Production Performance and Nitrogen Utilization in Dairy Cows Fed Low Or High Crude Protein Diets Containing Corn Dried Distillers Grains With Solubles and Provided Supplemental By-Pass Protein/Amino Acids

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Abstract

Feeding corn dried distillers grains with solubles in low crude protein (CP) diets improves nitrogen utilization efficiency, however it can also reduce metabolizable AA supply, especially Lys, which compromises lactation performance. Therefore, our objective was to determine the effects of feeding by-pass protein/rumen-protected AA supplements in low or high CP diets containing 10% corn dried distillers grains with solubles. Six multiparous Holstein cows (619.3 ± 49.8 kg BW; 26.8 ± 6.2 DIM) were subjected to a split-plot 3 × 3 Latin square design with 21 d periods. The whole-plot factor was dietary CP content; low (14.6%; LP) or high (16.6%; HP), and the subplot was by-pass protein/AA supplement; control (no supplement), Supplement A (0.11 kg/cow/d) or Supplement B (0.45 kg/cow/d). Supplement A and B differed in both ingredient and chemical composition, resulting in differing AA concentrations. Dry matter intake, milk and milk lactose and protein yield did not differ in cows fed the LP than HP diet. However, reducing dietary CP content resulted in a decrease in N intake and apparent total tract CP digestibility, and a tendency for a decrease in milk protein yield. Furthermore, cows fed the LP diet tended to excrete a lower amount of total urinary N and urea-N and excreted a lower amount of total N than cows fed the HP diet. Similarly, BUN concentration was lower, and MUN concentration tended to be lower when N intake was restricted. However, there was no supplement effect on nutrient intake and digestibility, milk and milk component yields, and all measures of N utilization. Overall, feeding CDDGS in a LP compared to a HP diet had a marginal effect on production performance, which possibly negated the potential benefits of providing supplemental bypass protein/RP-AA under our experimental conditions. However, feeding CDDGS in a LP than

HP diet improved N utilization efficiency, which may be beneficial from an environmental sustainability standpoint.

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Introduction

Historically, maximizing economic returns has been the major driver of the increase in milk production in the U.S. In recent years, however there has been a shift towards sustainable dairy production, with environmental issues taking center stage. This is reflected by the increase in environmental litigations and regulations as consumers are demanding greater environmental accountability for dairy producers especially for N emissions, including nitrates. Thus, it is increasingly important to minimize N excretion and properly manage manure on dairy farms.

In dairy cows, only 20 to 25% of total N intake is converted to milk N; therefore, up to 80% of feed N is excreted in urine and feces (Tamminga, 1992; Hristov et al., 2004). Because feed costs account for the bulk of operational expenses and protein is the most expensive component of most diets, this low efficiency of N use limits profitability. In addition, the 75 to 80% of fed N that is excreted as urine and fecal N can be an environmental pollutant. Therefore, enhancing N use efficiency is not only important in reducing feed costs, but is also a key in limiting N excretion and emissions, which could go a long way in changing consumer perception about U.S. dairy production being unsustainable.

The most effective method to reduce N excretion and improve utilization is lowering dietary CP content (Hristov et al., 2011). Unfortunately, this method may also impact production performance. For instance, some studies (Giallongo et al., 2015; Liu et al., 2000) have reported that limiting dietary CP does not result in a decrease in production performance. However, others (Lee et al., 2012; Barros et al., 2017) observed a decrease in milk yield, which makes it a difficult choice for producers. Therefore, the current industry

trend is to overfeed CP during early lactation to prevent the potential decrease in milk production, and this contributes to the low efficiency of N utilization (Broderick, 2003; Hristov et al., 2014).

Because protein is widely recognized as the most expensive macro-nutrient, dairy producers often seek cost-effective protein sources to reduce production costs. Therefore, corn dried distillers grain with solubles (CDDGS) is currently a widely used protein source as it is typically cheaper than traditional protein sources such as soybean meal (Kleinschmit et al., 2007). However, CDDGS, has a high CP content and its dietary inclusion can result in N supply exceeding the requirements, which leads to waste. In addition, like most corn byproducts, CDDGS is Lys-deficient (Lui et al., 2000; Kalscheur, 2006), and could remain an insignificant source of Lys, even when fed in a high CP diet. Lysine, Met and Phe have also been reported as limiting when CDDGS is fed even in high CP diets (Nichols et al., 1998; Liu et al., 2000). Therefore, although reducing N intake when feeding DDGS improves N use efficiency, it can exacerbate the metabolizable AA deficiency that compromises milk and milk protein yield (Chibisa and Mutsvangwa, 2013). However, supplementation of by-pass protein sources when feeding low CP diets can be effective in combating the potential loss in production performance while maintaining the improvement in N utilization efficiency (Sinclair et al., 2014; Robinson et al., 1995).

Chapter 1. Review of Literature

1.1 Sustainable dairy production

The traditional goal of the dairy industry has been to increase milk production and, thus, on-farm profitability. For instance, there has been a 12% increase in annual milk yield per cow over the past 10 years (USDA-NASS, 2018a). While maximizing lactation performance is still important, there has been a shift towards more sustainable dairy production in recent years. There are three pillars of sustainable dairy production; economic, environmental, and social (Van Cauwenbergh, 2006). From an economic standpoint, the major challenges that producers are currently facing are the volatile and rising feed costs and low milk prices (Tyner, 2010; USDA-NASS, 2018b) and this has created a greater need for an improvement in the efficiency of milk production. In addition to maximizing production performance in a manner that does not compromise animal health and welfare, producers must also address the environmental cost of production, particularly N emissions. Therefore, meeting these goals will ensure that consumers may begin to view US dairy production as being sustainable.

While there have been environmental regulations about animal agriculture since the early 1970's (US EPA, 1972), in recent years, regulations are becoming much more focused and stricter. Specifically, these regulations are targeting 'concentrated animal feeding operations' (**CAFO**), which are any operation where more than 700 animals are housed indoors for a portion of the year and under controlled feeding for more than 45 days annually (USDA-EPA, 1999). Even smaller operations, with fewer than 300 animals, can be recognized as a CAFO and subjected to related regulations on a case-by-case basis. Therefore, most dairies fall under the definition of CAFO, and are subjected to strict

regulations on manure application to land, runoff maintenance and removal, and total daily emissions (USDA-EPA, 1999). These guidelines on waste management have been put into place by the EPA and are enforced within each state.

There are currently no direct costs associated with N loss in the U.S., which is unlike in certain European countries, including the Netherlands, where dairy producers must adhere to a cap-and-trade N excretion system on farms (OECD, 2015). However, there has been an increase in litigations and regulations in the U.S., as consumers are demanding greater environmental accountability for dairy producers. For instance, in a recent environmental lawsuit, several dairies in Yakima Valley, WA, were sued for allegedly causing ground water pollution as a result of manure runoff. Following ground water tests in the area, the EPA determined that between 10 and 20% of the sampled wells had nitrate levels that exceeded acceptable levels (US EPA, 2012a). Ultimately, the producers lost the case, and had to sign a consent order to implement several new waste management practices and submit to more frequent and intensive monitoring. Legal fees alone were nearly two million U.S. dollars, on top of the costs to implement new management practices and sampling protocols; one of the dairy owners was forced to sell his operation (US EPA, 2016). This case clearly highlights the shift towards sustainable dairy production with environmental issues taking center stage; which introduces a new level of difficulty in meeting the demands of lactating cows, without excess N waste.

1.2 Meeting amino acid requirements in dairy cattle

Lactating dairy cows require AA for maintenance, growth, and reproduction. Therefore, it is imperative to provide an adequate amount of dietary protein to facilitate the production and absorption of both essential- (**EAA**) and non-essential amino acids (**NEAA**).

Traditionally, crude protein (**CP**) is used to determine protein quality of a diet, whereby feeds are analyzed for N and CP is calculated under the assumption that all AA contain approximately 16% N (AOAC, 1984). Crude protein is made up of two fractions; rumen degradable protein (**RDP**) and rumen undegradable protein (**RUP**). Rumen degradable protein is made up of true protein (**TP**) and non-protein nitrogen (**NPN**). When fed to cattle, TP is sequentially degraded to peptides, AA, and some ammonia, whereas NPN, such as urea-N, is hydrolyzed directly to ammonia-N (Hristov and Jouany, 2005). Some of the dominant proteolytic rumen microbes involved in this process are *Bacteroides ruminicola* and *Peptostreptococcus sp.* (Virtanen, 1966). Depending on fermentable energy supply, peptides, AA, and ammonia-N are the three forms of N used by rumen microbes for microbial protein synthesis (**MPS**). If ruminal fermentable energy is adequate, a large proportion of peptides and AA are used for MPS, and there is limited production of ammonia. For instance, McCarthy et al. (1989) reported that replacing barley with corn as the primary carbohydrates (**CHO**) of a diet significantly increased energy availability for MPS as starch degraded in the rumen increased by 1 kg/d ($P = 0.002$) with use of barley over corn. When energy supply is sufficient, a large amount of ammonia-N is also utilized for MPS. On the other hand, if energy supply is deficient, the capture of peptides, AA and ammonia-N into MPS is compromised, which reduces total MP (Hristov and Jouany, 2005).

Additionally, the rumen microbes may compensate for the limited energy supply by deaminating AA, which is a process that yields ATP that can be used for MPS. Deamination of free AA in the rumen involves enzymatic removal of the amine group, which is then converted to ammonia; the remaining portion of the AA is oxidized to produce ATP (Chen and Russell, 1991). While deamination will allow microbes to continue MPS, it is a much

less efficient method and can result in an increase in the ammonia-N concentration in the rumen (Lapierre et al., 2006; Chen and Russell, 1991).

Coupled with its limited capture for MPS, there is an increase in spillage of ammonia-N into blood across the rumen wall. Because it is neurotoxic, absorbed ammonia-N is transported to the liver where it is detoxified into urea-N. Blood urea-N (**BUN**) can then be either excreted in urine or recycled back to the gut through saliva or blood (Reynolds and Kristensen, 2008).

The utilization of microbial protein is an adaptive mechanism that allows cows to survive and produce milk even on extremely low CP diets (4.5% CP, DM basis; Virtanen, 1966). Because the modern dairy cow is producing approximately twice as much as the average dairy cow was in the early 1960's (USDA-NASS, 2018a), it has a much greater AA and N requirement. Microbial protein, ruminally undegradable protein (RUP; by-pass protein), and endogenous protein contribute to metabolizable AA supply in dairy cows. Of the 3 fractions, microbial protein is the most important because it provides up to 60% of metabolizable AA required to support milk production (Uddin, et al., 2015). In addition, it is also highly digestible in the small intestine; about 80% of AA in microbial protein flowing out of the rumen is absorbed (Rodriguez, et al., 2007). Furthermore, microbial protein is a high-quality protein as it possesses an AA profile nearly identical to that needed for tissue growth and milk synthesis (NRC, 2001; Lapierre et al., 2006). Despite its importance, microbial protein alone does not supply enough metabolizable AA to support milk protein synthesis in high-yielding dairy cows (NRC, 2001). Thus, feeding adequate RUP is essential to provide those AA, which may limit milk protein synthesis, primarily Lys, Met, and His, because of the extensive use of corn-silage and alfalfa hay-based diets (Lee et al., 2012b).

