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Evaluation of Models to Estimate Urinary Nitrogen and Expected Milk Urea Nitrogen[1]

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ABSTRACT

Milk urea nitrogen (MUN) has been introduced as a means to estimate urinary nitrogen (N) excretion and protein status of dairy cattle. For Holstein cows, the amount of urinary N excreted (g/d) was originally reported to be 12.54 × MUN (mg/dl), but recently urinary N (g/d) was reported to equal 17.64 × MUN (mg/dl). The objectives of the present study were to evaluate models to predict urinary N and expected MUN, by using older and newer data sets, and to quantify changes that may have occurred in MUN measurements over time. Two data sets were used for model evaluation. Data set 1 was from the spring of 1998 and data set 2 was from the spring of 1999. Similar cows and diets were used in both studies. By using data set 1, the newer model underestimated MUN by an average of 3.8 mg/dl, whereas the older model was accurate. By using data set 2, the older model overestimated MUN by 4.8 mg/dl, but the newer model was accurate. In the period between the two studies, the MUN measured appeared to decrease by an average of 4.0 mg/dl. By using current wet chemistry methods to analyze for MUN, urinary N (mg/dl) can be predicted as 0.026 × MUN (mg/dl) × body weight (kg). Because of changes in methodology that occurred in the fall of 1998, target MUN concentrations have decreased to 8.5 to 11.5 mg/dl for most dairy herds compared with previous target concentrations of 12 to 16 mg/dl.
(**Key words:** milk urea nitrogen, urinary nitrogen excretion, mathematical model evaluation)

Abbreviation key: MUN = milk urea nitrogen, **RMSPE** = root mean square prediction error, **UN** = urinary nitrogen, $_{\mathbf{P}}$**UN** = predicted urinary nitrogen.

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INTRODUCTION

Milk urea nitrogen (**MUN**) can be used as a rapid and noninvasive way to estimate urinary N excretion (**UN**) from dairy cows (Jonker et al., 1998). Conversely, by predicting UN for cows consuming ideal diets, target MUN concentrations can be derived (Jonker et al., 1999). Deviations from this target can identify overfeeding or underfeeding of protein or other issues related to feeding and management. For Holstein cows, the amount of N excreted, measured as grams per day (g/d), was 12.54 × MUN, measured as milligrams per deciliter (mg/dl) (Jonker et al., 1998). This model was consistent when evaluated against 18 studies published in the literature. However, more recent studies have suggested that there is a potential bias in this predictor. Kauffman and St.-Pierre (2001) reported UN equal to 17.64 × MUN for Holstein cows and 11.8 × MUN for Jerseys, and Sannes et al. (2000) found a similar bias for Holsteins. One of the differences among studies that may explain the different coefficients is that laboratory methods used to determine MUN have changed. Another consideration is that the appropriate coefficient depends on BW (Jonker et al., 1998; Kauffman and St.-Pierre, 2001).

On September 28, 1998, a hardware defect was discovered on the MUN analyzer that was being used by DHIA laboratories across the United States to run MUN samples for standard curves (P. Miller, National DHIA, 2000, personal correspondence). When this defect was corrected, MUN standards changed so that DHIA laboratories reported lower MUN values. However, the magnitude of the change was not reported. Thus, it has been exceedingly difficult to interpret MUN values based on models that were developed previously. Currently, DHIA laboratories use the average MUN reported by several different analyzers to develop standards for indirect methods.

The first objective of this study was to evaluate the older and newer models for predicting UN from MUN, and for predicting MUN from diet and production parameters by using both older and newer data. The second objective is to quantify the change in MUN measurements that occurred in the fall of 1998.

METHODS

Data Sets

Fortunately, our laboratory conducted two similar studies with lactating Holstein cows; one study (Kalscheur et al., 1999) was conducted before the change in standards, and the second study (Kalscheur et al., 2000) was conducted thereafter. The studies used similar Holstein cows, but the first study was a production trial with 48 cows (32 multiparous and 16 primiparous) in a 4 × 4 Latin square design, and the second trial included collection of ruminal and total tract digestibility data on three cows for four periods (incomplete Latin square). In both studies, similar diets were offered with four different levels of RDP, and CP ranged from 13.1 to 16.6% of diet DM. Average milk production was 32.8 kg/d in the first study and 24.9 kg/d in the second study. MUN ranged from 9.5 mg/dl for the low CP diet to 16.4 mg/dl for the high CP diet in the first study, and from 6.8 to 13.3 mg/dl for the second study.

