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Sustainable Dairy Fact Sheet Series

Dairy Cow Nitrogen Efficiency

Definition of terms

Amino-acids	AA	One of the 20 building blocks of protein. Amino-acids contain carbon, oxygen, hydrogen, and nitrogen and join together to form proteins. Amino-acids contain an amino group (NH_2) and a carboxyl group (COOH), both attached to the same carbon.
Blood urea nitrogen	BUN	Urea produced in the cow's liver that is released to the bloodstream and either transferred to the gastrointestinal tract or excreted by the kidneys into the urine.
Crude protein	CP	A laboratory measure of the total nitrogen in feed or biological samples (e.g., milk or blood) that typically includes both true proteins and non-protein nitrogen sources.
Metabolizable Protein	MP	Proteins that produce amino-acids available for transfer from the digestive system to the blood through the intestinal wall. The two main sources of proteins digested in the small intestine of a dairy cow are microbial protein synthesized in the rumen and the dietary rumen undegraded proteins.
Microbial crude protein	MCP	Nitrogen captured in rumen microbes as they grow during ruminal fermentation. Microbial crude protein includes both true protein and non-protein nitrogen mostly in the form of DNA.
Microbial true protein	MTP	True protein synthesized by rumen microbes as they grow during fermentation. Microbial true protein is a main source of metabolizable protein for the cow.
Milk urea nitrogen	MUN	Nitrogen in the form of urea measured in the milk. Milk urea nitrogen originates from and is in equilibrium with BUN.
Nitrogen use efficiency	NUE	Daily secretion of nitrogen in the milk (most often measured as "true milk protein") divided by daily consumption of dietary nitrogen.
Non-protein nitrogen	NPN	Nitrogen-containing compounds such as ammonia, urea, and amino-acids commonly found in feed and biological samples.
Protein	--	Molecular compound consisting of one or more chains of at least 50 amino-acids held together by peptide bonds. Proteins carry out many important biological functions. Most of the crude protein in milk is in the form of protein. On average, proteins contain 16% nitrogen.
Rumen degradable protein	RDP	Protein and non-protein nitrogen that can be degraded and produce ruminal ammonia as a result of microbial activity. Rumen degraded protein refers also to the fraction of dietary crude protein that may serve as a source of nitrogen for ruminal microbial growth.
Rumen undegradable protein	RUP	True protein that cannot be degraded in the rumen but protein can be partially digested in the small intestine.
Soluble protein	SP	The fraction of crude protein that is soluble in rumen fluid. It is often assumed that soluble proteins are readily degraded in the rumen.
True protein	TP	A synonym of protein.
Urea	--	An organic molecule soluble in water that is the main component in urine, with chemical formula $CO(NH_2)_2$.
Urinary urea nitrogen	UUN	Nitrogen in the form of urea excreted in the urine. Urinary urea nitrogen varies from approximately 50-90% of total nitrogen in urine.

Introduction

Nitrogen (N) is an important nutrient for dairy cows and is a major constituent of amino-acids, which are the building blocks of protein. The release of amino-acids from proteins in the digestive system followed by their absorption is key to providing animals with the amino-acids they need to build their own proteins, which are essential for all biological functions, including maintenance, growth, milk production, and pregnancy. Avoiding deficiencies or excess protein in the diet is important to optimize nitrogen use efficiency (NUE). Feeding diets deficient in protein may severely limit digestive efficiency, milk production, and milk protein production. On the other hand, feeding excess protein can result in inefficiencies, as the N that is not used for productive functions is excreted in urine. Additionally, the more N that is excreted in urine, the greater the environmental risks from undesirable N losses to air and water.

Although there are methods to measure a cows NUE, they are expensive and time consuming. Thus, scientists have developed indicators such as the concentration of urea N in the milk (milk urea N, or MUN) and urea in the blood of the cow (blood urea N, or BUN). These indicators reflect both N consumption and the excretion of urinary urea N (UUN), and thus they are useful tools in adjusting dairy rations to maximize NUE and minimize the risks of negative environmental impacts. One of the main strategies to increase NUE and reduce UUN excretion is to reduce the ingestion of crude protein (CP), but this must be done with caution to avoid underfeeding CP.