Several sources of RUP, including soybean meal (SBM) and CDDGS, are currently fed to lactating dairy cows to maximize metabolizable AA supply and, thus milk protein synthesis (Lee et al., 2012b; McCarthy et al., 1989). High quality sources of RUP are chosen for their resistance to ruminal degradation and high intestinal digestibility (Loerch, et al., 1983). When increasing dietary RUP, however, it is possible to compromise RDP supply, which reduces rumen N balance and, therefore, reduce MPS (Clark, et al., 1992; Gaillard et al., 2017). Additionally, digestibility of the total diet can impact passage rate through the rumen, reducing time for potential MPS and RUP breakdown. In a 12-year review of 108 studies, 76% reported impaired MPS that was related to use of RUP sources in lactating cow diets (Santos et al., 1989). Thus, it is important to first meet the N requirements of the rumen microbes, and then provide RUP as a source of additional AA.

If provision of RUP does not inhibit MPS, the next major consideration is intestinal digestibility. For a feed to bypass the rumen, it must be either less degradable or facilitate faster passage rate (Casper, et al., 1999). In the case of decreased degradability, there is a risk that the feed will not be adequately hydrolyzed once past the rumen, which limits the amount of nutrients available for absorption. For instance, fish meal has an ideal AA profile, but is much more expensive, whereas SBM is highly digestible and more affordable, although its AA profile is less complete (McCarthy, et al., 1989).

Another consideration in selection of RUP sources is complementarity in terms of AA supply relative to the contribution of microbial protein. For instance, fish meal is an excellent RUP source because it has a high Lys and Met content and can be used as a supplemental AA source (McCarthy et al., 1989). In general, most animal-based proteins have an ideal AA profile that compliment microbial protein. Because of their high cost,

however, they are often overlooked in favor of other sources such as SBM or CDDGS. While both SBM and CDDGS are often deficient in Met and Lys respectively, they are affordable and highly digestible (Nichols, 1998).

Besides microbial protein and RUP, endogenous protein can make a substantially contribution to metabolizable protein (**MP** supply). The primary source of endogenous protein in ruminants is the sloughed off tissue of the gastrointestinal tract (**GIT**). Digestive enzymes and juices also contribute to endogenous protein. Because of constant degradation of tissue protein as part of cellular turnover, the released AA can ultimately undergo deamination. The resulting ammonia-N is detoxified to urea-N, which may be recycled back to the GIT, where it also contributes to endogenous protein. Endogenous protein can contribute up to 20% of total MP flow (Lapierre, 2006; Lapierre and Lobley, 2001), which is influenced by numerous factors including forage quality, source, and digestibility. For instance, feeding hay rather than silage increased endogenous protein supply, likely because of increased scratch factor on the rumen (Ouellet et al., 2010).

1.3 Nitrogen utilization efficiency in dairy cattle

In ruminants, the capture of dietary N into saleable product, including milk protein, is low. For instance, Spek et al. (2013) reported the conversion of only 26% of dietary N into milk protein, whereas 35% was excreted as urine N, and 33% as fecal; leaving approximately 6% of N as an unknown pool. Therefore, up to 80% of consumed N can be excreted as N waste. The major contributor to the low efficiency of N utilization in dairy cows is the indiscriminate degradation of dietary protein by rumen microbes (Tamminga, 1992; Calsamiglia, 2010). The presence of rumen microbes is an adaptive feature of dairy cows which makes them so desirable in converting feed sources otherwise unavailable for

human consumption into highly nutritious animal products. Rumen microbes, however, will indiscriminately degrade all available RDP; this includes high quality dietary CP sources, which could otherwise serve as a good source of metabolizable AA following intestinal digestion (Pfeffer and Hristov, 2005). Similarly, variation in rumen pH and microbial activity, as well as digestive passage rates, can negatively influence N capture. Additionally, availability of fermentable energy in the diet determines N uptake and MPS (Cammell et al., 2000). Therefore, these factors offer an opportunity to improve N efficiency; for instance, manipulation of dietary energy and fiber content to optimize rumen health and therefore microbial activity and reducing dietary CP to maximize N efficiency (Calsamiglia et al., 2010).

The low efficiency of capture of dietary N into milk has both economic and environmental implications. From an economic standpoint and considering that protein sources are typically the most expensive feed component, the overall low N utilization efficiency of dairy cows results in reduced profit margins (Vandehaar et al., 2006). Wastage of dietary N is further exacerbated with the use of high CP diets, which reduces N utilization efficiency and therefore profitability (Godden et al., 2001). The loss of the majority of feed N in urine and feces can also contribute to environmental impact. Currently, there is no direct cost associated with N excretion on US dairy farms. However, the recent litigations alleging environmental pollution because of poor N waste management are an indication that the U.S. may be heading towards more consequential environmental regulations. For instance, the dairy producers sued in Yakima Valley had to spend over $2,000,000, even before implementing the N management practices mandated at the conclusion of the case

(US EPA, 2016). Therefore, improving the efficiency of N utilization has the potential to not only reduce feed costs, but also minimize environmental impact and those associated costs.

From an environmental standpoint, the greatest concern is with urine N as it contains 60 to 90% urea-N (Bristow et al., 1992), which is a labile form of N that can cause pollution. Although fecal N is less environmentally labile, the presence of microbial urease converts urine urea-N into ammonia-N (Bussink and Oenema, 1998). Upon release, which is dependent on the temperature, pH, and oxygen availability, ammonia-N can vaporize into the atmosphere and contribute to the production of haze and acid rain (Bussink and Onenema, 1998). Furthermore, atmospheric ammonia-N can transform to ammonium, contributing to small diameter particulate matter (< 2.5 μm), negatively impacting respiratory health in humans, which is a serious concern (VandeHaar and St-Pierre, 2006).

The proportion of ammonia-N that does not vaporize is absorbed into the soil and can undergo nitrification by soil microbes (e.g., *Nitrosomonas*) to form primarily nitrates, which are an excellent N source for plants (Frate, 2007). However, if nitrates are not taken up by plant roots, they leach through the soil and may end up in ground water. A high concentration of nitrates in groundwater (> 10 mg/L) is undesirable as it can compromise human health (US EPA, 2012a). For instance, excess nitrate concentrations can lead to blue baby syndrome, which occurs because of the ability of nitrates to cause oxidation of hemoglobin to methemoglobin thus, reducing the number of oxygen-carrying red blood cells in the body (Knobeloch, et al., 2000). The process of nitrification is catalyzed by warm temperature. Additionally, a high concentration of urea-N in the soil will lower the pH and results in elevated ammonia-N levels, catalyzing denitrification (Hristov et al., 2011). These factors contribute to the necessity of EPA regulations on manure land application rates. As

soil depth increases and conditions become more anaerobic, ammonia-N can be transformed to nitrites, which can also reach toxic levels in the soil and ground water (US EPA, 2012a). Nitrate and nitrites can undergo further transformation; denitrifying microbes, including *Clostridium spp.*, convert nitrate and nitrites to other nitrous compounds, including nitrous oxide that may be released into the atmosphere. Nitrous oxide is a powerful green-house gas that contributes to the destruction of the ozone layer (Van Cleemput and Samater, 1996). Nitrogen excretions pose several threats to environmental sustainability of dairy production and must be addressed to answer an increasingly conscious consumer.

1.4 Reducing the environmental costs of dairy production

To prevent the potential loss in production performance, there is currently widespread use of high CP diets (Chase et al., 2012 and NRC, 2001; 17.5 - 23%, DM basis) in the dairy industry. This is a result of the belief that dietary CP is directly correlated to MY. However, this is not entirely true because beyond a certain point, an increase in dietary CP does not result in an increase in milk yield (NRC, 2001; Ipharraguerre and Clark, 2005). In addition, high CP diets are fed because of the inclusion of safety margins to prevent a potential decrease in milk and milk protein yield related to a deficiency in MP. Ultimately, use of high CP diets further reduces the efficiency of N utilization in dairy cows.

There have been several studies on the improvement of N utilization efficiency by lowering of CP content in lactating dairy cow diets. For instance, in a study by Barros et al. (2017), diets containing 16.2, 14.4, 13.1 and 11.8% CP (DM basis) were fed to lactating dairy cows for twelve weeks; DMI and MUN concentration decreased linearly as dietary CP content decreased. While the change from 16.2 to 14.4% did not affect milk yield, it led to a decrease in milk protein concentration (Barros et al., 2017). Additionally, milk production

was 1.6 and 5.6 kg lower in cows fed the 13.1 and 11.8% CP diets, respectively, compared to the 16.2% CP diet. After reducing dietary CP from 16.7 to 14.8% (DM basis), Lee et al. (2011) reported a 2.9 kg decrease in MY. Similarly, in a study by Cyriac et al. (2008), reducing RDP by 3.7% (DM basis) resulted in a decrease in DMI and a tendency for a decrease in MY.

One of the major causes of the decrease in MY with lowered dietary CP is the potential decrease in DMI. Low CP diets can impede DMI by impairing rumen function. Specifically, when limiting RDP, rumen ammonia-N concentration is limited, which can negatively impact the microbial population, specifically fiber-fermenting microbes, which are the most sensitive to RDP supply (Russell, et al., 1992). Reduced growth and activity of the rumen microbes may further limit the rate of MPS and efficiency, reducing total MP (Lee et al., 2012; Barros et al., 2017). Furthermore, impaired fiber digestion caused by a CP deficient diet can result in a decrease in the rumen passage rate and prolonging gut fill, which decreases total DMI (Lee et al., 2012a). A decrease in DMI limits MY by reducing available nutrients for the synthesis of milk protein, fat and lactose, and the energy necessary to carry out these processes (Hristov et al., 2004). In addition, limiting N intake could also reduce RUP flow to post-ruminal sites. The potential impact of feeding a low CP diet on MP/AA supply can vary depending on the predominant source of CP used. For instance, Lys, Met and His tend to be the most limiting AA when low CP diets are fed because of the extensive use of corn- and alfalfa-based diets in North American (Lee et al., 2012b). However, it has also been reported (Chandler, 1989) that many RUP sources that are considered high quality (e.g., blood meal and corn gluten meal) are still limiting in some EAA.

Despite the documented loss in production performance (Barros et al., 2017; Lee et al., 2011; Cyriac et al., 2008), there are indications, that N utilization efficiency may be improved when feeding low CP diets. For instance, in the study by Barros et al. (2017), MUN concentration decreased by increments of about 20% with each unit of dietary CP reduction (13.3, 10.1, 8.05, and 5.97 mg/dL, respectively). In the study by Lee et al. (2011), BUN was lower by 36% (20.1 vs. 12.8 mg/dL) for cows fed the 14.8% compared to the 16.7% CP diet. Similarly, Cyriac et al. (2008) noted that reducing RDP by 3.7% (DM basis) resulted in a 10.9% increase in N efficiency, based on measures of milk N and N intake. The improvement in N utilization efficiency following a reduction in dietary CP content is likely related to a decrease in N waste. Specifically, less N in the rumen lowers the amount of ammonia-N absorbed across the rumen wall, providing less ammonia-N for the liver to detoxify into urea-N. Thus, BUN is decreased, and total urea-N available for excretion in the urine or feces is reduced (Hristov et al., 2005).

