
RUP[7]	6.76	6.85	6.56	6.47
NDF	34.6 ± 2.12	34.3 ± 1.13	32.4 ± 1.81	33.8 ± 2.48
ADF	22.0 ± 1.52	21.4 ± 1.53	20.4 ± 2.00	21.0 ± 2.08
Starch	16.2 ± 0.95	17.5 ± 1.43	19.3 ± 2.08	20.5 ± 2.52
Ether extract	3.47 ± 0.327	3.71 ± 0.264	3.55 ± 0.305	3.36 ± 0.498
NFC[8]	33.7 ± 0.89	34.3 ± 0.60	36.0 ± 2.92	36.3 ± 2.95
NE_L,[7] Mcal/kg	1.67	1.65	1.67	1.65

[1]SFC−SRU = steam-flaked corn (SFC) without slow-release urea (SRU) diet; SFC+SRU = SFC with SRU diet; HMC−SRU = high-moisture corn (HMC) without SRU diet; and HMC+SRU = HMC with SRU diet.
[2]Mixture of soybean meal and canola meal at 50:50 in a DM basis.
[3]Optigen® (Alltech Inc., Nicholasville, KY).
[4]Calcium salts of palm oil (EnerGII®, Virtus Nutrition, LLC, Corcoran, CA).
[5]Diamond V XP® (Diamond V Mills Inc., Cedar Rapids, IA).
[6]Formulated to contain (per kg DM): 226.7 mg of Se (from sodium selenite), 9,278.7 mg of Cu (from copper amino acid complex), 40,537.4 mg of Zn (from zinc amino acid complex), 38,653.4 mg of Mn (from manganese amino acid complex), 552.6 mg of Co (from cobalt carbonate), 1,234,585.2 IU of vitamin A, 152,808.1 IU of vitamin D, 3,815.1 IU of vitamin E, and 295 mg of Rumensin® (Elanco Animal Health, Greenfield, IN).
[7]Based on tabular value (NRC, 2001).
[8]NFC = 100 – CP – NDF – ether extract – ash.
[2]CG = type of corn grain in the diet (SFC vs. HMC); SRU = supplementation of SRU (−SRU vs. +SRU); and INT = interaction between CG and SRU.

current study. Because of the negative impacts of feeding HMC in high-forage diets, in the present study we reduced dietary inclusion rate of HMC from 17.0 to 14.4% DM. However, the 2.6% unit reduction of HMC inclusion apparently failed to avoid the negative results on DM and nutrient intakes, although amount of DMI reduction was slightly less in the present study compared with the previous report (1.75 vs. 2.30 kg/d, respectively) when HMC diets were tested with SFC diets (Eun et al., 2014). Allen (2000) reported that when ruminal starch digestion as % of DM increased, DMI of lactating dairy cows was reduced. It has been well established that HMC has shown greater ruminal and total-tract starch digestibility in dairy cow rations (Firkins et al., 2001; Ferraretto et al., 2014). The average drop in DMI reported by Allen (2000) with the increased starch digestion was 3 kg/d. While the depression in DMI in the current study averaged about half that amount (1.75 kg/d), potentially increased digestibility of starch in the HMC diets tested in the current study may have influenced DMI. The NRC (2001) also stated that when absorption of nutrients exceeds requirements, negative metabolic-feedback influences DMI. The HMC treatments had a greater digestible CG in them, which could have altered the ratio of nutrients that were absorbed. If this happened, there could have been a negative metabolic response that triggered the HMC-fed cows to reduce DMI. Allen (2000) also reported that feeds with a rapid rate of fermentation are anticipated to result in shorter meal length and size when those negative responses are triggered. Although HMC treatments had a greater starch concentration in the diet compared with SFC treatments (19.9 vs 16.9% DM) in the present study, this difference in the starch concentration did not affect DMI. Piccioli-Cappelli et al. (2014) reported no

difference in DMI in dairy cows fed low- vs. high-starch diet (20.1 vs. 25.9% DM). In addition, Fredin et al. (2014) also found no difference in DMI between low- vs. high-starch diets (18.2 vs. 26.5% DM).