Forms of nitrogen and their transformation in the cow's digestive system

Fat and protein are the main determinants of milk price. As a result, increasing the yield of these valuable components in milk can increase revenue. Dietary composition can strongly influence milk composition, and therefore many dairy farmers hire consultants to formulate rations that maximize milk production, fat yield, and protein yield. Deciding on the concentration of protein in the diet of a dairy cow has economic implications for the farmer, as commercial protein supplements are among the most expensive components of dairy diets. Despite the cost, protein supplementation of lactating cow diets is common around the world.

Crude protein is a laboratory measure of the total N in the feed, which comes from both true protein and non-protein N sources. True proteins (TP) are long chains of amino-acids that contain on average 16% N. In contrast, non-protein N sources are typically simpler compounds with variable N content, such as urea, ammonia, and DNA. The laboratory method to measure CP actually measures N content and not TP. Nutritionists have agreed to refer to CP content of a feed as its N content measured in the laboratory multiplied by 6.25 (i.e., 100/16) (Equation 1).

$$\mathbf{CP = N \times 6.25 \quad (Eq.\ 1)}$$

In equation 1, both CP and N are typically expressed as a percent of the dry matter. For example, if corn grain analysis reveals N content of 2.18% of the corn's dry matter, its CP is 2.18 x 6.25 = 8.0% CP (on a dry matter basis). However, not all N in the corn is in the form of TP, and the proportion of TP and non-protein N is quite variable among feeds.

When feeding a dairy cow, the N (or CP) consumed is classified using different terms to identify the portion that is degradable in the rumen, digestible in the small intestine, and indigestible as it moves through the cow's digestive system (Figure 1). The N-containing compounds that can be degraded by ruminal microorganisms are known as rumen degradable protein (RDP). The fraction (mostly TP) that cannot be degraded by rumen microorganisms passes to the small intestine where it is partially digested and eventually serves as a direct source of amino-acids to the cow. This protein that escapes rumen degradation is known as rumen undegradable protein (RUP). Each source of RUP has its own intestinal digestibility value. The amount of RDP and RUP varies considerably among feeds (dietary constituents), but the following relationship (Equation 2) holds true for all feeds:

$$\mathbf{CP = RDP + RUP \quad (Eq.\ 2)}$$

In equation 2, all terms can be expressed as a percentage of the dry matter intake if one focuses on characterizing a feed. They can also be expressed as daily amounts consumed by the cow (CP), degraded in the rumen (RDP), and escaping rumen degradation (RUP).

Although CP has traditionally been used to formulate dairy rations, amino-acids are what cows require as the building blocks for the synthesis of their own proteins. The proteins that produce amino-acids available for transfer from the digestive system to the blood through the intestinal wall are collectively called metabolizable protein (MP) by nutritionists. There are two main sources of MP: the dietary RUP (as described in the previous paragraph) and the microbial protein synthesized in the rumen during the fermentation of the feed consumed by the cow. In general, MP is a much better predictor of milk production than CP. Measuring MP is extremely difficult, but there are prediction models precise enough to determine reliable MP estimates.

The main two requirements that nutritionists seek to fulfill when formulating dairy rations are those of the ruminal microbes (N supplied as RDP) and those of the cow (amino-acids supplied as MP). Amino-acids are required for

