Fine-tuning Test-day MUN Records for DHI-related Variables

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Abstract
The liver of the cow is a cross-road of nitrogen metabolism in dairy cows. Urea is synthesized in the liver as a result of unused degradable protein in the rumen, the absorption of excess amino acids from rumen undegradable protein, and from the catabolism of amino acids that are not utilized for productive purposes. Dietary crude protein (CP, % of DM) is the one single dietary factor most closely associated with milk urea nitrogen (MUN). From a nutrition perspective, MUN of approximately 12 mg/dl associated with a diet of approximately 16.5% CP, indicates an optimal situation that does not penalize milk production, but avoids unnecessary losses of urinary nitrogen. Current data suggest also that MUN increases by approximately 2.0 mg/dl per percentage unit of increase in dietary CP in the range of 15.0% to 18.5%. Target values should be used cautiously to assess test-day MUN on farm because of the variations due to breed, parity, sample type (a.m. vs. p.m.), season, level of production, and in Holstein cows the interactions between milking frequency and season and between milking frequency and level of milk production. Target test-day MUN may not apply to records collected the first 35 days of lactation. During this period, MUN may be more reflective of the energy and protein balance of the cow than the adequacy of dietary inputs. The use of adjustment coefficients to standardize test-day MUN to a common base may facilitate interpretation and improve its dependability as a tool to fine-tune rations on commercial herds. For herds that manage DHI test-day MUN in combination with dietary data from frequent ration analysis, it might be possible to recommend target MUN in the range of 10-12 mg/dl. However in herds without such combined information, we would recommend not to “raise a flag” until MUN is above 14 mg/dl.

Introduction
In one of its potential applications to dairy farm management, milk urea nitrogen (MUN) can help producers avoid unnecessary (and sometimes costly) excess dietary crude protein (CP) while minimizing the excretion of environmentally vulnerable urinary nitrogen (Jonker et al., 2002). Considerable advances have been made in the last few years in our understanding of the utilization of CP, the prediction of urinary nitrogen, and the sources of variation in MUN. Progress has been made with controlled nutritional studies and with the analyses of DHI databases. Nutrition experiments have been useful in quantifying the impact of feed ingredients or diet composition on MUN and to define “target range” or “optimal” values. However direct application of these results to actual farm situations should be cautioned because of the series of non-nutrition-related factors affecting MUN as obtained through bulk tank, milk line, or monthly individual cow testing. In contrast, analyses of large DHI databases have shown a strong impact of time-dependent (e.g., season) and time-independent (e.g., breed) factors on MUN. Nevertheless, there are also major limitations in applying results of such analyses to individual farm for management purpose. Such analyses are often done without knowledge of any diet information. In addition, some of the DHI-collected factors are at least partially confounded with feeding practices.
Thus our first objectives were to review the sources of urea found in milk, to review the target MUN obtained from nutritional studies, and to review the lessons learned from a recent analysis of DHI database. Our second objective was to introduce the concept of “adjusted” or “management MUN” as a way to improve the usefulness of test-day MUN to fine-tune rations under farm conditions.

**Whole Body Nitrogen Balance and Urea Synthesis**

**Where does urea come from and what does it tell us?**

In cattle, the liver is a major organ in the metabolism of amino acids and urea production (Figure 1). The recent review of Lapierre et al. (2005) will be used to understand the flow of nitrogen and the origin of urea in dairy cows. These authors analyzed a composite database of 14 studies in which milk production average 30.3 kg/d and ranged from 15.9 to 47.7 kg/d. Nitrogen consumed and found in feces, milk and urine averaged, 494, 171, 157 and 155 g/d, respectively and the apparent amount of nitrogen digested (i.e., that “disappeared” from the digestive tract) was 321 g/d.

As asserted by the authors, diets were relatively well-balanced for energy and protein, yet nitrogen entering the blood from the digestive tract included about as much ammonia-N (176 g/d) compared with amino acids-N (184 g/d, Figure 1). The gastrointestinal tract uses considerable amounts of amino acids for its own turnover and thus considerable amounts of ammonia-N may appear in the blood even when diets are properly balanced. This contention is supported by the fact that on average only 65% of the AA-N available for absorption from the small intestine (as NRC-predicted metabolizable protein) is found the blood. In other words, as much as 35% of the AA-N available for absorption is “lost” most likely because of AA oxidation as they cross the intestinal wall.