Conversely, reducing dietary CP content has also been reported not to compromise milk production while maintaining the reduction in N waste (Leonardi et al., 2003). For instance, in a study by Giallango et al. (2015), lowering dietary CP content from 16.7 to 14.8% (DM basis) did not result in a decrease in MY. Milk urea-N, however, was 2 mg/dL lower in cows fed the 14.8 than 16.7% CP diet. Similarly, in a study by Colmenero and Broderick (2006), incrementally reducing dietary CP content (19.4, 17.9, 16.5, 15.0, and 13.5%) did not result in a reduction in MY but reduced MUN from 15.6 to 7.7 mg/dL. In all of these studies, there were also no differences in DMI; possibly because the low dietary CP content did not compromise rumen function. Others (Aguerre et al., 2010; Lee et al., 2012)

have also made similar observations, whereby MY is not compromised when feeding low CP diets.

Decreasing dietary CP content improves the efficiency of N utilization by reducing excess ruminal ammonia-N, which would otherwise escape MPS and be excreted as urine urea-N. A rumen ammonia-N concentration of 5 mg/dL is believed to be adequate for optimal MPS and can be achieved when feeding as little as 13% CP (DM basis), further supporting the potential success of use of low CP diets (Satter and Roffler, 1975). The impact of dietary CP on DMI, however, is inconsistent and the potential decrease in milk and milk protein yield is a major concern for producers as it reduces profitability given the component pricing system for milk. A common practice for producers to maximize profit margins, is use of alternative energy and protein feed sources, to reduce feed input costs.

1.5 Use of corn dried distillers grains with solubles in the dairy industry

Corn DDGS is currently a widely used feed ingredient in the dairy industry, especially in the pacific northwest, where it is readily available; and because of its high CP, especially RUP content, and overall affordability (Kleinschmit et al., 2006; Firkins et al., 1984). In fact, CDDGS can provide ≥ 55% RUP (Kleinschmit et al., 2007; DM basis). Although CDDGS has a high NDF content, it contains a low amount of lignin (NRC, 2001), which makes it highly digestible (Birkelo et al., 2004). Additionally, when compared to high starch energy sources, CDDGS provides a comparable amount of fermentable energy with reduced risk of acidosis. For instance, in a study by Ham et al. (1994), replacing corn grain with DDGS resulted in an increase in available energy (1.24 vs. 1.35 Mcal/kg, respectively; $P \leq 0.05$), but no change in rumen pH was reported. Additionally, CDDGS has been reported (Nichols et al., 1998; Liu et al., 2000) to be comparable to SBM as a protein and

energy source, although SBM tends to be more expensive. Given its high nutritive value, feeding corn DDGS has consistently been reported to increase production performance (Shingoethe et al., 2009).

Because it is typically cost-effective, it is not uncommon for a high amount of CDDGS to be fed to lactating cows on U.S. dairy farms (McCarthy, et al., 1989). Given its high CP content and limiting EAA, however, this practice can increase N wastage. Therefore, reducing total dietary CP may be an option in order to enhance N utilization efficiency when feeding CDDGS as a protein source. For instance, in a study by Chibisa and Mutsvangwa (2013), feeding DDGS in a low (15.2% DM basis) compared to a high (17.3%) CP diet resulted in significant improvement in measures of N utilization. Specifically, MUN was reduced by more than 21% whereas BUN was also lower by more than 20% for cows fed the low vs. high CP diet. However, Chibisa and Mutsvangwa (2013) also observed a decrease in MY and MPY (4.60 and 0.10 kg/d respectively) with reduced dietary CP, which was attributed to reduced nutrient supply, specifically metabolizable AA. In that study, although N digestibility was lower (5.30% decrease) for cows fed the low vs. high CP diet, apparent total tract ADF and NDF digestibility were not compromised, which may explain why DMI did not differ between diets.

It has been reported (Nichols et al., 1998; Liu et al., 2000) that Lys, Met, and Phe are the most limiting AA in diets containing corn-based byproducts, including CDDGS. This is particularly important for Lys as it is heat-sensitive and may be further degraded during the distilling process (Choi et al., 1949). Limiting AA in lactation rations determine potential milk yields, encouraging use of high CP diets to avoid milk loss. In a study by Nichols et al. (1998), Lys, Met and Phe were noted to be the most limiting AA even when feeding a high

CP diet (17.9% CP, DM basis) containing 20% CDDGS compared to that of SBM (18.7% CP, DM basis) to lactating cows. Additionally, supplementation of rumen-protected (**RP**) Met and Lys improved MY and MPY for cows fed the CDDGS diet, but not the SBM diet, suggesting that even at this elevated dietary CP content, the CDDGS diet was significantly limiting in Lys and Met. Furthermore, it was speculated that even with supplementation of RP Lys, the CDDGS diet was still Lys deficient, as there was no improvement seen in plasma Lys concentration. Similarly, in a study by Paz et al. (2013), it was observed that although supplementing RP-Lys in diets (16.7% CP; DM basis) containing an increasing amount of DDGS (0, 10, and 19.9%, DM basis) resulted in no effect on MY, plasma Lys concentration decreased by up to 11.8% as the DDGS inclusion increased. Similarly, in Liu et al. (2000), when feeding a diet containing 18.9% CDDGS (16.6% CP, DM basis) instead of a protein supplement blend (fishmeal and soybean meal; 16.5% CP, DM basis) and supplementing with RP-Lys + Met, Lys and Leu were consistently more limiting. Additionally, Lys and Leu were less efficiently extracted by the mammary gland in cows fed CDDGS (Nichols et al., 1998; Liu et al., 2000). Furthermore, even with RP-Lys + Met, the CDDGS diet was Lys limiting, which suggested that supplementation was still inadequate.

Overall, reducing dietary CP content is the most effective way to improve the efficiency of N utilization in dairy cows. Feeding CDDGS, however, even in high CP diets can result in a deficiency of Lys, Met, and Phe, it may be necessary to provide supplemental by-pass protein/AA. Information on the impact of supplementing by-pass protein/AA sources in low CP diets containing CDDGS on production performance and the efficiency of N utilization is still limited.

1.6 Use of by-pass protein sources and rumen-protected amino acids

There has been success in the use of RP-AA and by-pass protein sources to increase production performance in several studies (Lee et al., 2012; Noftsnger and St-Pierre, 2003). For instance, in a study by Lee et al. (2012a), when feeding a high (15.6%, DM basis) vs. low (14.0%, DM basis) CP diet supplemented with RP Met or RP Lys, MY did not differ across diets, but N excretion was significantly lower for cows fed the 14% CP (DM basis) diet. Milk protein yield, however, was lower for cows fed the low CP diet supplemented with RP Lys. Similarly, Schwab et al. (1992), supplemented Met, Lys, Met + Lys, or Casein in diets throughout four stages of lactation (CP ranged from 13.5 to 16.0%, DM basis); during peak lactation (16.0% CP), only the Lys + Met supplementation improved MY, suggesting Lys was the first limiting AA, and that supplementation may increase overall production performance. This trend was observed in the other three stages of lactation as well; as CP content decreased, RP Met + Lys supplementation consistently improved production performance.

Even when RP amino acid supplementation does not improve MY, it often increases MPY (Lee et al., 2012a). For instance, in a study by Armentano et al. (1996), supplementation of increasing levels of RP Met in a 19.5% CP (DM basis) diet resulted in a linear increase in MPY; from 1,191 g without RP Met to 1,250 g with 11.5 g RP Met. Furthermore, in a study by Zang et al. (2019), supplementation of RP His on a low (15.1%, DM basis) CP diet resulted in a linear increase in plasma His concentration; while plasma Arg, Leu, Lys, and Phe decreased, suggesting a more complete absorbed AA profile available for milk protein synthesis. Additionally, in a study by Ariolla et al. (2014), supplementing moderate (18.75%) and high (21.26% CP, DM basis) CP diets with RP Met,

RP Lys + Leu, RP Met + Lys, RP Met + Leu, and RP Met + Lys + Leu at least in part improved MPY for the lower CP diet; although CP level did not affect MY. While studies in current literature report predominately positive outcomes with supplementation of RP-AA and by-pass protein sources, there is still little information on the interaction of RP-AA/by-pass protein supplementation in low CP diets containing CDDGS.

1.7 Hypothesis and objective

We hypothesized that the supplementation of by-pass protein in cows fed a low compared to a high CP diet would at least limit the decrease in milk and milk protein yield while maintaining an improvement in N utilization efficiency. The objective of this study was to evaluate the effects of feeding a low vs. high CP diet containing 10% CDDGS, supplemented with 2 by-pass protein/rumen-protected AA sources (Supplement A and B; the names of supplements cannot be disclosed as it is proprietary information; however, chemical composition data is reported in later sections), on production performance and the efficiency of N utilization.

Chapter 2. Feeding high and low crude protein diets, containing 10% corn dried distillers grains, and supplemented with by-pass protein products.

2.1 Materials and Methods

The experiment was conducted at the University of Idaho Dairy Center (Moscow, ID) from November 15th, 2017 to January 24th, 2018. All procedures were preapproved by the Institutional Animal Care and Use Committee at the University of Idaho (Protocol #2017-37). Cows were cared for according to the Dairy Center's pre- and post-calving management protocol. Briefly, dry cows are kept at pasture for approximately 45 days before transfer to dry-lot sheds for close-up observation. Fresh multiparous cows are given a Ca bolus at calving, and body temperature is monitored for the first 10 d post-calving. Any incidence of fever is treated with antibiotics and cows are continuously monitored. Furthermore, fresh cows were milked 2 times/d.

2.1.2 Animals and experimental design

Six multiparous Holstein cows (619.3 ± 49.8 kg BW; 26.8 ± 6.2 DIM) were randomly assigned to a split-plot, 3 × 3 Latin square design. The whole-plot factor was dietary CP content; and the subplot was by-pass protein/RP-AA supplement. Each experimental period lasted 21 d, with 18 d for dietary adaptation and 3 d for data and sample collection. Cows were housed in individual tie-stalls for the entire study.

2.1.3 Treatments and feeding management

At the beginning of the study, the cows (n = 6) were assigned to one of two dietary protein concentrations including HP (formulated to contain 17.5% CP; DM basis) and LP (15.5% CP), and to one by-pass protein/RP-AA supplement (top-dressed); CON (no supplement; control), Supp A (0.11 kg of Supplement A/cow/d) or Supp B (0.45 kg of

Supplement B/cow/d). Specifically, three cows were offered a basal LP diet and provided either no supplement, Supplement A, or Supplement B at morning feeding. The other three cows were offered a basal HP diet and provided either no supplement, Supplement A, or Supplement B at morning feeding (Table 3). The basal diets contained 10% CDDGS (DM basis) and the forage:concentrate ratio was 49:51 (Table 1). Single batches of dietary ingredients, including CDDGS were used for the entire study. Diets were fed as a TMR, for *ad libitum* intake (approximately 5% refusals), prepared twice daily with 50% fed at 0630 h and 50% at 1830 h. Water was always available throughout the study.