The interactions seen in increased intakes of DM, OM, and CP due to SRU in SFC but not HMC were unexpected, and the mechanism whereby supplementing SRU increased the intakes is difficult to explain. It is known that urea can be fed to lactating dairy cows up to a concentration of 1.0% of the total ration without negative effects on DMI (Kertz, 2010). In the current study, SRU was included at a rate of 0.46% DM in the SFC+SRU. Considerable controversy exists whether DMI is affected by supplementing SRU in dairy diets. For example, Galo et al. (2003) reported no effect of supplementing SRU at 16% and 18% CP in lactation diets on DMI. In contrast, Neal et al. (2014) reported decreased intakes of CP and NDF because of SRU supplementation in a high-forage lactation diet. Additionally, Golombeski et al. (2006) found a decrease in DMI when SRU was added in a typical TMR containing ground CG and greatly fermentable sugar. When formulating a diet, use of SRU can create space in the diet to be filled with other ingredients, which may have improved intakes of DM and CP under SFC in the present study, but the potential effect would have disappeared under HMC because of its improved ruminal fermentability.

Neither type of GC nor SRU supplementation affected milk production except that cows fed HMC-based diets tended to decrease ECM yield ($P = 0.08$) compared to those fed SFC-based diets (Table 2). Milk fat concentration and yield were similar across dietary treatments, while milk TP concentration decreased with feeding HMC.

Table 2. Intake of DM and nutrients, milk yield, and feed efficiency of lactating Holstein dairy cows fed with different types of corn grain without or with slow-release urea

	Diet[1]					Significance of effect[2]		
	SFC		HMC					
Item	−SRU	+SRU	−SRU	+SRU	SEM	CG	SRU	INT
Intake (kg/d)								
DM	20.8	22.5	20.5	19.3	1.40	0.02	0.72	0.06
OM	18.5	20.2	18.2	17.5	1.26	0.02	0.45	0.07
CP	3.65[b]	4.00[a]	3.59	3.28	0.292	0.02	0.87	0.04
NDF	7.03	7.56	6.51	6.38	0.551	< 0.01	0.45	0.21
ADF	4.47	4.73	4.10	3.93	0.371	< 0.01	0.79	0.20
Yield (kg/d)								
Milk	39.2	39.3	38.5	38.8	2.03	0.32	0.73	0.84
3.5% FCM	38.2	37.5	35.2	35.9	2.95	0.14	0.99	0.65
ECM	38.5	38.2	36.0	36.4	2.46	0.08	0.97	0.80
Milk composition (%)								
Fat	3.34	3.06	2.89	3.01	0.348	0.22	0.70	0.32
True protein	2.90[b]	2.95[a]	2.90[a]	2.83[b]	0.111	< 0.01	0.50	0.01
Lactose	4.70	4.74	4.77	4.77	0.078	0.10	0.58	0.52
Milk component yield (kg/d)								
Fat	1.30	1.24	1.14	1.17	0.153	0.19	0.89	0.58
True protein	1.14	1.18	1.12	1.10	0.038	0.06	0.72	0.24
Lactose	1.86	1.91	1.85	1.86	0.105	0.36	0.49	0.61
Efficiency								
Milk yield/DMI	1.83	1.83	2.07	2.03	0.187	0.08	0.89	0.89
3.5% FCM yield/DMI	1.80	1.68	1.86	1.85	0.169	0.23	0.51	0.52
ECM yield/DMI	1.80	1.72	1.91	1.88	0.153	0.18	0.57	0.75

[a,b]Means within a row with different superscripts differ ($P < 0.05$).
[1]SFC−SRU = steam-flaked corn (SFC) without slow-release urea (SRU) diet; SFC+SRU = SFC with SRU diet; HMC−SRU = high-moisture corn (HMC) without SRU diet; and HMC+SRU = HMC with SRU diet.
[2]CG = type of corn grain in the diet (SFC vs. HMC); SRU = supplementation of SRU (−SRU vs. +SRU); and INT = interaction between CG and SRU.
[3]Net energy used for maintenance, BW change, and milk.