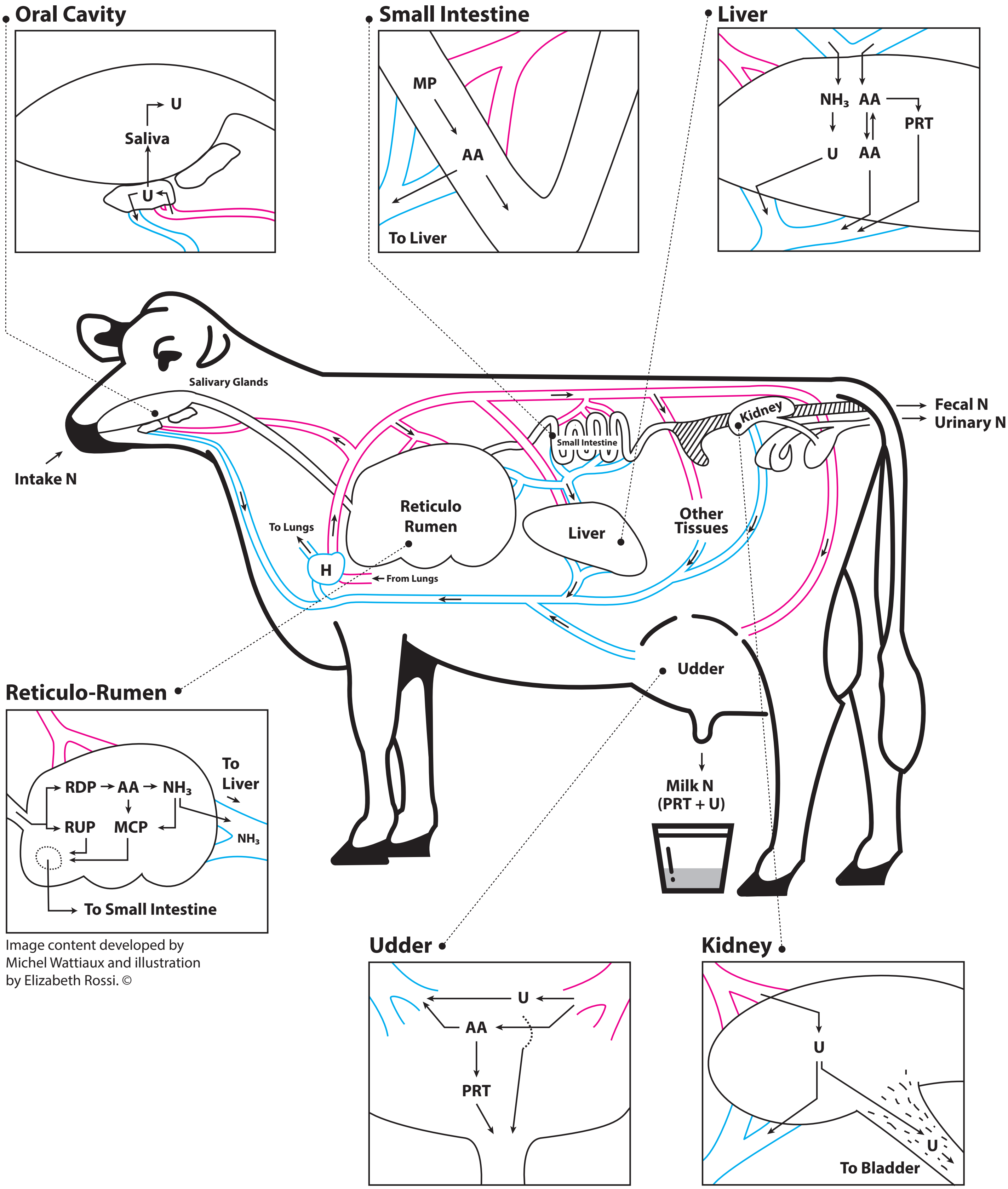

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Figure 1. Protein and urea N in the digestive system of the cow. Organs: B: Bladder, H: Heart, K: Kidney, LI: Large intestine, LV: Liver, RR: Reticulo-rumen, SI: Small intestine, SG: Salivary gland, UT: Uterus. Forms of nitrogen: AA: Amino-acid, FN: Fecal nitrogen, MCP: Microbial crude protein, MP: Metabolizable protein, NI: Nitrogen intake, NH3: Ammonia, PRT: Protein, RDP: Rumen degradable protein, RUP: Rumen undegradable protein, U: Urea, UN: Urinary nitrogen. Illustration by Michel Wattiaux and Elizabeth Rossi.

maintenance, tissue growth, lactation, and reproduction (NRC 2001). Except for maintenance, which is required on a constant basis throughout the cows life, all these bodily functions depend on the stage of reproduction, lactation cycle, and parity of the cow. Total daily cow requirements can be estimated as the sum of these four body functions and quantified with a factorial approach presented by NRC (2001).

For efficient milk protein synthesis, the profile of the supplied amino-acids in the MP should ideally match the profile of the amino-acid in protein being synthesized. Researchers have identified the profile of different protein sources and concluded that microbial protein has the most similar profile to milk protein compared to any feed ingredient used as supplemental protein in the diet (NRC 2001). This is true especially in regard to methionine and lysine, which typically are the most limiting amino-acids for milk production. The optimum ratio of lysine to methionine is 3:1 at concentrations of 7.2% for lysine and 2.4% for methionine in MP, respectively (NRC 2001). As a result, nutritionists should formulate diets for lactating cows that focus not only on commercial sources of RUP or supplemental amino-acids, but also on the rumen's ability to ferment the carbohydrates. This is because these energy sources are driving the microbial protein synthesis in the rumen, which considerably influences the amount and quality of MP supply in the intestine.