2.1.4 Data collection and sampling

All cows were weighed before the morning feeding on two consecutive days at the beginning of each period. Additionally, cows were body condition scored at the start of each period by two observers. To determine DMI, the TMR offered and refused were weighed and recorded daily for each cow. Samples of dietary ingredients were collected weekly on two consecutive days for particle size determination using the Penn State Particle Separator as described by Kononoff et al. (2003). Samples were also dried for 72 h in a forced air oven at 55°C in preparation for CP analysis, which was conducted weekly to ensure that the 2% difference in dietary CP content was maintained for the entire study. Weekly silage DM content was determined to adjust the forage inclusion level (as-fed basis) if it deviated by >3% from the previous week's average. Samples of TMR and refusals were also collected on d 19, 20, and 21 of each period. The TMR and refusal samples were composited by cow per period, dried for 72 h in a forced air oven at 55°C, and ground through a 2-mm screen (Retsch Cutting Mill SM 200, Retsch, Haan, Germany) for later analyses. Cows were milked twice daily at 0600 and 1800 h. Milk samples were collected into containers with bronopol

as a preservative at 0600 and 1800 h from d 19 to 21. Samples were stored at 4ºC before shipping to Dairy One Cooperative Inc. (Ithaca, NY) for analysis of fat, protein, lactose, SCC, MUN, and solids (Milkoscan; Fourier Transform Infrared Spectroscopy).

Spot urine samples were collected from all cows at 0900, 1500 and 2100 h on d 19, 0300, 1200 and 1800 h on d 20, and 0000 and 0600 on d 21. To prevent loss of NH_3-N, 50-mL aliquots of the urine collected at each time point were immediately added to 3 mL of 2*M* H_2SO_4. Thereafter, a 1-mL subsample of the acidified urine was diluted in 9 mL of distilled H_2O. All collected samples were composited by cow per period and stored at -20°C for later analyses. Fecal grab samples were collected at the same time as spot urine samples and stored at 4ºC. On the last day of each period, collected fecal samples were composited by cow and dried to a constant weight in a forced air oven at 55°C. Dried fecal composites were ground through a 2-mm screen (Retsch Cutting Mill SM 200, Retsch, Haan, Germany) and stored in sealed bags for later analyses.

Blood samples were collected by jugular venipuncture on d 21 at 3 h after morning feeding into 10 mL evacuated tubes (Becton Dickinson Vacutainer Systems, Franklin Lakes, NJ) containing either a clot activator or sodium heparin. After collection, blood samples were immediately transported to the laboratory for centrifugation (645 *g* for 20 min at 4°C). Plasma and serum were harvested and frozen at -20°C for later analyses.

2.1.5 Sample analysis

The ingredient, TMR, refusal and fecal samples were analyzed for analytical DM (AOAC, 1991; method 930.15), ash (AOAC, 2006; method 942.05), total N (Kjeltec 8400, FOSS Analytics; Hillerod, Denmark; AOAC, 1990; method 976.05), ADF (AOAC, 2005; method 973.18) and NDF (Mertens, 2002; with amylase and sodium sulfite). Composite

samples (d 19 – 21) of TMR and refusals were particle sized with a 2 screen (19mm and 8mm, respectively) Pennsylvania state particle separator (Jones and Heinrichs, 2013) for determination of particle length distribution, and to screen for sorting behavior. Sorting was calculated (per Leonardi and Armentano, 2003) by comparing actual intake of each fraction with predicted intake. The iNDF content of TMR, refusal, and fecal samples was also determined according to Valente et al. (2011). Briefly, samples were weighed into nylon bags (F57, Ankom Technology, Macedon, NY), distributed in a large mesh bag, and incubated for 288 h in the rumen of two cannulated lactating Holstein cows. After incubation, all bags were rinsed under tap water, and then washed three times using a washing machine (1-minute wash and 2-minute spin cycles). Residues were then analyzed for NDF as previously described. Samples of dietary ingredients were also sent to the University of Missouri Experimental Station Chemical Laboratories for amino acid analysis (AOAC Official Method 982.30, 2006).

Prior to analysis, plasma and serum samples were thawed at room temperature. Thereafter, commercial kits were used to analyze for NEFA (Caymen Chemcial Co., Ann Arbor, MI), βHBA (Caymen Chemical Co., Ann Arbor, MI), glucose (Caymen Chemical Co., Ann Arbor, MI), and PUN (Arbor Assays; Ann Arbor, MI). Dilute acidified urine composite samples were also thawed at room temperature before analysis. Thereafter, samples were then analyzed for creatinine (Caymen Chemical Co., Ann Arbor, MI), urea-N (Arbor Assays; Ann Arbor, MI) and total N (Kjeldahl method). Purine derivatives (allantoin and uric acid; Verbic et al., 1990) were analyzed via HPLC/MS using a method adapted from Stentoft et al. (2014). Briefly, separation was carried out using a reversed-phase column (2 mm × 250 mm, 5 μm particle size,; C18, Phenomenex, Torrance, CA) and a 5%

methanol mobile phase. Flow rate was 200 µL/min with an injection volume of 5 µL. Column temperature was maintained at 30° C and sampler temperature was at 4° C.

2.1.6 Calculations

Urine output was estimated using creatinine concentration, with the constant of 29 mg/kg BW (Valadares et al., 1999). Creatinine concentration and BW for each animal in each period were used in the following equation:

$$\textbf{Urine output, kg/d} = (29 \times BW^{0.75}) \div \textbf{Creatinine concentration, mg/dL}$$

The TMR and fecal iNDF data was used to estimate fecal output, according to the following equation:

$$\textbf{Fecal output, kg} = (\textbf{iNDF intake} \times \textbf{\% iNDF indigestibility}) \div \textbf{Fecal \% iNDF}$$

Apparent total tract digestibility was then calculated using the following equation:

$$\textbf{Digestibility, \%} = [(\textbf{Nutrient intake, g} - \textbf{Nutrient output, g}) \div \textbf{Nutrient intake, g}] \times 100$$

Apparent N balance was calculated as the difference between N intake and N excretion (fecal + urine + milk). To estimate purine absorption, excretion of purine derivatives (allantoin and uric acid) was used, as described in Chen and Gomes (1992), in the following equation:

$$PD_{excreted}\textbf{, mmol/d} = 0.85(PD_{absorbed}) + (0.385\ BW^{0.75}),$$

where 0.85 is the recovery of absorbed purines and 0.385 $BW^{0.75}$ represents purine excretion from endogenous sources. Microbial N flow was then calculated using estimated PD absorption according to the following equation:

$$\textbf{Microbial N, g/d} = 70(PD_{absorbed}) \div (0.116 \times 0.83 \times 1{,}000),$$

where 70 represents the known N content of purines, 0.83 is the digestibility of purines, and 11.6:100 is the purine-N:total N ratio in rumen microbes.

2.1.7 Statistical analysis

All data were analyzed as a split-plot 3 × 3 Latin square design using the MIXED procedure of SAS (SAS version 9.4; SAS Institute Inc., Cary, NC). The model included the following independent variables: cow, period, basal diet (LP vs. HP), supplementation (CON, Supp B, Supp A), and basal diet × supplementation interaction. The period, basal diet and supplementation were considered fixed, and cow was considered random. Significance was declared at $P < 0.05$ and trends at $0.05 < P < 0.10$. Results are presented as least square means.

2.2 Results

2.2.1 Diets and chemical composition

The ingredient and chemical composition data of the basal diets are presented in Table 1. Diets were formulated to contain 15% (LP) or 17% CP (HP) on a DM basis. To reduce dietary CP content, a portion of canola meal in the high CP pellet was replaced with soybean dust in the low CP pellet (Table 2). Although CP content was lower (14.6% and 16.6% CP) than the formulation targets, we were able to maintain the 2% difference between the diets throughout the study. The OM, NDF, and ADF content of the basal diets were comparable between the HP and LP diet. As for AA composition, the HP pellet contained a greater amount of Leu, Lys, Phe, Thr, and Glu than the LP pellet, which reflects the substitution of canola for soybean protein (Table 2, 3). Furthermore, Supp A had greater concentrations of Lys, Met, and His than Supp B, because of the use of different blends of plant and animal by-pass protein/RP-AA sources.

2.2.2 Production measures

There was no dietary CP content × supplement interaction ($P \geq 0.22$) for all measurements. Dry matter intake did not differ across dietary CP content ($P = 0.45$) and by-pass protein/RP-AA supplement ($P = 0.78$; Table 4). Milk yield and ECM also did not differ ($P \geq 0.27$) in cows fed the LP compared to HP diet. Similarly, provision of a by-pass protein/RP-AA supplement had no detectable effect ($P \geq 0.88$) on milk or milk component yields. Although dietary CP content had no detectable effect ($P \geq 0.19$) on milk fat and lactose yield, milk protein yield tended to be lower ($P = 0.10$) in cows fed the LP than HP diet. Neither dietary CP content nor by-pass protein/RP-AA supplementation had an effect

($P \geq 0.46$) on feed efficiency. Similarly, there was no dietary CP content or by-pass protein/RP-AA supplement effect on N efficiency ($P \geq 0.86$).

2.2.3 Intake and apparent total tract nutrient digestibility

There was no dietary CP content × supplement interaction ($P \geq 0.18$) for all nutrient digestibility measurements. Dietary CP content did not have an effect ($P \geq 0.32$; Table 5) on OM, NDF, and ADF intake. Similarly, there was no detectable basal diet effect ($P \geq 0.18$) on apparent total-tract digestibility of DM, OM, NDF, and ADF. Crude protein intake and apparent total-tract CP digestibility, however, were lower ($P = 0.01$) in cows fed the LP than HP diet. There were no differences ($P \geq 0.30$) in intake or apparent total tract digestibility of DM, OM, NDF, ADF, or CP detected across by-pass protein/RP-AA supplements.

2.2.4 Measures of nitrogen utilization

With the exception of total urine ($P =0.04$) and urine urea-N output ($P = 0.05$), there was no dietary CP content × supplement interaction for all other measures of N utilization (Table 6). Nitrogen intake, total N excretion and BUN concentration, however, were lower ($P \leq 0.02$) for cows fed the LP compared to that of the HP diet. Similarly, there was a tendency for total urinary N output ($P = 0.07$), urinary urea N excretion ($P = 0.05$), urine uric acid ($P = 0.06$) and MUN concentration ($P = 0.07$) to be lower for cows fed the LP than HP diet. There was no dietary CP effect ($P \geq 0.28$) on milk N output, fecal DM (g/d) and N excretion (g/d and % of N intake), total urine (kg/d), urine N (% of N intake) and urea-N (% of total urine N) output. Apparent N balance, total urinary purine derivative excretion, and microbial N flow also did not differ ($P \geq 0.41$) for cows fed the LP compared to the HP diet. In addition, there was no by-pass protein/RP-AA supplement effect ($P \geq 0.12$) on all measures of N utilization. There was a diet × supplement interaction for total urine output

(Figure 1; $P = 0.04$); the cows fed the LP diet excreted less urine per day than the cows fed the HP diet when receiving no supplementation (CON), whereas urine output did not differ across dietary CP content when supplements were provided.