Supplementing SRU increased milk TP concentration under SFC but decreased under HMC, resulting in a CG × SRU interaction. Yield of milk TP tended to decrease (P = 0.06) because of feeding HMC. The overall results in milk production in response to

feeding HMC agree with the ones reported by Eun et al. (2014) where the authors did not find any effect of feeding HMC on milk yield and composition. Decreases in milk TP concentration and yield due to feeding HMC coincide with reduced CP intake. In addition, the CG × SRU interaction on milk TP is also attributed to the result of CP intake. Akay et al. (2004) reported a decreased milk protein concentration, but an increase in milk yield of 3.7 kg/d, resulting in an increased milk protein yield when a similar SRU product to the one tested in the present study was supplemented in diets containing 47.5% forage and 58.5% concentrate on a DM basis. Neal et al. (2014) did not find any response on milk composition when the same SRU product was supplemented in a similar high-forage diet tested in the current study.

Utilization of HMC in the diet had a tendency ($P = 0.08$) to increase dairy efficiency based on milk yield over SFC utilization (2.05 vs. 1.83; Table 2), but based on 3.5% FCM and ECM yields it did not differ between HMC and SFC. The dairy efficiency values are consistent with the ones reported by Spurlock et al. (2012) with the values greater than 1.80 for the first 150 DIM. The greater milk yield-based dairy efficiency values for HMC diets can be attributed to reduced DMI while not decreasing milk yield. Improved feed digestion is one of the most important factors affecting dairy efficiency. In fact, Eun et al. (2014) reported increased NDF digestibility by feeding HMC-containing diets and resulted in similar milk yield even with reduced DMI, causing the overall improvement in dairy efficiency in their study. However, supplementing SRU did not influence dairy efficiency in the present study. Giallongo et al. (2015) also reported no difference in dairy efficiency when SRU replaced 70% of soy-bypass protein used in the

diet. In contrast, Neal et al. (2014) reported greater dairy efficiencies when a SRU-supplemented diet was compared to a SBMCM-based control diet. In the previous study, reduction of DMI but increase in milk yield due to SRU supplementation led to the improved dairy efficiencies (Neal et al., 2014).

BW Change and Net Energy Partitioning

All diets resulted in BW gain during the course of the trial with the exception of SFC+SRU (Table 3). Cows fed HMC diets gained more BW than those fed SFC diets, whereas supplementing SRU tended to reduce BW gain regardless of type of CG (P = 0.07). The BW responses due to CG and SRU were mirrored directly in net energy calculations; feeding HMC diets caused increased net energy values for BW gain, while SRU supplementation resulted in a tendency to decrease net energy use for BW gain (P = 0.09). Net energy used for milk tended to decrease (P = 0.08) by cows fed HMC diets compared to those fed SFC diets. In contrast, combined values of BW gain and milk as well as total net energy values (maintenance + BW gain + milk) tended to increase (P = 0.10) by HMC diets, but these tended to decrease due to SRU supplementation (P = 0.10). Net energy partitioned into maintenance was similar across dietary treatments. Cows fed HMC diets shifted more net energy into BW compared with those fed SFC diets, whereas supplementing SRU tended to decrease (P = 0.10) a portion of net energy partitioned into BW gain under both SFC and HMC diets. Feeding HMC diets resulted in a less portion of net energy channeled into milk compared with SFC diets, but combined portion of net energy partitioned into BW and milk did not differ across diets.

The shift in net energy utilization with decreased DMI due to feeding HMC diets observed in this study suggests that the HMC diet had an advantage in net energy that was partitioned toward body tissue during early to mid-lactation with a slight reduction on milk energy. Knowlton et al. (1998) showed that feeding HMC increased starch digestion in lactation diets compared with dry corn, and the increased starch digestion resulted in increased BW gain of cows fed HMC compared to those fed dry corn (51.5 vs. 22.1 kg, respectively) while showing no difference in milk production. When feed intake decreases, dairy cows typically mobilize body tissue to support their potential milk production (NRC, 2001). Energy utilization is affected by several variables; Taylor and Allen (2005) stated that the capacity of the mammary gland to use nutrients for milk is influenced by hormone secretion and clearance, insulin resistance of tissues, and nutrient demands of various tissues, which are all influenced by the stage of lactation and milk production. Oba and Allen (2003) reported that HMC-fed cows gained more BW than those fed ground corn. It has been well documented that an increase in concentrates in the diet can increase plasma glucose and insulin (Marett et al., 2014). Boerman et al. (2015) and McCarthy et al. (2015) reported that insulin was found to be an integral part of feed intake regulation and energy partitioning in the body. Therefore, enhanced ruminal starch fermentation by feeding HMC may have triggered the insulin response which affects net energy partitioning by cows fed high-forage diets in early to mid-lactation.