Once absorbed, ammonia and amino acids flow to the liver. The liver has the ability to remove essentially all of the ammonia-N collected from the digestive tract before the blood reaches the general circulation. The hepatic output of ammonia-N into the general circulation is essentially zero. The data indicated that on the average the liver removes 190 g/d of ammonia-N, but exports 260 g/d of urea-N. The greater export of urea-N compared with the removal of ammonia-N indicates that significant amounts of urea come presumably from the hepatic catabolism of amino acids. As suggested in Figure 1, the release of amino acids by the liver is about half its daily uptake. The negative balance of amino acids across the liver may be explained by either loss of amino acids to deamination (with corresponding formation of ammonia-N and subsequently, urea) or the synthesis of protein (e.g., albumin) and enzymes. The take-home message from these observations is that from a ration balancing viewpoint, excess dietary nitrogen not just in the form of RDP, but also in the form of RUP may contribute substantially to urea production by the liver. This contention has been confirmed recently by others (Huhtanen et al., 1997; Vanhatalo et al., 2003).

The urea produced by the liver can either be recycled into the gastro-intestinal tract or be excreted in the urine (Figure 1). The authors estimated that on the average 47% of the urea produced by the liver is returned to the gut (i.e., the rumen, caecum and large intestine). Correspondingly, this data indicated that on average about a third of the nitrogen entering the digestive tract is not of dietary origin. In reality, these recycling figures are underestimating the total recycling to the gut because they do not account for the urea-N...
returning to the rumen with saliva. The recycling of urea to the digestive tract through arterial blood and saliva explains why the total amount of nitrogen entering the blood from the digestive tract \((176 + 184 = 360 \text{ g/d})\) is greater than the amount apparently digested \((321 \text{ g/d}, \text{Figure 1})\). One should notice also that on a daily basis, the amount of hepatic urea-N not recycled to the digestive tract \((260 - 105 = 155 \text{ g/d})\) is equal to the nitrogen found in the urine \((155 \text{ g/d})\).

In summary, it appears that the high rate of metabolism of the digestive tissue requires substantial amino acids and yields substantial amounts of ammonia. In addition, and more importantly, this review indicated that a substantial amount of urea produced by the liver originated from amino acids removed from circulation (i.e., the liver is a major site of amino acid oxidation). In other words, amino acids that are not utilized for productive purpose are rapidly deaminated by the liver. When this occurs, the carbon chain is used either as an energy source (i.e., oxidation), or it is used in the synthesis of glucose (i.e., neoglucogenesis). Thus, blood urea should be thought of as an indicator of overall nitrogen status of the cow rather than a simple reflection of excess RDP in the diet.

**Blood urea N, Plasma urea and Milk urea N.**

As a small and highly water-soluble molecule, urea diffuses readily in most tissues within the body. In a review of the literature, Westwood et al., (1998) found that the correlation between plasma urea-N and MUN ranged from 0.77 to 0.99 \((n=12 \text{ experiments})\). Broderick and Clayton (1997) explored the relationship between blood urea-N and plasma urea-N and found that both yielded essentially the same value. This result indicates that the permeation of urea through the membrane of the red blood cell is essentially unrestricted. In contrast, the diffusion of urea from the blood into the milk has a 1- to 2-hour lag-time (Gustafsson and Palmquist, 1993). This lag-time is secondary to another 1.5-to 2.0-hour lag-time between peak blood urea-N and rumen ammonia-N. These time delays have been used to explain the decline in MUN observed for longer feeding-to-milking interval in a.m. samples relative to shorter feeding-to-milking intervals more typical of p.m. sampling (Godden et al., 2001).

### Target MUN — A Nutritional Perspective

Researchers in various countries have predicted MUN in absence of excess CP in the diet (Table 1). Optimal MUN were 10.3 and 11.7 mg/dl when cows were fed diets balanced according to the recommended guidelines of the Dutch system (Hof et al., 1997) and the Scandinavian (Nousiainen, et al., 2004) system, respectively. In the U.S., target MUN for lactating dairy cows fed according to the NRC (1989) recommendations were first established by Jonker et al., (1999) and later adjusted to a range of 8.5 to 11.5 mg/dl by Kohn et al. (2002). These results agreed with those of Roseler et al (1993).