2.2.5 Plasma AA and metabolite concentration

For EAA, there was a dietary CP content × by-pass protein/RP-AA supplement interaction ($P \leq 0.02$) for plasma His, Ile, Leu and Val concentrations, and a tendency for a dietary CP content × by-pass protein/RP-AA supplement interaction for plasma Lys ($P = 0.06$) and Met concentrations ($P = 0.05$; Table 7). Specifically, the plasma His, Ile, Leu, and Val concentrations were lower for cows fed the LP than HP diet when provided with Supp B (Figure 2; $P \leq 0.02$). Additionally, plasma Ile, Leu, and Val concentrations were greater for cows fed the LP than HP diet when provided with Supp A ($P \leq 0.02$). There was no diet effect ($P \geq 0.17$), however, on plasma Arg, Thr and Trp concentrations. Except for plasma Gly and Tyr concentrations, which were greater ($P \leq 0.03$) for LP than HP cows, there was no diet effect ($P \geq 0.16$) on other NEAA. There was a dietary CP content × by-pass protein/RP-AA supplement interaction ($P \leq 0.02$) for total plasma EAA and BCAA concentrations, and a tendency for a dietary CP content × by-pass protein/RP-AA supplement interaction ($P = 0.07$) for plasma total AA concentration. Specifically, plasma total BCAA and EAA concentrations were higher for cows fed the LP than HP diet when provided with Supp A, and plasma BCAA was lower for cows fed the LP than HP diet when provided with Supp B (Figure 2; $P \leq 0.02$). However, there was no diet effect ($P \geq 0.40$) on plasma total urea-N cycle AA, sulfur AA and NEAA concentrations. Similarly, diet had no detectable effect ($P \geq 0.12$) on plasma 3-methylhistidine and carnosine concentrations and plasma glucose concentration ($P \geq 0.27$).

2.3 Discussion

There is widespread use of CDDGS in lactating cow rations in the U.S., because it is cost-effective compared to traditional protein sources. Feeding CDDGS, however, especially in the high CP diets (16.5 to 18.5%, DM basis) that are typically fed during early lactation increases urinary and fecal N excretion and, thus, reactive N emissions. Consequently, there is growing interest in the development of nutritional strategies that reduce feed costs, maximize milk production, and limit N excretion when feeding CDDGS in lactating cow diets. Although restricting N intake reduces N wastage, it can compromise metabolizable AA supply and, thus, production performance. Therefore, our objective was to determine the effects of feeding supplemental by-pass protein sources (Supplement B and Supplement A) in high (16.6%; DM basis) vs. low (14.6%) CP diets containing 10% (DM basis) CDDGS on production performance and measures of N utilization efficiency.

Except for plasma AA concentrations and urinary excretion data, there were no interactions between dietary CP and supplemental RP-AA/bypass protein for all measurements in this study. Therefore, most of the discussion will focus on the main effects of dietary CP and supplemental RP-AA/bypass protein. Although feeding a low compared to a high CP diet resulted in a decrease in N intake, milk and milk component yields did not differ across diets (Table 4; Table 5). Giallongo et al. (2015) and Weigel et al. (1997) also did not observe a decrease in production performance when limiting N intake. Others (Lee et al., 2012a, Haque et al., 2012 and Fagundes et al., 2017), however, have reported a decrease in milk and milk protein yield when N intake was limited. Lee et al. (2012a) reported a 1.5 kg decrease in DMI when dietary CP content was reduced from 15.7 to 13.5% and this was attributed to a RDP deficiency that compromised ruminal microbial digestion and, thus,

passage rate. Similarly, Barros et al. (2017) reported a linear decrease in DMI with decreasing dietary CP content (16.2, 14.4, 13.1, and 11.8% CP, DM basis). In the current study, we did not measure nutrient digestion in the rumen. However, because apparent total tract DM and fiber digestibility did not differ across diets it is plausible that ruminal digestion was also not compromised, thus explaining DMI and milk production being similar for cows fed the low compared to high CP diet. Others, (Giallongo et a., 2015; Colmenero and Broderick, 2006) also did not report a decrease in DMI when lactating cows were fed low (13.5 to 15.8 %) than high CP (16.5 to 19.4%, DM basis) diets. In a study by Chibisa and Mutsvangwa (2013), feeding a low compared to high CP diet resulted in a 3.0 kg/d and 140 g/d decrease in milk and milk protein yield, respectively. This loss in production performance was attributed to a decrease in metabolizable protein supply caused by a decrease in ruminal NH_3-N concentration that compromised microbial protein synthesis. In the current study, although it was not measured, the observed decrease in BUN, MUN and UUN suggests ruminal NH_3-N concentration may have been lower in cows fed the low than high CP diet. However, microbial N supply estimated using urinary purine derivative excretion did not differ across diets. Therefore, the decrease in RDP supply when N intake was reduced possibly did not result in an NH_3-N deficiency that could have caused a substantial decrease in MP supply to limit milk production.

Because others (Nichols et al., 1998; Liu et al., 2000) have reported Lys, Met, and Phe to be limiting in high CP diets containing CDDGS, we expected metabolizable Lys and Met supply to be lower in cows fed the 14.6 compared to 16.5% CP diet. Therefore, we also expected the provision of supplemental RP AA/by-pass protein to be more beneficial for cows fed the 14.6 than 16.5% CP diet. We did not observe a supplement effect for any

measures of production performance. This contrasts with others (Socha et al., 2005 and Broderick et al., 2009) who reported an improvement in production performance in cows fed a low compared to a high CP diet supplemented with RP Lys, Met and His. Because we did not observe a decrease in milk and milk component yield, it is possible that the Lys and Met deficiency in cows fed the 14.6 than 16.6% CP diet was not substantial enough to compromise production performance. Therefore, this could explain the lack of a supplement effect in the current study.

As expected, limiting N intake in the current study resulted in a decrease in urinary urea-N excretion, which possibly accounts for the decrease in total urine output as Maltz and Silanikove, (1996) reported a positive correlation between urine urea-N excretion and total urine production. For cows fed the LP diet and provided with either Supp A or B, however, urine urea-N tended to increase and total urine output increased, which resulted in similar urine output levels as cows fed the HP diet. This suggests that supplementation of by-pass protein/RP-AA sources resulted in an increase in the amount of AA hydrolyzed, releasing ammonia-N, possibly because a portion of the supplemented AA provided were not being utilized for anabolic processes such as milk protein synthesis, tissue growth, and repair.

In the present study, we expected that the LP diet would be limiting in some EAA, and therefore, provision of Supp A or B would increase the metabolizable AA supply, resulting in increased MY and/or MPY. We measured plasma AA concentration as an indirect indicator of AA supply. There were no differences in the plasma AA concentrations for cows fed the HP and LP diets with no supplementation. Plasma His, Ile, Leu, and Val concentrations, however, were lower for cows fed the LP than HP diet receiving Supp B. Additionally, plasma concentration of Ile, Leu, and Val were higher in cows fed the LP than

HP diet when provided with Supp A, although plasma His did not differ. Similar reductions in plasma His, Arg, Ile, Leu, Lys, Val, and Phe have been observed with supplementation of low CP diets (Piepenbrink et al., 1996 and Kröber et al., 2000). These observations could possibly be related to AA supply relative to requirements for protein synthesis. Provision of increasing levels of RP-His to a low compared to a high CP diet (14 vs. 18 % CP, DM basis) resulted in a decrease in plasma His, which was attributed to an increase in the extraction and utilization for protein synthesis; suggesting His was a limiting AA, and supply was increased with supplementation (Piepenbrink et al., 1996). Similarly, besides a decrease in several plasma EAA concentrations, when providing RP-His in a low CP (15.1%, DM basis) diet, Zang et al. (2018) also noted an increase in plasma and muscle His concentrations, suggesting that supplementation could have partially corrected an imbalanced metabolizable AA supply. In lactating cows, the plasma AA concentration is influenced by numerous factors besides dietary supply. For instance, post-absorptive use including mammary gland extraction for milk protein synthesis, body protein mobilization, and AA catabolism are all processes that have an impact on plasma AA concentration; however, they were not measured in the present study. Therefore, a potential increase in the availability of limiting AA when feeding Supp B in the LP than HP diet could possibly explain the decrease in plasma His, Ile, Leu and Val concentrations. The potential of an improved absorbed AA profile with use of Supp B over Supp A is not surprising; despite the fact that Supp A had higher concentration of almost all EAA, Supp B was fed at four times the inclusion level (Table 2; 113 vs. 453 g/d). The different inclusion rates for each supplement were used based on product recommendations; likely because of the greater EAA concentration of Supp A. Specifically, Met concentration is four times higher in Supp

A than B. Based on the amount of each supplement provided, however, Supp B supplied more than twice as much His, Ile, Leu, and Val each day (Table 2, 3).

2.4 Conclusion

Reducing dietary CP content from 16.6 to 14.6% when feeding CDDGS to cows during early lactation did not result in a decrease in DMI, MY, and milk component yield possibly because metabolizable protein supply was not compromised. Therefore, this may in part explain the lack of a benefit observed when providing by-pass protein/RP-AA supplements on production performance, despite the changes in the plasma AA profile. In addition, feeding CDDGS in a LP vs. HP diet also resulted in decreased MUN and UUN, which is suggestive of an improvement in the efficiency of N utilization. Provision of supplements to cows fed the LP diet, however, resulted in an increase in both total urine and urea-N output, which possibly indicates that at least a portion of the supplemented AA were not captured for anabolic purposes and the N was excreted as waste.

Chapter 3. Summary and Implications

In our study, we anticipated that reducing dietary CP from 16.6% to 14.6% when feeding CDDGS would improve the efficiency of N utilization, but also possibly reduce production performance. Whereas we did observe a decrease in BUN, MUN, and UUN, indicating less N wastage, there was no negative effect on production performance when feeding the low CP diet, except for a decrease in milk protein composition. This contradicts with reports from others (Chibisa and Mutsvangwa, 2013; Barros et al., 2017), who showed that decreasing dietary CP decreased MY while improving N utilization efficiency. Considering DMI was not affected in our study, it is possible that feeding the LP diet did not result in impaired rumen fiber digestion, and therefore did not decrease DMI and MY. This likely means that we did not restrict MP supply to a great extent when feeding the LP diet. Therefore, this may, in part, explain why we did not observe any supplement effects on production performance measures. We observed, however, a dietary CP × supplementation interaction for urine output and some plasma EAA levels, which suggests that supplementing the LP diet influenced AA supply and metabolism. The changes, however, were possibly not substantial to impact MY.

There were some limitations to our experimental design and execution, which may have resulted in some of our unanticipated observations. For instance, we were limited to use of 6 Holstein cows in a 3 × 3 split-plot experimental design, because of budgetary constraints. While the experimental power is limited with use of a low number of animals, others (Lascano and Heinrichs, 2011; Suarez-Mena et al., 2013) have used a similar approach as a strategy to obtain useful estimates. Furthermore, we were unable to study ruminal N metabolism, such as measurement of ammonia-N concentration. While it was our

intention to collect rumen samples for analysis, we were forced to reconsider this plan during our first sampling period. Unfortunately, the rumen sampling hose we had was not rigid enough such that it proved difficult to penetrate the rumen mat. Therefore, the samples we collected had varying degrees of saliva contamination, which prevented us from analyzing them. It is not uncommon, however, to use other measures, such as MUN and UUN, as indirect measures of ruminal metabolism (Jonker et al., 1998). For instance, the ammonia-N lost from the rumen is detoxified to urea-N in the liver, which then can be excreted as MUN and UUN; therefore, there is a positive correlation between those variables. Lastly, this study was conducted using cows in early lactation, which is a period when animals are experiencing numerous stressors. Therefore, this can result is wide variation in measurements taken during this period as different animals cope with the stressors to varying degrees. We chose to work with cows during early lactation, however, because it is during this phase that producers mostly often over-feed protein because of the high level of milk production (NRC, 2001).