Noteworthy is that the calculated NE_L values of the diets were greater than those estimated by NRC (2001) for cows fed at 3.0 times net energy maintenance intake (Tables 1 and 3). Robinson (2007) reported the lack of a relationship between the

Table 3. Change of BW and calculated net energy values and partitioning of lactating Holstein dairy cows fed with different types of corn grain without or with slow-release urea

	Diet[1]					Significance of effect[2]		
	SFC		HMC					
Item	−SRU	+SRU	−SRU	+SRU	SEM	CG	SRU	INT
BW (kg)								
Initial	691	698	692	689	25.7	0.53	0.74	0.42
Mean	692	696	712	695	25.3	0.12	0.27	0.07
Change (kg/d)	0.08	-0.07	0.96	0.31	0.283	< 0.01	0.07	0.24
Calculated net energy values (Mcal/d)								
Maintenance	10.9	10.8	11.0	10.8	0.297	0.25	0.16	0.15
BW change	0.18	-0.22	4.86	1.20	1.612	< 0.01	0.09	0.28
Milk	25.8	25.1	23.9	24.4	1.73	0.08	0.85	0.45
BW + milk	25.9	24.8	28.8	25.7	1.94	0.10	0.10	0.51
Total[3]	36.9	35.7	39.8	36.8	2.01	0.10	0.10	0.42
NE_L (Mcal/kg DMI)	1.72	1.60	2.13	1.89	0.150	< 0.01	0.13	0.62
Net energy partitioning (% energy intake)								
Maintenance	30.0	30.8	27.8	30.0	0.016	0.16	0.16	0.48
BW change	0.49	-0.61	12.1	3.30	0.039	< 0.01	0.10	0.24
Milk	70.1	70.4	60.2	66.7	0.034	< 0.01	0.16	0.12
BW + milk	70.6	69.7	72.2	70.0	0.016	0.18	0.16	0.52

[a,b]Means within a row with different superscripts differ ($P < 0.05$).
[1]SFC−SRU = steam-flaked corn (SFC) without slow-release urea (SRU) diet; SFC+SRU = SFC with SRU diet; HMC−SRU = high-moisture corn (HMC) without SRU diet; and HMC+SRU = HMC with SRU diet.
[2]CG = type of corn grain in the diet (SFC vs. HMC); SRU = supplementation of SRU (−SRU vs. +SRU); and INT = interaction between CG and SRU.
[3]Net energy value used for maintenance, BW change, and milk.

deviations of the actual calculated vs. predicted NE_L concentration of 92 diets extracted from publications. In the present study, increased NE_L by feeding HMC diets was used to support BW gain, but not for milk production. However, the increased NE_L values by feeding HMC may have contributed to improved dairy efficiency by HMC-fed cows. On the other hand, increased DMI due to SRU supplementation was not translated into any

benefit on BW gain and net energy partitioning. Therefore, SRU-supplemented diets in the current study may have resulted in reduced DM and nutrient digestibilities and consequently increased mobilization of body tissue to support potential milk production, leading to a reduction in energy utilization efficiency reflected by decreased total net energy.