Using the NRC (2001) guidelines, Wattiaux and Karg (2004) found that predicted MUN at zero RDP balance was not influenced by forage source (corn silage vs. alfalfa silage) in 55% (DM basis) forage diets, but would increase from 12.1 to 13.1 mg/dl when RDP balance increased from 0 to 100 g/d (0.22 lb/d). Considering that 2.18 kg (6.16

### Table 1. “Optimal” or “target” milk urea nitrogen (MUN, mg/dl) as found by various authors.

<table>
<thead>
<tr>
<th>Authors</th>
<th>MUN</th>
<th>Comments</th>
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<tbody>
<tr>
<td>Roseler et al., 1993</td>
<td>11.6</td>
<td>For RDP:RUP ratios of 80:80, 100:100, 100:120, and 120:120 MUN was 5.6, 11.6, 14.4 and 17.8 mg/dl, respectively.</td>
</tr>
<tr>
<td>Hof et al., 1997</td>
<td>10.3</td>
<td>When predicted surplus available N was zero according to Dutch feeding system.</td>
</tr>
<tr>
<td>Kohn et al., 2002</td>
<td>8.5-11.5</td>
<td>Adjusted from an earlier model derived from the 1989 NRC (Jonker et al., 1999).</td>
</tr>
<tr>
<td>Nousiainen et al., 2004</td>
<td>11.7</td>
<td>Obtained from a meta-analysis of 50 Scandinavian trials (grass-based diet only).</td>
</tr>
<tr>
<td>Wattiaux and Karg, 2004</td>
<td>12.1</td>
<td>When NRC-predicted RDP and RUP balance were zero. No effect of forage source.</td>
</tr>
<tr>
<td>Broderick, 2003</td>
<td>12.4</td>
<td>Found a disparity between MUN levels determined by infrared scanning and a colorimetric assay</td>
</tr>
</tbody>
</table>
lbs) of RDP was required daily by the cows producing 48 kg (106 lbs) of milk in this trial, an excess of less than 4% RDP \((0.22 \times 100/6.16)\) would increase MUN by 1 mg/dl. In contrast, with a 100 g/d excess of RUP, MUN was predicted to increase by only 0.3 mg/dl. In this trial, reducing CP from 17.5 to 16.4% of diet DM did not alter milk production (48 kg/d) or true protein production in milk (1.30 kg/d) but lowered nitrogen intake by 65 g/d (700 vs. 635 g/d) and lowered MUN by 1 unit (12.7 vs. 11.7 mg/dl).

In another recent experiment, MUN averaged 15.9, 12.4 and 9.2 mg/dl and milk production averaged 34.6, 34.3 and 33.1 kg/d when dietary CP was reduced from 18.4% to 16.7% and to 15.1% of diet DM, respectively Broderick (2003). These results indicated that a MUN of 12.4 mg/dl reflected a diet that did not penalize milk production. When MUN was 9.2 mg/dl, the adequacy of nitrogen supply became borderline as milk production was reduced (although milk protein yield was not affected, data not shown). In contrast, when MUN was 15.9 mg/dl the diet did not support additional milk or milk protein production, but led to increased urinary nitrogen excretion (data not shown).

Simple regression showed a tight linear relationship between MUN and dietary CP within experiments, but substantial variations exist among experiments (Figure 2). In the range of 15 to 18.5% dietary CP, the rate of increase in MUN per percentage unit of dietary CP was 2.4, 2.0 and 1.6 mg/dl in Roseler et al., (1993), Broderick (2003), and Wattiaux and Karg (2004), respectively. By comparison, the meta-analysis of Nousiainen et al. (2004) yielded an estimate of 1.7 mg/dl (Figure 2).