Model Evaluation

Measurements of urinary N were available only for the second study, so only this data set was used to evaluate predictions of urinary N. For a wide range of diets studied previously, the true digestibility of CP was 0.83 and metabolic fecal N was 97 g/d (Jonker et al., 1998). Thus, one model for predicted urinary N (**$_p$UN**), measured as grams per day (g/d), was as follows:

$$_{p}\text{UN} = \text{NI}(0.83) - \text{MN} - 97 \qquad [1]$$

where NI is nitrogen intake (g/d) and MN is milk nitrogen (g/d). This model assumes retained N used for growth or reserves is negligible for mature cows. The National Research Council (2001) assumes that metabolic fecal N is a function of BW, but the added complexity could not be justified based on the data used by Jonker et al. (1998) to develop the model. Because it is often difficult to obtain accurate measurements of NI on farms, another model was developed to predict urinary N from MUN. Jonker et al. (1998) reported that UN could be predicted as follows:

$$_{p}\text{UN} = 12.54 \times \text{MUN} \qquad [2]$$

where MUN is expressed as milligrams per deciliter (mg/dl). Kauffman and St.-Pierre (2001) revised the model to be as follows:

$$_{p}\text{UN} = 17.64 \times \text{MUN} \qquad [3]$$

for Holstein cows, and a more robust model that was accurate for both Holsteins and Jerseys was developed as follows:

$$_{p}\text{UN} = 0.0259 \times \text{BW} \times \text{MUN} \qquad [4]$$

where BW represents body weight in kilograms (kg).

In addition to models that predict UN, models have been developed to predict MUN from diet and management (Jonker et al., 1999). The purpose of these models is to identify when MUN deviates from an expected value and thus indicates a potential management problem. Expected MUN, by using three different models, was predicted from diet and production parameters for all observations in both data sets, and these values were compared with measured MUN. For all models, the true digestibility of 0.83 and metabolic fecal N of 97 g/d were assumed and thus $_p$UN was defined as in equation 1. Predicted MUN depended on the model used and was $_p$UN divided by the coefficient 12.54 (Jonker et al., 1998) or 17.64 (Kauffman and St.-Pierre, 2001) or 0.0259 × BW (Kauffman and St.-Pierre, 2001).

Statistics were performed by using JMP (2000). Just as when the original models were developed to predict UN from MUN, the observed UN was regressed against observed MUN, and if the intercept was not significantly different from 0, it was set to 0 and the slope coefficient was determined. For the first data set, actual UN measurements were not available, so $_p$UN (equation 1) was regressed against MUN. Equation 1 provides an estimate of UN that is independent from the equations that use MUN.

The accuracy of each model was further assessed by calculating the residuals (predicted minus observed UN or MUN). The root mean square prediction error (**RMSPE**) was determined and decomposed into mean bias, slope bias, and residual error (Bibby and Toutenburg, 1977). The linear bias for UN was derived by regressing residuals versus *predicted* UN. In contrast, linear bias for MUN was derived by regressing residuals versus *observed* MUN. Ordinarily, such bias is estimated by regressing against predicted values, but that would have resulted in a positive bias because the coefficients were derived by regressing UN versus MUN, and the target MUN values derived by reversing the equation and solving for MUN (Draper and Smith, 1981).

Changes Over Time

All of the models that predict MUN account for most of the factors known to affect MUN, including N intake, milk production, and milk protein percentage. In addition, use of equation 4 adjusts for BW effects. Thus, equation 4 was used to correct for differences in these variables between the two data sets. The changes in MUN measurement that occurred between the two studies were quantified as the change in bias of equation 4 from one study to the next. This approach is justified based on the following relationships. Bias was determined based on the difference in predicted versus observed MUN. For the first data set, this is expressed as follows:

$$\text{Bias}_{old} = \text{Predicted} - \text{MUN}_{old} \quad [5]$$

where Bias_{old} represents bias of the model on the older data set, and MUN_{old} represents observed MUN with the older data set. For the second data set,

$$\text{Bias}_{new} = \text{Predicted} - \text{MUN}_{new}. \quad [6]$$

Bias_{new} represents the bias of the model on the new data set, and MUN_{new} represents the observed MUN of the new data set. Thus, subtraction of equation 6 from equation 5 yields $\text{MUN}_{new} - \text{MUN}_{old}$ or the difference between the newer and older methods.