Characteristics of Ruminal Fermentation

Dietary treatments did not influence ruminal minimum and mean pH (Table 4), and mean pH of at least 6.24 was maintained across the diets. Feeding HMC tended to increase ($P < 0.10$) daily episodes toward pH < 5.8 and the resultant duration (h/d) compared with feeding SFC, indicating that a rate of HMC fermentation was relatively faster than that of SFC. However, these results would have minimal effects on physiological conditions in the rumen, because diurnal fluctuation of the ruminal pH showed a very typical pattern, with the highest pH values observed just before morning feeding and the lowest pH values around 12 h after the feeding (Figure 1), which is very similar to the patterns from cows fed 20 or 40% HMC reported by Vagnoni and Broderick (1997) and Eun et al. (2014). Although there were some daily episodes of pH < 5.8, the ruminal pH averaged on an hourly basis was maintained above 6.0 in the current study except the HMC+SRU at 12 h. Therefore, some effects of statistical tendencies due to feeding HMC would have biologically minor consequences on microbial physiology. Although fermentation acids or proteolysis degrade prolamin-zein proteins during the ensiling process of HMC and lead to greater and more rapid ruminal starch fermentation

in HMC (Hoffman et al., 2011), its effects on ruminal pH would not be detrimental when cows are fed HMC in an appropriate forage proportion in their diets (Eun et al., 2014). No effect of supplementing SRU on ruminal pH is consistent with the finding by