Fate of rumen degradable protein

The RDP is the fraction of the CP a cow consumes that is degraded by rumen microbes. When the CP reaches the rumen, ruminal microorganisms degrade any N-containing compound into simpler structures with ammonia being the end-product of degradation. In turn, the ammonia produced within the rumen is used by microbes to synthesize their own proteins. Some microbes, however, can also incorporate pre-existing amino-acids. In many feeding situations (especially when including high-CP legume feed ingredients), rumen microbes produce more ammonia than they can use. The excess ammonia is absorbed into the bloodstream to be processed by the liver. The liver is extremely efficient at converting ammonia to urea. Thus, the blood that brings nutrients from the digestive system to the liver is rich in ammonia, but the blood that drains out of the liver into the general circulation is rich in urea. This conversion is sometimes referred to as "ammonia detoxification" because ammonia in the general circulating blood is known to have devastating toxic effects responsible for rapid death of the animal.

There are two possible fates for BUN. First, it can be recycled into the digestive tract (diffusing from the blood to the saliva or through the arteries that bring nutrients to the digestive tissues) and serve as a source of "internal" RDP for rumen microbes. Second, it can be captured by the kidney and excreted as UUN. Nutritionists have found recently that BUN is a useful indicator of excess RDP in the diet and other N transformation in the body of the cow.

Fate of metabolizable protein

Microbial true protein (MTP) synthesized in the rumen and the dietary CP that escapes rumen degradation (RUP) eventually reach the small intestine and are digested by enzymes from the cow's digestive system. The undigested N will eventually be lost in the fecal material. However, the digested amino-acids, which constitute the MP supply of a diet to the cow, are absorbed into the bloodstream and transported to the liver. A few amino-acids pass through the liver, but most are captured and processed. The liver uses amino-acids to synthesize many proteins with important biological functions once they are released into general circulation. Finally, the liver may use amino-acids as an energy source to produce urea N. Thus, the profile of amino-acids entering the general blood circulation is very different from the profile of amino-acids absorbed from the digestive tract.

Amino-acids can be: 1) used to maintain the body of the cow, 2) used for growing a fetus (if the dairy cow is pregnant), or 3) taken up by the mammary gland to produce milk proteins. The amino-acids that are not used by these peripheral tissues are eventually returned to the liver.

Nitrogen use efficiency

Evaluating how well the dairy cow converts dietary N into milk N can be done by analyzing two important indicators: nitrogen use efficiency (NUE) and milk urea nitrogen MUN (see below). Nitrogen use efficiency is the ratio of N in the milk divided by the daily N consumed. Studies indicate that NUE may range from less than 20% to a theoretical maximum of approximately 45%. The remaining N (60-80%) is excreted in the manure, with approximately half in fecal material and half in the urine (Hutjens and Chase 2012). Figure 2 shows how the intake N is partitioned between milk, feces, and urine in lactating dairy cows in the U.S. Interestingly, a typical dairy cow in the U.S. excretes more urea N in urine than N in milk. This highlights the importance of different strategies to improve the use of N to meet cows' needs but reduce excesses that they excrete.

Higher NUE values suggest a better conversion efficiency of ration N into milk protein N. Consequently, a high NUE value also indicates a lower excretion of N into the environment via manure. However, high NUE values could also mean that there is a deficiency of dietary protein, which can lower milk production. Figure 3 serves as a guideline to interpret NUE values.