Given these limitations, there are opportunities to generate more useful information in this area of research. We conducted a metabolism study, with only a small group of animals. Therefore, a large production study, possibly at the farm scale, would increase statistical power and provide useful estimates. Additionally, we only tested two supplementation rates. Therefore, there is potential for testing other inclusion levels for each of the supplements to determine the optimum rate to use, based on differing EAA profiles, as well as absorption efficiencies. For AA supplementation overall, there is great potential in the industry for a better understanding of meeting specific AA demands; even if it is still not a realistic method for most producers because of cost. Gaining more knowledge on the use of these by-

pass protein/RP-AA sources could enable greater adoption of the use of LP diets, which would improve both environmental and economic sustainability of the US dairy industry.

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Tables and Figures

Table 1. Ingredient and chemical composition of the basal low and high CP diets

	Dietary CP content	
Item	Low (LP)	High (HP)
Ingredient, % of DM		
Barley silage	28.0	28.2
Barley grain	13.3	14.3
Rolled corn	12.0	12.9
Canola meal	1.67	11.9
Alfalfa hay	10.7	10.7
Grass hay	10.3	9.7
Corn DDGS	10.0	10.0
Soybean hulls	11.3	0.00
TopPeak[1]	1.00	1.00
ADE premix[2]	1.15	0.87
MagnaFat[3]	0.45	0.45
Salt	0.05	0.04
DM, %	57.0	57.0
OM, % of DM	90.1	89.6
CP, % of DM	14.6	16.6
ADF, % of DM	54.6	53.7
NDF, % of DM	43.7	41.6
NE_L, Mcal/kg	1.41	1.51
Protein supply and balance[4]		
RDP, g/d	2,670	2,961
RUP, g/d	1,886	2,287
MP, g/d	2,905	3,244
MP balance	45.6	292
Lys, g/d	158	174
Lys balance	-39.0	-30.3
Met, g/d	54.5	62.7
Met balance	-14.0	-8.10
Lys:Met ratio	2.90	2.77

[1]Liquid supplement (Performix Nutrition Systems; Agri Beef Co., Boise, ID) contained 0.5% fat, 8.27% CP (0.21% as NPN), 6% Ca, 0.16% P, 2% K, 1.07% Mg, 0.35% S, and 4.10% salt.

[2]ADE premix (Performix Nutrition Systems; Agri Beef Co., Boise, ID) contained 14.4% CP, 20.4% Ca, 0.47% P, 0.66% K, 0.69% Mg, 0.36% Mg, and 7.61% Z, 540 kIU/kg vitamin A, 86.4 kIU/kg vitamin D, and 0.214 kIU/kg vitamin E.

[3]MagnaFat (Energy Feeds International; San Leandro, CA) contained 82.4% crude fat (total CA long-chain fatty acids; 42% palmitic, 3% stearic, 35% oleic, and 9% linoleic), 4% unsaponifiable matter, and 5% moisture.

[4]Protein supply and balance data calculated using CNCPS.

Table 2. Amino acid profile of dietary ingredients and by-pass protein supplements

Item	Dietary Ingredient								
	Barley silage	Alfalfa hay	Grass hay	Corn grain	Barley grain	Low CP pellet[1]	High CP pellet[2]	Supp A	Supp B
CP, %	9.60	21.3	7.50	9.10	10.9	23.1	32.1	--	--
DM, %	94.0	95.0	95.6	78.5	90.2	88.1	88.6	--	--
EAA, W/W%[3]									
Arg	0.23	0.86	0.26	0.30	0.50	1.08	1.61	3.28	4.53
His	0.12	0.23	0.11	0.20	0.21	0.51	0.78	4.16	2.22
Ile	0.36	0.90	0.27	0.25	0.36	0.91	1.21	1.10	2.24
Leu	0.37	1.06	0.47	0.77	0.66	1.98	2.44	8.20	6.25
Lys	0.32	0.71	0.34	0.24	0.43	0.94	1.50	8.84	4.15
Met	0.11	0.27	0.11	0.13	0.17	0.34	0.56	3.51	0.79
Phe	0.37	1.06	0.30	0.32	0.46	0.87	1.24	4.19	3.37
Thr	0.20	0.60	0.27	0.24	0.33	0.81	1.20	2.15	2.59
Trp	0.06	0.25	0.05	0.06	0.08	0.18	0.32	0.74	0.59
Val	0.48	1.02	0.36	0.34	0.51	1.01	1.52	5.81	4.90
NEAA									
Ala	0.39	0.72	0.40	0.49	0.46	1.17	1.50	5.14	4.75
Asn	0.44	1.44	0.61	0.47	0.63	1.60	1.99	7.09	6.02
Cys	0.20	0.44	0.08	0.16	0.22	0.53	0.72	0.80	1.75
Glu	0.68	1.45	0.67	1.18	2.09	3.19	5.08	6.33	7.78
Gly	0.41	1.01	0.32	0.28	0.42	1.03	1.40	4.01	6.43
Orn	0.16	0.29	0.01	0.00	0.00	0.02	0.02	0.05	0.12
Pro	0.41	1.08	0.56	0.58	2.09	1.47	2.03	3.23	5.28
Ser	0.35	1.14	0.25	0.29	0.37	0.94	1.17	2.91	3.97
Tau	0.14	0.19	0.14	0.18	0.18	0.14	0.11	0.03	0.08
Tyr	0.24	0.68	0.13	0.18	0.24	0.75	0.90	1.61	1.90

[1]Low CP pellet contains 38.0% soybean dust, 32.2% CDDGS, 10.7% canola meal, 10.7% barley grain, 5.37% ADE supplement, 1.61% salt, and 1.29% MagnaFat.

[2]High CP pellet contains 48.8% canola meal, 32.2% CDDGS, 10.74% barley grain, 5.37% ADE supplement, 1.61% salt, and 1.29% MagnaFat

[3]W/W% = grams per 100 grams of ingredient.

Table 3. Amino acid profile of each dietary treatment combination.

	Dietary treatments[1]					
	LP			HP		
	CON	Supp A	Supp B	CON	Supp A	Supp B
EAA[2], W/W%[3]						
Arg	1.60	1.64	1.80	2.09	2.13	2.30
His	0.76	0.81	0.86	1.01	1.06	1.11
Ile	1.57	1.58	1.67	1.85	1.86	1.95
Leu	2.83	2.92	3.11	3.26	3.35	3.54
Lys	1.54	1.64	1.73	2.06	2.16	2.25
Met	0.57	0.61	0.60	0.77	0.81	0.81
Phe	1.60	1.65	1.76	1.95	1.99	2.10
Thr	1.23	1.26	1.35	1.60	1.62	1.71
Trp	0.31	0.32	0.33	0.44	0.45	0.46
Val	1.89	1.96	2.11	2.37	2.43	2.59
NEAA						
Ala	1.93	1.99	2.15	2.24	2.30	2.46
Asn	2.52	2.60	2.79	2.88	2.96	3.15
Cys	0.89	0.90	0.97	1.07	1.08	1.15
Glu	4.81	4.89	5.17	6.57	6.64	6.92
Gly	1.78	1.82	2.07	2.12	2.17	2.41
Orn	0.25	0.25	0.26	0.25	0.25	0.26
Pro	2.64	2.68	2.88	3.16	3.20	3.40
Ser	1.62	1.65	1.80	1.83	1.87	2.01
Tau	0.43	0.43	0.43	0.40	0.40	0.41
Tyr	1.19	1.20	1.27	1.32	1.34	1.41

[1]Basal treatment = high CP (HP) or low CP (LP); supplemental treatment = CON (No supp), Supp A (113g), or Supp B (453g).

[2]EAA = Essential AA, NEAA = non-essential AA

[3]W/W% = grams per 100 grams of ingredient.

Table 4. Production performance for cows fed a high-CP or low-CP diet containing corn DDGS and top-dressed with either no supplement (CON), supplement A (Supp A), or supplement B (Supp B).

	CP content			By-pass protein/RP-AA supplement				*P*-value[1]		
Variable	Low	High	SEM	CON	Supp A	Supp B	SEM	CP	SP	CP × SP
DMI, kg/d	24.6	25.7	1.01	25.1	25.8	24.6	1.24	0.45	0.78	0.43
Milk yield, kg/d	41.1	44.3	2.52	42.0	42.7	43.3	3.09	0.39	0.96	0.24
ECM[2], kg/d	39.5	43.9	2.65	40.4	42.8	41.8	3.36	0.27	0.87	0.26
Feed efficiency[3]	1.60	1.70	0.09	1.61	1.62	1.71	0.11	0.46	0.76	0.59
N efficiency[4]	29.7	29.8	1.40	29.2	29.6	30.5	1.78	0.97	0.86	0.27
Milk protein, %	2.71	2.93	0.07	2.87	2.80	2.80	0.08	0.04	0.78	0.91
Milk protein yield, kg/d	1.11	1.29	0.08	1.20	1.20	1.20	0.09	0.10	0.99	0.31
Milk fat, %	3.44	3.25	0.12	3.30	3.28	3.45	0.15	0.27	0.69	0.55
Milk fat yield, kg/d	1.40	1.49	0.11	1.39	1.46	1.48	0.14	0.58	0.88	0.48
Milk lactose, %	4.79	4.88	0.05	4.87	4.79	4.85	0.06	0.17	0.67	0.22
Milk lactose, kg/d	1.96	2.34	0.14	2.05	2.16	2.08	0.18	0.19	0.89	0.29
Plasma glucose, mg/dL	84.8	75.0	6.01	75.4	80.2	84.0	7.36	0.27	0.72	0.49

[1]CP = dietary CP content, SP = by-pass protein/RP-AA supplement, and CP × SP = dietary CP content × by-pass protein/RP-AA supplement interaction.

[2]ECM = [0.327 × milk yield (kg/d)] + [12.95 × milk fat yield (kg/d)] + [7.2 × milk protein yield (kg/d)].

[3]Feed efficiency = [CM ÷ DMI].

[4]N efficiency = [(milk N ÷ N intake) × 100].

Table 5. Nutrient intake and apparent total tract nutrient digestibility for cows fed a high-CP or low-CP diet containing corn DDGS and top-dressed with either no supplement (CON), supplement A (Supp A), or supplement B (Supp B).

	CP content			By-pass protein/RP-AA supplement				*P*-value[1]		
Variable	Low	High	SEM	CON	Supp A	Supp B	SEM	CP	SP	CP × SP
Intake, kg/d										
OM	22.3	23.8	1.01	23.2	23.3	22.7	1.23	0.32	0.93	0.88
NDF	11.3	11.7	0.59	11.7	11.8	10.9	0.72	0.65	0.64	0.86
ADF	15.3	15.3	0.35	15.2	15.4	15.3	0.43	0.90	0.92	0.98
CP	3.61	4.40	0.18	4.03	4.10	3.89	0.22	0.01	0.80	0.85
ATTD[2], % of intake										
DM	50.9	57.9	2.42	57.5	53.7	51.9	28.4	0.12	0.38	0.99
OM	55.1	60.7	2.55	61.6	56.4	55.6	3.13	0.15	0.38	0.92
NDF	41.7	49.4	3.52	48.3	45.3	43.0	4.30	0.15	0.69	0.81
ADF	54.2	59.6	2.72	59.4	55.3	56.1	33.3	0.18	0.67	0.86
CP	43.9	56.8	2.98	54.5	50.6	45.9	36.5	0.01	0.30	0.78

[1]CP = dietary CP content, SP = by-pass protein/RP-AA supplement, and CP × SP = dietary CP content × by-pass protein/RP-AA supplement interaction

[2]ATTD = apparent total tract digestibility

Table 6. Measures of N utilization for cows fed a high-CP or low-CP diet containing corn DDGS and top-dressed with either no supplement (CON), supplement A (Supp A), or supplement B (Supp B).