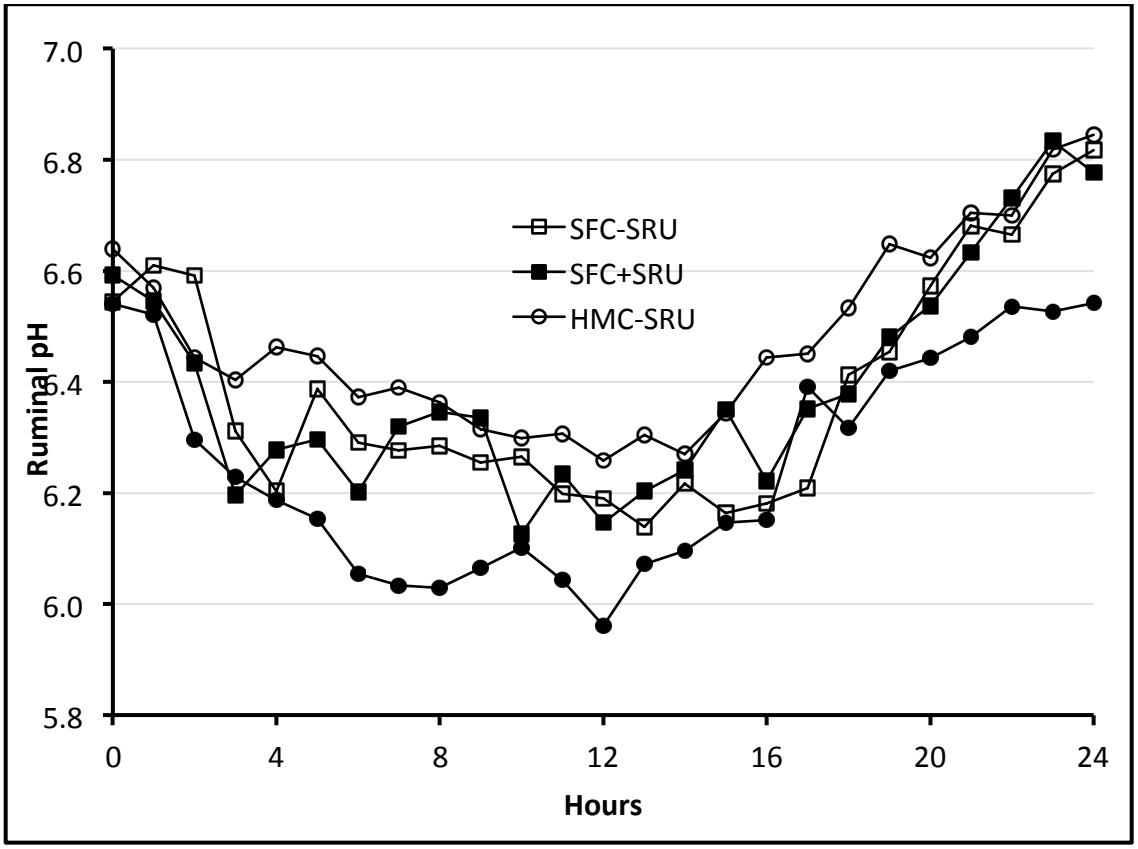

Figure 2. Effects of type of corn grain [steam-flaked (SFC) vs. high-moisture (HMC)] and supplementation of slow-release urea (SRU) product [without (–SRU) vs. with SRU (+SRU)] on diurnal variation of ruminal pH. The pH values were recorded every 30 sec over a 48-h period. Least squares means for culture pH were 6.44, 6.42, 6.47, and 6.24 for SFC–SRU, SFC+SRU, HMC–SRU, and HMC+SRU, respectively. Hour 0 represents time at first feeding at 6:00 AM.

Tikofsky and Harrison (2006), in which there was no change in ruminal pH detected in rumen-stimulating fermentors when SRU was supplemented in high-forage diets consisting of 25% corn silage and 25% AH (DM basis).

Type of CG in the diet did not affect total volatile fatty-acid (**VFA**) concentration (Table 4), but SRU supplementation decreased the total VFA concentration only under HMC diet, causing an interaction between CG and SRU which was likely due to the effect of SRU on DMI (Table 2). Given the stable and consistent ruminal pH pattern across the diets, no effect of VFA due to feeding different CG was expected. In general, increasing ruminal fermentability of grain typically yields increased VFA concentration with a greater propionate proportion. Although HMC may have been fermented more quickly than SFC in the rumen, reduced DMI with feeding HMC may have moderated the potential effects of feeding HMC on ruminal VFA profiles in the present study. Dietary treatments did not affect molar proportions of individual VFA and acetate-to-propionate ratio except molar proportion of butyrate which was decreased by feeding HMC or SRU. Xin et al. (2010) reported increased acetate proportion but decreased butyrate proportion due to SRU addition in a low-CP diet (13.1% DM) under continuous culture fermentation and raised a possibility of interconversion between acetate and butyrate in the rumen due to SRU. In the present study, decreased butyrate proportion was not associated with any change on acetate proportion. Thus, diet composition such as CP concentration may influence ruminal VFA composition with SRU supplementation.

Concentration of ruminal NH_3-N did not differ because of CG processing and SRU (Table 4), but it tended to increase ($P = 0.07$) due to SRU supplementation under HMC,

resulting in a tendency for CG × SRU interaction. Ruminal NH_3-N concentration is a result of balance between production (proteolysis) and assimilation (De Visser et al., 1997), and thus any efforts to maximize N utilization in the rumen should involve an optimal balance between the 2 metabolic processes. Yet, it is believed that energy is the

Table 4. Ruminal fermentation characteristics of lactating Holstein dairy cows fed with different types of corn grain without or with slow-release urea

	Diet[1]							
	SFC		HMC			Significance of effect[2]		
Item	−SRU	+SRU	−SRU	+SRU	SEM	CG	SRU	INT
Minimum pH	5.83	5.78	5.94	5.77	0.149	0.60	0.22	0.47
Mean pH	6.44	6.42	6.47	6.24	0.119	0.44	0.20	0.27
Maximum pH	7.04	7.01	7.00	6.95	0.082	0.55	0.63	0.89
pH < 5.8								
Daily episodes	7.00	1.50	12.3	17.5	8.330	0.09	0.98	0.36
Duration (h/d)	0.54	0.44	2.85	3.42	1.989	0.10	0.87	0.82
Area (pH × min)	2.65	3.43	16.8	27.5	15.41	0.15	0.64	0.69
Total VFA (m*M*)	116	121	124[a]	116[b]	6.7	0.66	0.64	0.05
Individual VFA[3]								
Acetate (A)	60.3	60.5	60.3	61.7	2.39	0.19	0.12	0.20
Propionate (P)	23.5	24.2	24.8	23.9	2.48	0.51	0.87	0.29
Butyrate (B)	12.1	11.7	11.2	10.6	0.57	< 0.01	0.02	0.78
Valerate	1.95	2.00	1.89	2.04	0.431	0.96	0.50	0.73
Isobutyrate	0.25	0.24	0.19	0.24	0.158	0.38	0.42	0.24
Isovalerate	1.62	1.24	1.42	1.29	0.175	0.47	0.02	0.23
A:P	2.57	2.50	2.43	2.58	0.346	0.75	0.15	0.24
NH_3-N[4] (mg/100 mL)	8.67	8.14	7.88	9.18	1.410	0.80	0.46	0.09