In addition to CP, the adequacy of (fermentable) energy in the diet has been suggested as a dietary characteristic that may have a profound impact on MUN. To our knowledge no experiments have been designed to study specifically the impact of non-fiber carbohydrates (NFC) on MUN, however Broderick (2003) reported significant increases in MUN from 11.5 to 12.7 and 13.3 mg/dl when dietary NDF increased from 28 to 32 and 36 %, respectively. Also, incorporation of liquid molasses in the diet reduced MUN from 11.2 to 10.2 mg/dl (Broderick and Radloff, 2004), while substituting citrus pulp for corn grain in the concentrate mix increased MUN by 1.5 mg/dl (18.8 to 20.3 mg/dl; Broderick et al., 2002). In the latter study, physical form of the corn grain did not influence MUN.

In summary, under common feeding conditions of the Midwest, it appears that MUN of approximately 12 mg/dl associated with a diet of approximately 16.5% CP, is an optimal situation that does not penalize milk production, but avoid unnecessary losses of urinary nitrogen. In addition, current data suggest that MUN increases by approximately 2.0 mg/dl per percentage unit of increase in dietary CP (% of DM) in the range of 15 to 18.5 %. Finally, CP (% of diet DM) is the single most reliable diet characteristic associated with MUN. All other measures displayed lower levels of association with MUN, even those attempting to quantify “excess” nitrogen intake and those estimating the relationship between energy and protein in the diet (see for example Broderick and Clayton, 1997 or Nousiainen et al., 2004).

**Target MUN — Comparing Nutritional and DHI Databases**

The analysis of variation in MUN as collected monthly on individual cow by DHI (i.e., test-day MUN) showed that at least two-third of the total variation was attributed to test-day associated factors (e.g. season, stage of lactation, nutrition and other management changes), with the remaining variation associated with cow – or herd – associated factors (Rajala-Schlultz and Saville, 2003; Wattiaux et al., 2005). Total variation in MUN was found to be lower in high producing
Holstein herds than in low producing herds (Rajala-Schultz, 2003) and in Jersey and Brown Swiss herds compared with Holstein herds (Wattiaux et al., 2005).

As MUN is arguably an indicator of nitrogen utilization, we wanted to explore the relationship between test-day MUN and test-day protein yield from a DHI-database and from data obtained in nutritional studies. The DHI database was from 294,031 records, on 54,454 Holsteins distributed in 533 Midwest herds (Wattiaux et al., 2005). For the nutritional studies, the database of Broderick and Clayton (1997) was modified to include only the averages of a group of cows (n=8 to 32) fed an experimental diet based on alfalfa silage and (or) corn silage (n=91). The relationship between test-day protein yield and test-day MUN in the range of 7 to 24 mg/dl in both datasets is presented in Figure 3. In the DHI database, the relationship was curvilinear with maximum test-day protein yield observed for test-day MUN of 16 mg/dl. Although the figure does not imply a cause and effect relationship, it appeared that protein yield remained within 50 grams of its maximal daily value (1.03 kg/d) while MUN ranged from 10 to 22 mg/dl. In contrast, the database of nutritional studies showed a drastically different pattern with the highest protein yield observed in cows fed diets in which lowest MUN (< 12 mg/dl) were recorded. However, the experimental diets resulted in protein yields that remained within a narrow range (0.92 to 1.0 kg/d) but in MUN that varied considerably (12 to 24 mg/dl). Thus, both the DHI dataset and the dataset of nutritional studies indicated that protein yield remained relatively “flat” across a wide range in MUN. This observation confirmed that MUN can be used as a monitoring tool to minimize dietary CP without penalizing milk protein yield.