As previously mentioned, one common strategy to minimize excreted N is to reduce the dietary CP content. Researchers have found that a 1% reduction in CP can reduce urinary

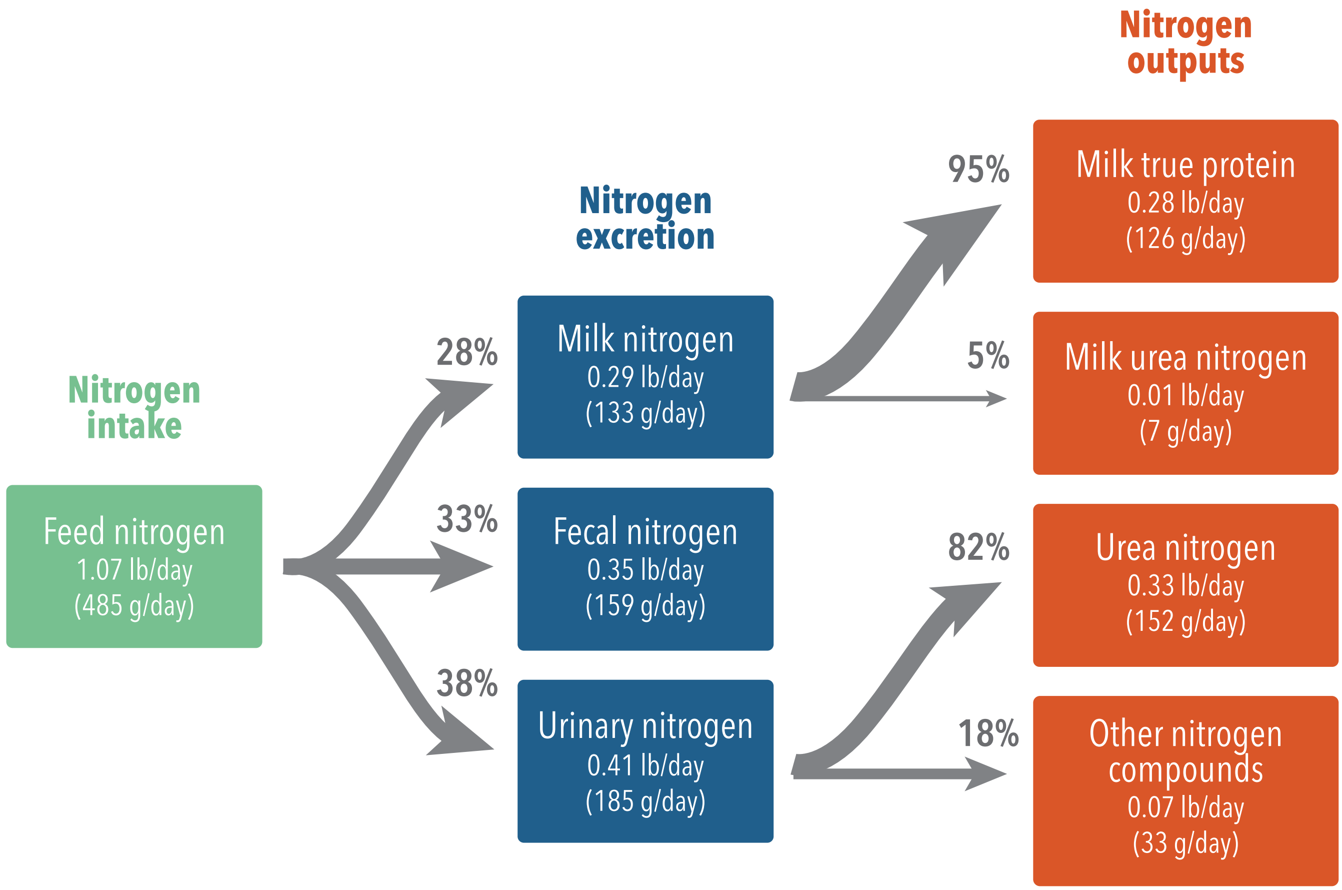

Figure 2. Nitrogen content in feed, milk, and manure from a lactating dairy cow in the U.S. (Adapted from Spek et al. 2013).