	CP content			By-pass protein/RP-AA supplement				*P*-value[1]		
Variable	Low	High	SEM	CON	Supp A	Supp B	SEM	CP	SP	CP × SP
N intake, g/d	578	704	28.8	645	655	623	35.3	0.01	0.80	0.85
Milk N output, g/d	170	202	18.2	185	182	192	15.8	0.28	0.80	0.31
Fecal excretion										
DM, kg/d	11.7	10.5	0.90	10.1	11.0	12.3	1.14	0.36	0.41	0.28
N, g/d	315	333	27.9	292	317	362	35.4	0.65	0.37	0.29
N, % of N intake	45.3	54.4	4.89	45.0	53.2	51.2	6.36	0.23	0.58	0.49
Urinary excretion										
Total output, kg/d	35.6	34.5	2.75	37.0	33.9	34.2	2.66	0.78	0.42	0.04
N, g/d	245	319	25.5	289	269	288	35.5	0.07	0.90	0.50
Urea-N, g/d	139	185	14.8	157	157	172	20.5	0.05	0.79	0.05
N, % of N intake	40.3	43.5	3.35	43.3	44.0	43.5	4.11	0.55	0.99	0.71
Urea-N, % of total urine N	59.4	56.6	4.22	53.1	59.1	61.8	4.09	0.67	0.12	0.17
Total N excretion, kg/d										
N, g/d	462	561	26.6	511	533	491	33.7	0.02	0.66	0.52
N, % of N intake	80.9	80.1	5.31	79.1	82.4	79.9	6.73	0.92	0.92	0.84
N balance[2]	117	143	39.7	134	123	134	50.4	0.65	0.98	0.83
Urinary PD excretion[3], mmol/d										
Allantoin	232	213	11.6	230	218	218	14.68	0.27	0.77	0.18
Uric acid	43.0	61.0	6.17	53.7	53.0	49.3	7.83	0.06	0.91	0.79
Total	274	246	22.9	284	272	225	28.0	0.41	0.32	0.85
Microbial N flow, g/d	164	161	11.5	170	160	157	14.6	0.88	0.79	0.19
BUN, mg/dL	8.78	12.6	0.80	10.1	9.60	12.4	1.01	0.01	0.17	0.39
MUN, mg/dL	11.9	14.5	0.72	12.8	12.7	14.0	0.89	0.07	0.55	0.49

[1]CP = dietary CP content, SP = by-pass protein/RP-AA supplement, and CP × SP = dietary CP content × by-pass protein/RP-AA supplement interaction

[2]N Balance = [N intake (g/d)] - [milk N (g/d) + fecal N (g/d) + urine N (g/d)].

[3]PD = purine derivative

Table 7. Plasma concentration of EAA and NEAA for cows fed a high-CP or low-CP diet containing corn DDGS and top-dressed with either no supplement (CON), supplement A (Supp A), or supplement B (Supp B).

	CP content			By-pass protein/RP-AA supplement				*P*-value[1]		
Variable	Low	High	SEM	CON	Supp A	Supp B	SEM	CP	SP	CP × SP
EAA										
Arg	14.7	11.1	1.95	13.8	12.4	12.6	2.39	0.22	0.91	0.85
His	7.25	7.93	0.81	7.43	7.73	7.60	0.64	0.59	0.81	0.02
Ile	14.3	14.4	0.88	13.7	14.7	14.7	1.08	0.93	0.72	0.02
Leu	24.0	22.0	1.21	21.9	24.3	22.8	1.48	0.26	0.53	0.02
Lys	10.3	9.48	0.46	9.97	10.1	9.62	0.56	0.22	0.83	0.06
Met	2.75	2.91	0.09	2.83	2.89	2.78	0.11	0.23	0.78	0.05
Phe	8.77	6.66	0.48	7.19	8.55	7.40	0.59	0.01	0.25	0.45
Thr	8.13	7.87	1.16	8.02	7.50	8.49	0.95	0.88	0.54	0.17
Trp	7.34	7.40	0.51	7.78	7.41	6.92	0.42	0.94	0.11	0.28
Val	29.8	31.0	1.19	30.0	31.0	30.2	1.46	0.48	0.88	0.01
NEAA										
Ala	16.4	15.6	0.76	16.3	15.8	15.8	0.94	0.47	0.92	0.68
Asn	4.34	4.44	0.59	4.45	4.25	4.48	0.35	0.80	0.88	0.78
Cit	15.5	17.0	2.93	18.2	15.4	15.2	2.39	0.74	0.26	0.87
Cystathionine	0.23	0.28	0.03	0.25	0.25	0.26	0.02	0.30	0.93	0.74
Cys	0.24	0.16	0.12	0.26	0.14	0.19	0.15	0.63	0.86	0.95
Glu	6.07	6.42	0.49	5.96	6.46	6.32	0.48	0.65	0.65	0.59
Gln	30.4	31.5	1.98	32.2	30.8	29.9	2.42	0.70	0.80	0.64
Gly	18.0	13.7	1.24	16.8	14.9	15.8	1.52	0.03	0.68	0.79
Homocysteine	0.61	0.71	0.05	0.65	0.64	0.69	0.05	0.26	0.59	0.32
Orn	5.23	4.73	0.30	5.02	4.76	5.16	0.37	0.26	0.74	0.17
Pro	8.62	7.77	0.60	8.42	7.80	8.37	0.74	0.34	0.81	0.99
Ser	6.23	5.57	0.43	6.22	5.89	6.18	0.41	0.16	0.76	0.78
Tau	6.37	6.12	052	6.41	6.49	5.86	0.59	0.77	0.70	0.80

Tyr	10.2	7.54	0.56	9.03	8.75	8.78	0.69	0.01	0.95	0.50
3-Methylhistidine	0.46	0.39	0.04	0.41	0.45	0.42	0.04	0.22	0.74	0.87
Carnosine	1.34	2.32	0.32	1.90	1.73	1.86	0.35	0.12	0.92	0.77
Total BCAA[2]	68.1	67.5	3.45	65.6	69.8	67.9	3.97	0.90	0.74	0.01
Total EAA	127	121	5.40	123	126	123	6.62	0.41	0.92	0.02
Total urea cycle AA[3]	35.5	32.9	2.23	36.9	31.9	33.6	2.73	0.42	0.45	0.41
Total Sulfur AA[4]	10.2	10.3	0.53	10.5	10.4	9.7	0.65	0.95	0.67	0.77
Total NEAA	129	122	5.86	130	122	124	7.18	0.40	0.68	0.91
Total AA[5]	256	242	6.60	253	248	247	8.04	0.22	0.88	0.07

[1]CP = dietary CP content, SP = by-pass protein/RP-AA supplement, and CP × SP = dietary CP content × by-pass protein/RP-AA supplement interaction

[2]Branched-chain AA = Ile + Leu + Val.

[3]Urea Cycle AA = Arg + Cit + Orn.

[4]Sulfur AA = cystathionine + Cys + homocysteine + Met + Tau.

[5]Total AA = total EAA + total NEAA.

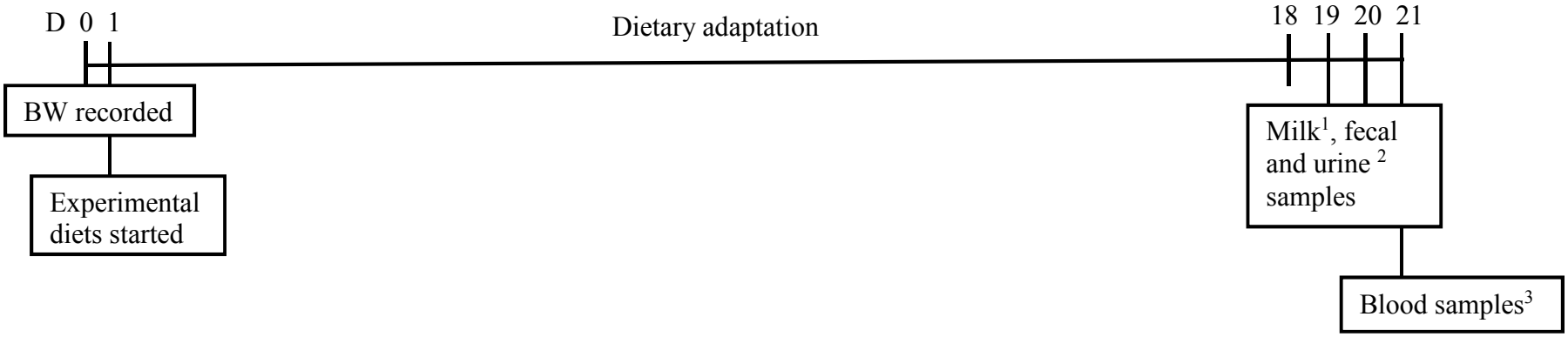


Figure 1. Sampling timeline for each 21-d period, with 18 d of dietary adaptation. [1]Milk samples collected at morning and evening milking as composites of all four quarters. [2]Grab fecal and spot-urine samples were collected at 0, 3, 6, 9, 12,15, 18, and 21 hours post-feeding. [3]Blood samples were collected 3 hours post-feeding.

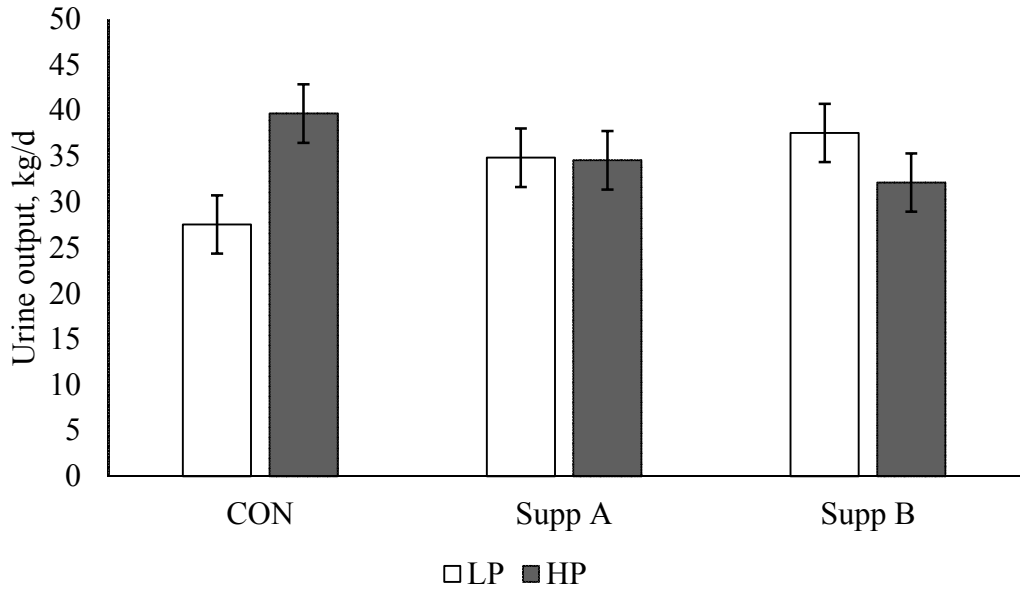
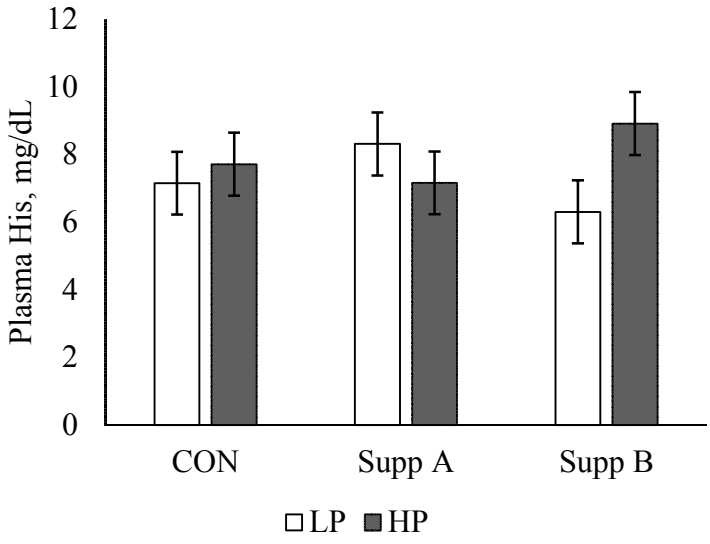