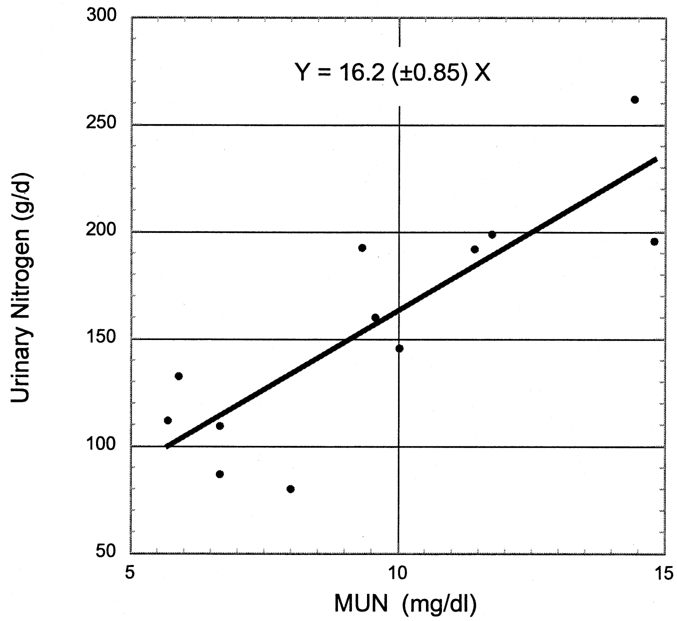

Figure 1. Milk urea nitrogen (MUN) versus urinary nitrogen for the second study.

RESULTS

Model Evaluation

Because the first data set did not record UN, the potential accuracy of the models that used MUN to predict UN was only briefly investigated by using this data set. The $_{p}$UN from equation 1 was used as the reference because it was considered to be the most accurate and to be independent of the other predictions. The $_{p}$UN (equation 1) was regressed against observed MUN for this data. The coefficient was not significant and was forced to 0. The slope coefficient was 12.45 (SE = 2.9), which was not different from 12.54 reported by Jonker et al. (1998) when regressing UN versus MUN. Of the three models that use MUN to predict UN, equation 2 was the most similar to predictions made by equation 1. Because the data set did not contain actual UN measurements, further evaluation of UN prediction was not pursued by using this data.

By using the second data set, UN (g/d) was regressed against MUN (mg/dl) as was done to develop the coefficients (Figure 1). The intercept was not significant and was forced through 0. The slope of the line was 16.2 (SE = 0.85), which indicates that 16.2 × MUN should be a good predictor of UN. This coefficient is greater than the 12.54 proposed by Jonker et al. (1998) but not different from the 17.64 proposed by Kauffman and St.-Pierre (2001). Urinary N (g/d) divided by kilograms of BW was regressed against MUN (mg/dl) as shown in Figure 2. Again the intercept was not different from 0 and therefore was forced through 0. The slope of the line was 0.026 (SE = 0.001), which was not different from the 0.0259 proposed by Kauffman and St.-Pierre (2001). When regressing UN versus MUN and MUN ×

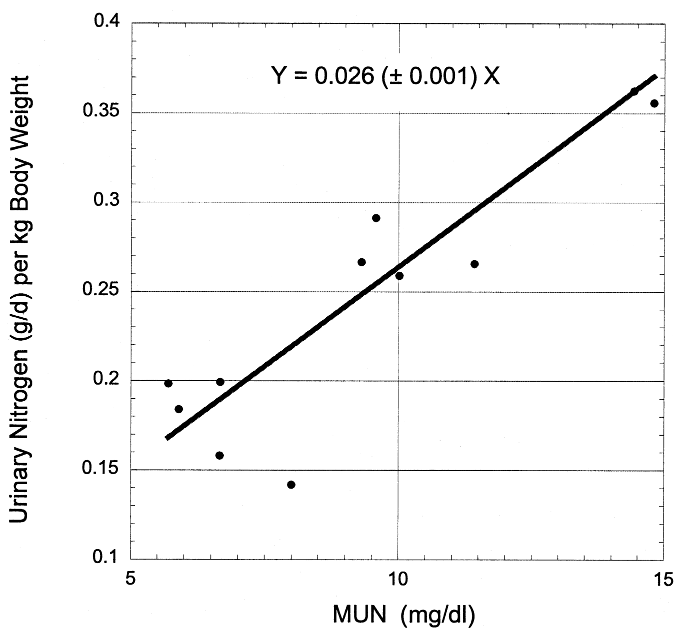

Figure 2. Milk urea nitrogen (MUN) BW versus urinary nitrogen for the second study.

Table 1. Predicted urinary nitrogen (UN) for different models compared with observed UN for the second data set (Kalscheur et al., 2000; n = 12).

Model[1]	Mean	Mean bias[2]	Residual error[3]	RMSPE[4]
Observed	155.6	(SD = 54.3)		
NI (0.83) – MN – 97	179.5	23.9**	24.2	34.0
12.54 × MUN	119.4	–36.2**	28.6	46.2
17.64 × MUN	167.9	12.3	29.1	31.6
0.0259 × BW × MUN	152.4	–3.2	21.0	21.2

[1]NI = Nitrogen intake (g/d), MN = milk nitrogen (g/d), MUN = milk urea nitrogen (mg/dl).

[2]Mean bias = Mean of residuals (predicted minus observed).

[3]Residual error was determined as standard deviation of residuals (predicted – observed) times √ {(n – 1)/n}.

[4]RMSPE = Root mean square prediction error = √ {(mean bias)^2 + (residual error)^2}.