Figure 3. Interpretation of nitrogen use efficiency (NUE) values (adapted from Chase 2007).

urea N by 15% when CP constitutes 13-19% of the dry matter intake (Wattiaux and Ranathunga 2016). The challenge for dairy farmers and dietary consultants is to minimize CP content in the diet without sacrificing milk yield. Values for NUE in commercial dairy farms generally range from 20-35%, where maximum reported values can reach 40-45% (Chase 2011). Many factors affect NUE, with feed ration CP content as one of the most studied (Huhtanen and Hristov 2009). For example, NUE generally decreases as ration CP increases. Colmenero and Broderick (2006) observe that an increase in CP does not change milk N, but rather increases total manure N excreted, mostly in the form of urinary N. Varga (2010) shows a substantial NUE improvement after reducing the CP content in the dairy ration from 18% to 16%. Although NUE

<table>
<tr><th>Stage of lactation</th><th>< 9</th><th>9 - 11</th><th>12 - 14</th><th>> 15</th></tr>
<tr><td>Early (0-30 days)</td><td rowspan="3">Lack of dietary protein
Intake and milk yield may be sub-optimal</td><td rowspan="3">Most desirable</td><td rowspan="2">Acceptable</td><td rowspan="2">Excess dietary protein</td></tr>
<tr><td>Peak and post peak (31-150 days)</td></tr>
<tr><td>Mid to late (>150 days)</td><td colspan="2">Check RDP, RUP and/or adjust NFC</td></tr>
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Figure 4. Interpretation of milk urea nitrogen (MUN) for Holstein herds. RDP: rumen degradable protein; RUP: rumen undegradable protein; NFC: non-fiber carbohydrates (Miller, Shaver, and Wattiaux 2004).

can be useful in dietary decision-making for dairy producers, its estimation can be challenging, as it requires knowing the N content in both the feed and the milk, which can be expensive and time-consuming information to obtain.

Milk urea nitrogen (MUN)

The overall efficiency of N utilization in dairy cattle can alternatively be predicted by MUN. Urea is present in the milk in very small concentrations (approximately 3-25 mg/dL), as a result of diffusion of BUN through the secretory cells of the mammary gland. Thus, MUN is highly correlated with BUN. In many situations, MUN can be used to acquire a general estimate of the NUE of the herd. However, it has become common practice to track and adjust N efficiency based on MUN, as it can be obtained very inexpensively and reliably from milk samples processed through dairy herd records organizations or through the processing plant.

High MUN levels typically indicate low NUE and high UUN (Hutjens and Chase 2012), both of which are undesirable. On the other hand, when MUN is too low, milk production and milk protein can be reduced, which is also undesirable from a profitability standpoint. Traditionally, recommended MUN values range from 10-14 mg/dL (Ishler 2017). More recently, researchers recommend that MUN values range from 8-12 mg/dL to reduce ammonia levels in the rumen (Ishler 2017). Figure 4 shows an interpretation of the MUN values for Holstein herds depending on the stage of lactation.

Formulating ration to increase NUE and maintain MUN in the optimal range

Although CP plays a significant role in developing dairy rations, it should not be the only consideration when developing diet formulations because it gives little information about the key variables that affect milk production (e.g., RDP and MP). Formulating low-CP diets could reduce milk production and protein content if the CP content in the diet is too low, especially if MP is not considered.

NRC (2001) advises use of MP rather than CP for protein formulation and utilization because MP is a better predictor of milk production. Diets based only on CP could overfeed or underfeed protein, as the MP needs of the cow could be met with different CP contents depending on the dietary constituents (Block 2014). By balancing for MP, rations can be adjusted to CP contents as low as 14-15% CP and maintain high levels of milk production. On the contrary, when MP is not balanced, there is potential for environmental contamination from N losses to the environment, as well as economic losses from reduced milk production or from excess costs of unneeded protein feed supplements.

Effectively balancing rations based on MP can be challenging because it requires the use of specialized software to predict MP requirements and supplied MP under a wide variety of situations (Block 2014). Both the farmer and nutritionist need to agree and commit to this approach with consistent measurements and ration adjustments. Consistency in feed can facilitate this process and minimize risks and monitoring needs (Chase 2011).

As indicated above, one of the key factors for efficient N use is to provide rumen fermentable carbohydrates, which act as energy sources for the microbes to convert RDP and rumen ammonia into microbial protein. MUN values are highly variable and can be influenced by different feeding practices. Some of the feeding practices contributing to high MUN values (low NUE) include:

- Feeding excess total CP. For example, cows grazing in plentiful pasture areas or changing to hay crop silage that is higher in protein or soluble protein.
- Feeding excess RDP and soluble protein. For example, changing from heat-treated soybeans to raw soybeans.
- Feeding insufficient fermentable carbohydrates such as starch. For example, feeding fresh corn silage, which may have less available starch, instead of fermented corn silage.
- Feeding grains that have larger particle size, as this will reduce the rate of fermentation in the rumen. For example, coarsely ground corn grain.