According to Nousiainen et al. (2004), the most desirable MUN is heavily dependent on the criteria being considered for optimization. These authors observed that efficiency of nitrogen utilization (milk N/intake N) increased as MUN declined to less than 5 mg/dl, but protein yield increased as MUN increased up to about 20 mg/dl. According to these results high efficiency of N utilization is incompatible with high protein yield. In other words, maximum or close to maximum protein yield would be possible only at the expense of efficiency of N utilization. To explore whether these relationships hold true in feeding conditions of the Midwest, we used the modified nutritional database of Broderick and Clayton (1997) described above to determine the change in milk N/intake N and concomitant change in protein yield with MUN (Figure 4). The data to create Figure 4 was generated by averaging milk N/intake N and protein yield of any dietary treatment that led to average MUN within 1 mg/dl of each other and categorized incrementally between 10 and 24 mg/dl. Thus, the 91 dietary treatments fell into one of 15 categories (the number of trials per data...
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point averaged 7, but ranged from 1 to 15). Results illustrated in Figure 4 contrasted drastically with those reported by Nousiainen et al., (2004) [the reader is invited to compare Figure 4 of this paper to Figure 2 of Nousiainen et al. (2004)]. In the modified data of Broderick and Clayton (1997) both milk N/Intake N and protein yield decreased linearly with an increased MUN \( (r^2 = 0.80 \text{ and } 0.55, \text{ respectively}) \) and the correlation between mean protein yield and mean efficiency of N utilization was -0.83 \( (P=0.002) \). Thus, for the type of diets prevailing in the Midwest, we concluded that the lower the MUN is (down to 10 mg/dL), the higher the protein yield and the higher the efficiency of N utilization.

Adjusting Test-day MUN for Fixed Effects and Interactions

The recent studies of Arunvipas et al. (2003) and Godden et al., (2001) focused on the association between DHI-related measurements from commercial farms in Canada. Similar studies were published for commercial dairies in western states (Johnson and Young, 2003), Ohio (Rajala-S Schultz and Saville, 2003) and more recently for 5 other midwest states (Wattiaux et al., 2005).

In the latter experiment, we chose to focus not only on main effects (e.g., parity, season, milk production level), but also possible interactions that may significantly influence test-day MUN. We used a dataset from Ag Source DHI (Verona, WI) that included records from Illinois (2,200), Michigan (2,758), Wisconsin (377,658), Minnesota (5,840) and Iowa (12,273). The dataset included records of Holsteins, Jerseys and Brown Swiss (and other breeds) collected over a period of 29 months starting January 1999 and ending May 2001. Laboratory measurements were performed by Ag Source (Menomonie, WI), using the combiFoss 5000 that included the MilkoScan™ 4000 for determination of milk components and MUN by infrared analysis. Overall, mean test-day MUN was 12.7, 14.6 and 14.4 mg/dL with 24, 45 and 42% of records above 14.5 mg/dL in Holsteins, Jerseys and Brown Swiss in single-breed herds, respectively. Additional details on breed comparison were published recently (Wattiaux, 2005) and will not be discussed further here.

Interestingly, for the Holstein data, test-day MUN in the first few days after calving increased, peaked 7-10 days after calving, declined to a local minimum 28-35 days after calving, and slowly increased again thereafter (Figure 5). The sharp increase in MUN the first days after calving should be interpreted carefully as the data comes from colostrum and transition milk. However, this rise and the subsequent pattern of change in the few weeks after calving may also reflect the changing metabolism of the cow. A recent study indicated that MUN in the first 3 week of lactation was positively correlated with production efficiency calculated as fat-corrected milk production (FCM) divided by dry matter intake (DMI), but negatively correlated with DMI (Wattiaux and Karg, 2004), suggesting that MUN was more reflective of the energy and protein balance of the cow than the adequacy of dietary inputs in the first weeks after calving. It may be possible that the height of the peak and the rate of decline afterward may be indicative of the amount and extent of hepatic deamination of dietary amino acids or body protein mobilization to meet cows’ need for gluconeogenesis.

Because Holsteins test-day MUN may not reflect dietary adequacy in early lactation, only records for days in milk >35 were subjected to statistical analysis. We used proc GLM (SAS, 1999) with a model including fixed effects only and a dataset of 96 observations obtained as the means of 5 selected factors arranged in a \( 2 \times 2 \times 2 \times 3 \times 4 \) factorial. Factors were parity (1 vs. > 1), type of sample (a.m. vs. p.m.), frequency of milking (2 vs. 3×), yield of test-day fat-corrected milk (FCM) classified into 1 of 3 categories (FCMc; low = bottom third, medium = middle third and high = top third), and season (winter = December to

![Figure 5. Change in early lactation test-day milk urea nitrogen (●, MUN) and 4% fat-corrected milk (▲, FCM) in Holsteins. When visible, vertical bars are SE.](image)
February, spring = March to May, summer = June to August, and fall = September to November). The data was eventually analyzed separately for primiparous and multiparous cows and a stepwise backward elimination was used to remove non-significant terms (main effects and interactions) from each model.