[1]SFC−SRU = steam-flaked corn (SFC) without slow-release urea (SRU) diet; SFC+SRU = SFC with SRU diet; HMC−SRU = high-moisture corn (HMC) without SRU diet; and HMC+SRU = HMC with SRU diet.
[2]CG = type of corn grain in the diet (SFC vs. HMC); SRU = supplementation of SRU (−SRU vs. +SRU); and INT = interaction between CG and SRU.
[3]Expressed as mol/100 mol.
[4]Ruminal ammonia-N.

most limiting factor in microbial growth (Bach et al., 2005), and consequently it was expected that a potential increase of ruminal starch fermentation in HMC would decrease NH_3-N concentration due to improved N use by ruminal microbes coupled with the accelerated HMC fermentation. We previously observed decreased NH_3-N concentration by feeding HMC (Eun et al., 2014), and it could be attributed to increased utilization of ruminally degraded N and consequently increased microbial protein yield. Rumen bacteria can utilize more NH_3-N for microbial protein synthesis in the presence of readily available energy such as HMC (NRC, 2001). However, the increased NH_3-N concentration due to SRU under HMC prevented the potential benefit of HMC on improvement of N utilization for microbial production in the present study. It is unclear why SRU supplementation in HMC diet increased the ruminal NH_3-N concentration, but it indicates an evidence of asynchronous condition in ruminal fermentation between HMC and SRU in our diets tested. The SRU product tested in the current study is designed to release urea slowly, but its degradation rate has been shown to change depending on the type of diet. For example, Holder (2012) indicated that when SRU was fed to Holstein steers in high-forage diets, the in situ rate and extent of ruminal degradation of SRU was increased compared with a high-concentrate diet. Thus, it would be better to supplement SRU in a greater dietary portion of concentrate in the diet to elicit improved utilization of dietary N in HMC-based diets.

Nitrogen Excretion and Utilization

Intake of N was found to be decreased when HMC was fed in the diet without an

effect of SRU (Table 5), but a CG × SRU interaction was noticed as SRU combined with SFC increased N intake, while SRU with HMC deceased N intake. Milk N decreased when feeding HMC with no effect of SRU supplementation. Neither CG nor SRU had an effect on N utilization efficiency for milk production in the current study with dietary treatments not influencing MUN and urinary-urea N concentrations. Excretion of urinary N was similar across diets, but feeding HMC diets decreased fecal N excretion compared with feeding SFC diets. In addition, supplementing SRU increased fecal N excretion under SFC, but it was decreased by SRU with HMC, leading to an interaction between CG and SRU. Consequently, manure N excretion and urinary N-to-fecal N ratio followed the same pattern shown in the fecal N excretion. Dietary treatments did not influence milk N-to-manure N ratio.

Although there were positive effects of N excretion with their reductions with feeding HMC, it is clear that the effects were resulted from SRU supplementation, but not HMC per se as indicated by interactions between CG and SRU on fecal and manure N excretions. Increased DMI by the SFC+SRU excreted more N into feces in the current study, suggesting that N digestion should decrease in this treatment. This increase in fecal N also resulted in greater manure N excretion when SRU was supplemented with SFC. As N intake increases in lactating dairy cows, manure N output also increases (Yan et al., 2006). It is unclear how SRU supplementation may have decreased N digestion under SFC diet. Supplementing SRU in SFC diet would not interfere with ruminal N metabolism, as it did not affect NH_3-N concentration and other ruminal fermentation parameters. However, supplementing SRU in SFC diet may have shifted digestion

Table 5. Nitrogen utilization of lactating Holstein dairy cows fed with different types of corn grain without or with slow-release urea