There are other factors that affect MUN at a broader level:

- Breed: Holstein cows have been shown to have lower MUN (12 mg/dL) than Jersey (14 mg/dL) and Brown Swiss (14.8 mg/dL) in a study in the Midwest (Wattiaux, Nordheim, and Crump 2005).
- Season: MUN values are generally higher during the summer due to heat stress (Hutjens and Chase 2012). However, some studies attribute these changes to change in seasonal feeding practices and other management practices (Wattiaux, Nordheim, and Crump 2005).
- Sampling schedule: Sampling in the evening generally results in higher MUN than sampling in the morning. This is likely attributable to the feeding schedule, as MUN values are higher 3-5 hours after feeding (Hutjens and Chase 2012).
- Milking frequency: Lactating cows that are milked three times a day have higher MUN values than herds milked twice a day (Wattiaux, Nordheim, and Crump 2005).
- Genetics: A recent study suggests that MUN variations may be attributed to genetics due to a high heritability of MUN (Aguilar et al. 2012).

Herds can have different optimal MUN values depending on their characteristics and practices (normal values range from 8-16 mg/dL). MUN testing can be an important tool for monitoring changes in feeding and management practices that affect the nutritional content of the milk. When beginning MUN testing, it is important to establish a baseline that is representative of the herd. Considerable changes (e.g., more than three sampling points) are an indication that a management or feeding practice is affecting the N efficiency of the herd (Hutjens and Chase 2012). Look for weekly averages, as daily MUN values can vary significantly. Table 1 presents some feeding recommendations to maintain MUN values at efficient ranges.

Table 1. Recommended ranges of different feeding variables in the dairy ration to maintain efficient MUN levels (Hutjens and Chase 2012).

Feeding variables	Recommended range
Crude protein (CP)	15-18% of ration
Rumen degradable protein (RDP)	60-65% of total CP
Rumen undegradable protein (RUP)	35-40% of total CP
Soluble protein (SP)	50% of RDP
Starch levels	24-28% of ration dry matter
Sugar levels	4-6% of ration dry matter
True milk protein to fat ratio (protein/fat)	>75% (a value less than this suggests a low MUN)
Manure consistency	High MUN results in looser manure, low MUN results in firmer manure

Negative impacts of nitrogen losses to the environment

Although fecal N is relatively stable and can serve as an effective soil amendment to maintain long-term soil fertility, urinary N (mostly UUN) (Figure 2) is unstable and readily convertible to ammonia. The ammonia is quickly lost to the atmosphere, representing a potential source for environmental contamination and human health risks. Ammonia emissions can travel long distances before settling on the soil. Deposition of ammonia from the air back to the Earth's surface can contribute to eutrophication of aquatic ecosystems (lakes and rivers), which promotes algal growth that can potentially affect aquatic populations. In addition, deposited ammonia can be transformed into nitrous oxide, a potent greenhouse gas (GHG) that is 265-298 times more potent than carbon dioxide (IPCC 2014).

Ammonia emissions can have negative impacts on the health and productivity of animals as well as on human health. For example, ammonia can irritate eyes and lungs, with the latter representing a major risk for people with respiratory problems. Ammonia can also react with other compounds in the atmosphere to form particulate matter that can penetrate deep into the lungs causing health impacts. Limiting N losses by reducing N excreted by the cow can reduce these negative impacts.

Given that a reduction in CP directly affects excreted N in manure and urine, low-CP diets can decrease ammonia emissions significantly. Kebreab et al. (2002) estimate that ammonia emissions could be decreased by 20% in the U.S. if dairy farms lower the CP content in feed to 16%.

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Acknowledgement

This material is based upon work that is supported by the National Institute of Food and Agriculture, U.S. Department of Agriculture, under award number 2013-68002-20525. Any opinions, findings, conclusions, or recommendations expressed in this publication are those of the author(s) and do not necessarily reflect the view of the U.S. Department of Agriculture.

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Graphic design: Elizabeth Rossi, University of Wisconsin Environmental Resources Center.

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USDA United States Department of Agriculture | National Institute of Food and Agriculture

Publication Numbers: UWEX A4131-09
GWQ 081