Figure 2. Urine output for cows fed a low-CP or high-CP diet containing corn DDGS and top-dressed with either no supplement (CON), supplement A (Supp A), or supplement B (Supp B). Urine output was lower for cows fed the LP than HP diet without supplementation ($P = 0.04$); however, provision of supplement resulted in no difference in urine output between cows fed the LP vs. HP diet ($P = 0.78$).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10
8
6
4
2
0
Plasma His, mg/dL
CON
Supp A
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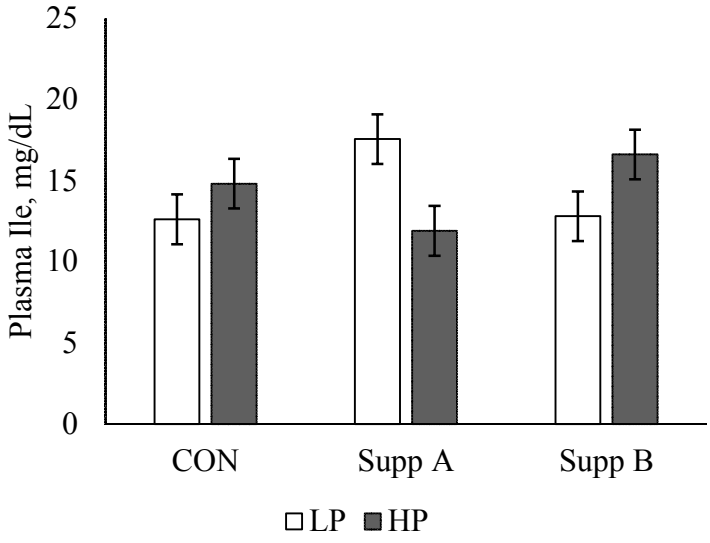
LP
HP


25
20
15
10
5
0
Plasma Ile, mg/dL
CON
Supp A
Supp B
LP
HP

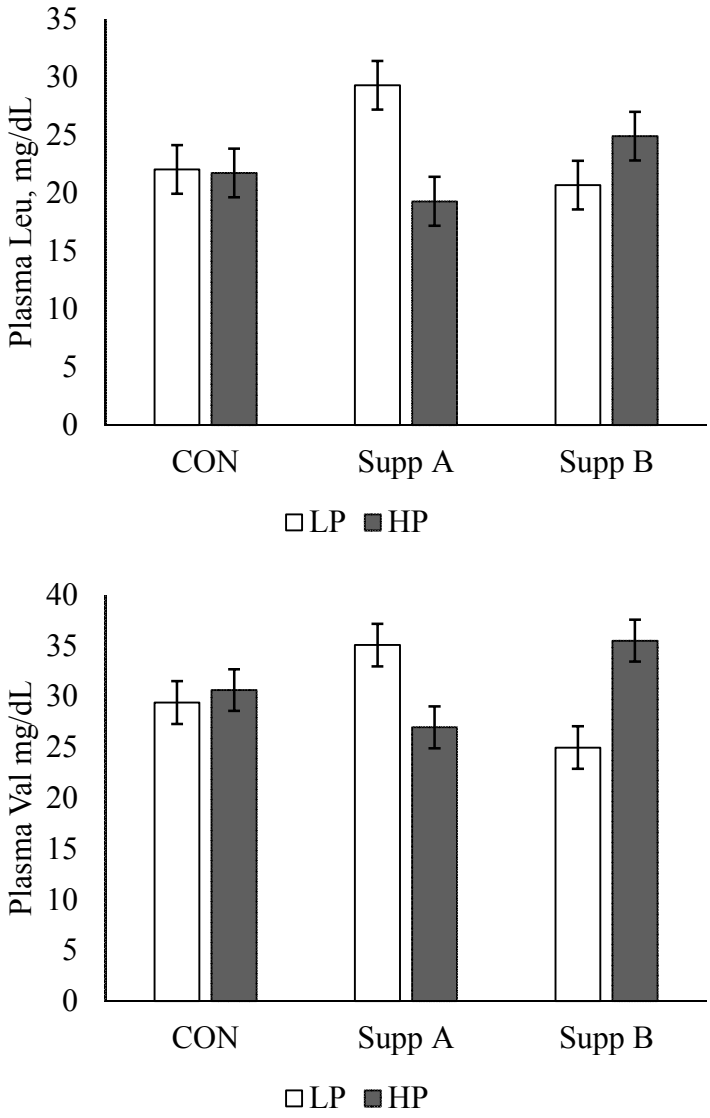


Figure 3. Plasma His, Leu, Ile and Val concentrations for cows fed a low-CP or high-CP diet containing corn DDGS and top-dressed with either no supplement (CON), supplement A (Supp A), or supplement B (Supp B). For cows fed both the LP and HP diets and did not receive any supplement (CON), plasma AA concentration did not differ ($P \geq 0.22$). However, plasma His, Ile, Leu, and Val concentrations were consistently lower ($P \leq 0.02$) in cows fed the LP than HP diet and provided Supp B, whereas, plasma Ile, Leu, and Val concentrations were higher ($P \leq 0.02$) for cows fed the LP than HP diet and provided Supp A.

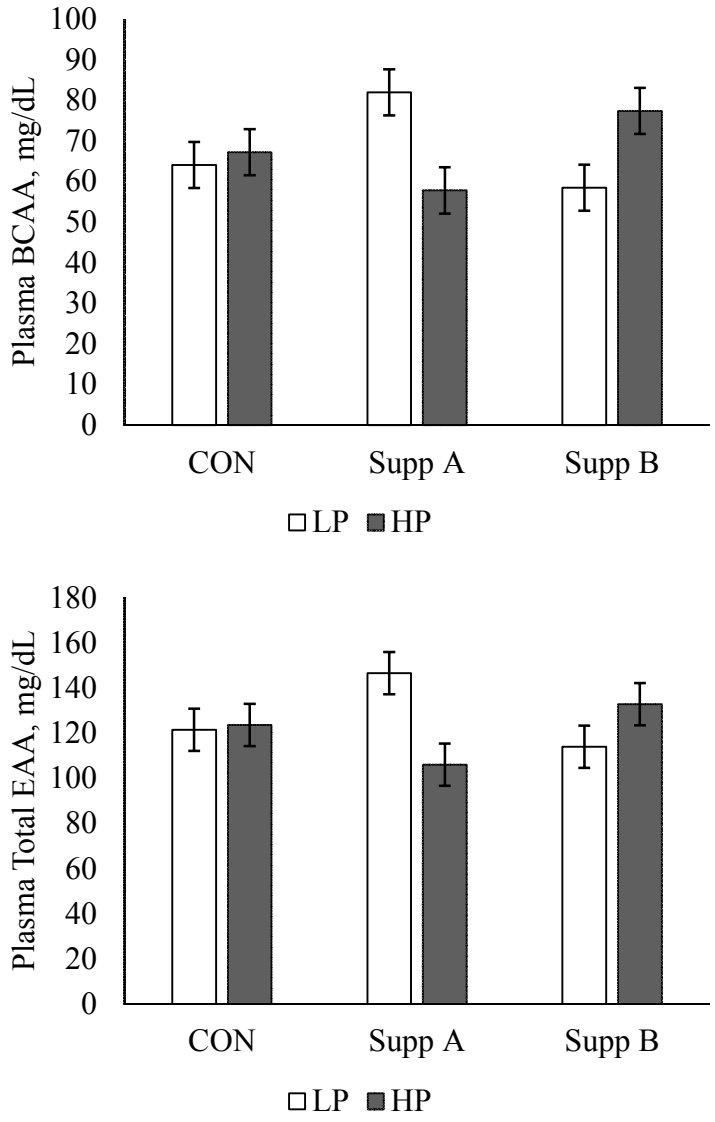


Figure 4. Plasma BCAA and EAA concentration for cows fed a low-CP or high-CP diet containing corn DDGS and top-dressed with either no supplement (CON), supplement A (Supp A), or supplement B (Supp B). For both total BCAA and total EAA, plasma concentrations were higher for cows fed the LP than HP diet when provided with Supp A ($P \leq 0.02$), and total BCAA concentration was also lower for cows fed the LP than HP diet when provided with Supp B ($P = 0.02$).

Appendices

Research protocol approval

University of Idaho
Institutional Animal Care and Use Committee

Date: August 09, 2017
To: Gwinyai Emmanuel Chibisa
From: University of Idaho
Institutional Animal Care and Use Committee
Re: IACUC-2017-37 *Supplementation of dietary ruminally-protected amino acids to enhance nitrogen utilization in dairy cows*

Your animal care and use protocol for the project shown above was reviewed and approved by the Institutional Animal Care and Use Committee on 08/09/2017.

The original approval date for this protocol is: 08/09/2017
This approval will remain in effect until: 08/08/2018
The protocol may be continued by annual updates until: 08/08/2020

Federal laws and guidelines require that institutional animal care and use committees review ongoing projects annually. For the first two years after initial approval of the protocol you will be asked to submit an annual update form describing any changes in procedures or personnel. The committee may, at its discretion, extend approval for the project in yearly increments until the third anniversary of the original approval of the project. At that time, the protocol must be replaced by an entirely new submission.

Craig McGowan, IACUC Chair

University of Idaho
Institutional Animal Care and Use Committee

Date: September 01, 2017
To: Gwinyai Emmanuel Chibisa
From: University of Idaho
Institutional Animal Care and Use Committee
Re: Protocol IACUC-2017-37 *Supplementation of dietary ruminally-protected amino acids to enhance nitrogen utilization in dairy cows*

Your requested amendment of the animal care and use protocol listed above was reviewed and approved by the Institutional Animal Care and Use Committee on 09/01/2017.

This amendment request was submitted for review on: 08/31/2017 10:19:12 AM PDT
The original approval date for this protocol was: 08/09/2017
This approval will remain in effect until: 08/31/2018
The protocol may be continued by annual updates until: 08/08/2020

Federal laws and guidelines require that institutional animal care and use committees review ongoing projects annually. For the first two years after initial approval of the protocol you will be asked to submit an annual update form describing any changes in procedures or personnel. The committee may, at its discretion, extend approval for the project in yearly increments until the third anniversary of the original approval of the project. At that time, the protocol must be replaced by an entirely new submission.

Craig McGowan, IACUC Chair