Parity had a distinct effect on the pattern of change in test-day MUN with increasing FCM yield (Figure 6). In primiparous cows, MUN remained constant (12.6 ± 0.03 mg/dl) for FCM d•42 kg/d, but declined linearly at a rate of 0.050 ± 0.008 mg/dL per kg as FCM increased from 43 to 60 kg/d. In contrast, MUN in multiparous cows increased linearly by 0.061 ± 0.003 mg/dl per kg as FCM increased to 28 kg/d and by 0.027 ± 0.001 mg/dl per kg as FCM increased from 29 to 58 kg/d. However, in the range of 59 to 82 kg/d, MUN declined at a rate of 0.057 ± 0.006 mg/dl per kg of FCM. The exact nature of the parity difference remains unclear, but suggests differences in the fate of metabolizable protein as the need and supply of energy (carbon) and amino acids vary due to parity and level of milk production. No other report indicated a decline in MUN for the highest milk production levels as reported here in both primiparous and multiparous cows. The reason for this decline is unclear, but warrants further investigation in the efficiency of utilization of dietary CP in unusually high producing dairy cows.

In both parity groups, the stepwise analysis resulted in final models that included sample type, season and FCMc in the multiparous, but not primiparous cow model. Milking frequency was not significant, but milking frequency × season and milking frequency × FCMc were significant. The nature of these interactions differed for each parity group. This analysis lead to the establishment of reliable mean predicted difference among the 96 factorial combinations. Thus the expected difference in test-day MUN for a record in any of the 96 factorial combinations could be used to adjust all records to a common base. In Figure 7, zero (i.e., the base of adjustment) was set for the test-day MUN of cows in high FCMc (the top producing cows), milked 3x a day on a p.m. sampling schedule in the fall (12.84 and 13.51 mg/dl in primiparous and multiparous cows, respectively). All data points (i.e., factorial combinations) with a positive deviation must be adjusted downward whereas data points with a negative deviation must be adjusted upward to place them on par with the base.

Overall, test-day MUN was 1.0 (13.1 vs. 12.1 mg/dl) and 0.9 (13.3 vs. 12.4 mg/dl) greater for p.m. vs. a.m. samples in primiparous and multiparous cows, respectively. As can be seen in Figure 7, these differences did not vary (i.e did not interact)
with milking frequency or FCMc. In contrast the significant interaction between milking frequency and season and milking frequency and FCMc lead to separate adjustment factors for each combination of these factors. Positive numerical adjustment was the greatest (+2.4 mg/dl) for multiparous cows in the bottom third production group (low FCMc) milked 3x per day on an a.m. sampling schedule in the spring. On the other hand, the largest negative numerical adjustment (-1.1 mg/dl) was for records of cows producing in the top third production group (High FCMc) milked 3x per day on a p.m. sampling schedule in the summer. These adjustments have been implemented in the April 2005 release of MUN reports from Ag Source-CRI, in which the adjusted test-day MUN are referred to as “management” MUN.

Conclusions

The synthesis of urea by the liver, blood urea nitrogen and thus MUN should be thought of as an overall indicator of the nitrogen status of the cow rather than a simple reflection of excess RDP in the diet. Nutritional studies indicated that MUN of approximately 12 mg/dl when cows are fed 16.5% CP, (% DM basis) diets reflect an optimal situation. However these results are not directly transferable to assess test-day MUN from DHI testing because there was large but predictable variation in MUN due to breed, parity, sampling type, season, level of milk production, and in Holsteins, the milking frequency x season interaction and the milking frequency x level of milk production interaction. In absence of specific nutritional information, the partitioning of the variation in MUN between nutritional and non-nutritional factors (within pre-defined homogenous groups of records, such as within a season or within a production level) will remain difficult. In the mean time, the removal of systematic biases via the adjustment of test-day MUN to a common base may improve its dependability to assess adequacy of the diet. For herds that combine test-day MUN from DHI records with frequent ration analysis, it might be possible to recommend target MUN in the range of 10-12 mg/dl. However in herds without such combined information we recommend not to “raise a flag” until MUN is above 14 mg/dl.

Citations