	Diet[1]					Significance of effect[2]		
	SFC		HMC					
Item	−SRU	+SRU	−SRU	+SRU	SEM	CG	SRU	INT
N intake (g/d)	605	652	602	538	36.8	0.03	0.74	0.04
Milk N (g/d)	191	198	188	185	7.21	0.04	0.71	0.23
Milk N:N intake[3]	0.31	0.31	0.31	0.35	0.022	0.25	0.40	0.25
MUN (mg/100 mL)	12.2	11.8	12.1	12.6	0.66	0.35	0.87	0.28
Urinary-urea N (mg/100 mL)	517	546	517	538	31.4	0.88	0.33	0.88
Urinary N excretion[5] (g/d)	217	213	213	225	11.8	0.55	0.58	0.25
Fecal N excretion[6] (g/d)	198	242	206	129	33.6	0.04	0.49	0.02
Manure N excretion[7] (g/d)	415	454	418	353	36.6	0.07	0.63	0.06
UN:FN[8]	1.12	0.89	1.06	1.84	0.502	0.04	0.23	0.08
MkN:MaN[9]	0.46	0.46	0.47	0.54	0.051	0.31	0.39	0.40

[1]SFC−SRU = steam-flaked corn (SFC) without slow-release urea (SRU) diet; SFC+SRU = SFC with SRU diet; HMC−SRU = high-moisture corn (HMC) without SRU diet; and HMC+SRU = HMC with SRU diet.
[2]CG = type of corn grain in the diet (SFC vs. HMC); SRU = supplementation of SRU (−SRU vs. +SRU); and INT = interaction between CG and SRU.
[3]Efficiency of use of feed N to milk N.
[4]Ruminal ammonia-N.
[5]Predicted using the following equation: 0.026 × MUN, mg/100 mL × BW, kg (Wattiaux and Karg, 2004).
[6]Predicted using the following equation: N intake, g/d – urinary N excretion, g/d – milk N, g/d.
[7]Manure N, g/d = urinary N excretion, g/d + fecal N excretion, g/d.
[8]UN:FN = urinary N to fecal N ratio, where urinary N and fecal N are expressed in g/d.
[9]MkN:MaN = milk N to manure N ratio, where milk N and manure N are expressed in g/d.

from the rumen to the hindgut, causing a reduction of total-tract CP digestion and a resultant increase in the fecal N excretion.

Decrease in N digestion in the SFC+SRU can be translated into negative effects on the environment. It has been well documented that excess ammonia and N in the manure is detrimental to the environment due to the volatilization of ammonia that is released

into the environment through biochemical reactions (Burgos et al., 2010). Meanwhile, the reduction of N excreted when SRU was fed with HMC was a direct result of reduced DM and N intakes. Neal et al. (2014) reported reduced N intake and its direct impacts on fecal and manure N excretion reductions with supplementing SRU.

Data not shown. Blood parameters of plasma glucose, NEFA, and BHBA were taken at the end of each period. These parameters were not found to be out of the normal range and so no data was reported.

CONCLUSIONS

As a practical means of optimizing nutrient utilization in high-forage lactation diets, in the present study we tested HMC to increase energy supply and SRU to additionally improve N utilization by lactating dairy cows. The HMC fed at 14.3% DM decreased DMI, but it allowed cows to partition more net energy into BW gain, while increasing NE_L values, which contributed to improving dairy efficiency. Although we could not explain why the cows tested in this study shifted net energy more into BW gain under reduced DMI by feeding HMC, it would be valuable to investigate if use of HMC can minimize cows to mobilize tissue energy while maintaining their potential milk production during transition period. Supplementation of SRU in HMC diets successfully replaced 44% of SBMCM without any negative impact on lactational performance, but SRU supplementation with SFC resulted in increased fecal N excretion possibly due to decreased CP digestion. Overall, the SRU supplementation did not contribute to improving synchronous ruminal fermentation coupled with HMC, and a relatively great proportion of forages in our diets (60% DM) may have diluted a potential effect of SRU in ruminal fermentation. These collective results demonstrate that feeding HMC with SRU can be a practical option in high-forage lactation diets to maintain or improve nutrient and energy utilization efficiency and minimize negative environmental impacts.

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