**Bias differed from 0 at $P < 0.01$.

BW, the latter term was significant ($P < 0.05$), which indicates that inclusion of BW explained more variation than MUN alone.

Results from further evaluation of models for predicting UN, by using the data from the second study, are shown in Table 1. Use of assumptions regarding protein digestibility and ignoring N retention (equation 1) resulted in RMSPE of 34.8 g/d, which was 19% of the mean UN. This model overestimated UN by 23.9 g/d, on average. Regression of residuals versus predicted UN resulted in a significant ($P < 0.05$) slope (linear bias) of 0.24, which accounted for 40% of the residual error (r^2 of regression of residuals was 0.40). Because the model ignores retained N, as a means of simplification, some overestimation was expected. The original models of Jonker et al. (1998) underestimated UN by 36.2 g/d on average and had an RMSPE of 47 g/d. This bias highlights the problem of using the model with current methodology. The mean bias was reduced when using the revised coefficient with the recent data set, and the residual error was reduced by using the BW adjusted model. The RMSPE was 22.1 g/d with the newer data set, or 14% of the mean UN. The linear bias was not significant for any of the models that use MUN to predict UN. For the prediction of urinary N, the older model (equation 2) accurately predicted the older data and the newer models (equations 3 and 4) accurately predicted the newer data.

The evaluation of models for predicting MUN by using the older data is shown in Table 2. For this data set, the older model of Jonker et al. (1999) showed no mean bias and had an RMSPE of 4.3 mg/dl. Newer models were negatively biased with improved residual error. No linear bias was apparent for the model of Jonker et al. (1999), but a negative linear bias was significant ($P < 0.01$) for the models of Kauffman and St.-Pierre (2001). Predicted minus observed MUN was lower for higher MUN values. Because the coefficient in the model of Kauffman and St. Pierre (2001) was too high for these data, it would have biased the MUN estimate more for the samples with higher MUN. Nonetheless, the linear bias had a numerically small effect. The slopes from the regression of residuals versus predicted were –0.25 and –0.17 for the revised models without and with adjustment for BW respectively, and linear bias accounted for 25 or 17% of total error about the mean bias for each model respectively.

The evaluation of models for predicting MUN by using the newer data is shown in Table 3. For this data set, the older model of Jonker et al. (1999) was biased by 4.8 mg/dl, but the newer models were not. Residual error was also reduced with the newer models. In particular, inclusion of BW reduced the residual error considerably. The RMSPE for the best model was 2.7 mg/dl or 24% of the mean. There were no linear effects observed when regressing residuals against observed MUN.

Changes Over Time

The difference in bias between the two studies was taken to represent the change in MUN after accounting for diet and production effects accounted for with the latest model, which includes BW. The mean bias was –2.4 mg/dl (Table 1) with the first data set and 1.6 mg/dl (Table 3) for the second data set. The difference in mean bias was therefore 4.0 mg/dl, which indicates that the MUN decreased by a mean of 4 mg/dl. An alternative explanation follows. Predicted MUN changed from 10.5 to 11.1 because of slight changes in intake and production between the two studies, and yet observed MUN changed from 12.9 to 9.5 mg/dl because of changes in methods of MUN analysis. Because the observed MUN decreased by 3.4 mg/dl (12.9 – 9.5) instead of increasing by 0.6 mg/dl (11.1 – 10.5) as the model predicted, it appears that the change in methods between the two studies resulted in a mean decrease in measured MUN of 4.0 mg/dl (3.4 + 0.6).

Table 2. Predicted milk urea nitrogen (MUN, mg/dl) for different models compared with observed MUN by using the first data set (Kalscheur, 1999; n = 186).

Model[1]	Mean	Mean bias[2]	Residual error[3]	RMSPE[4]
Observed	12.9	(SD = 3.2)		
$_{p}$UN/12.54	12.8	–0.1	4.3	4.3
$_{p}$UN/17.64	9.1	–3.8**	3.2	4.9
$_{p}$UN/(0.0259 × BW)	10.5	–2.4**	3.4	4.1

[1] $_{p}$UN = NI (0.83) – MN – 97, BW = body weight (kg).

[2]Mean bias = Mean of residuals (predicted – observed).

[3]Residual error was determined as standard deviation of residuals (predicted – observed) times √ {(n – 1)/n}.

[4]RMSPE = Root mean square prediction error = √ {(mean bias)^2 + (residual error)^2}.

**Bias differed from 0 at $P < 0.01$.

There was a linear bias when the newest model was used with the older data set. The regression of residuals versus observed MUN resulted in the following relationship:

$$\text{Predicted} - \text{MUN}_{old} = -0.17 \times \text{MUN}_{old} - 0.24 \quad [7]$$

where MUN_{old} represents observed MUN on the older data set. For the newer data set, because the mean bias was 1.6 and there was no linear bias, the following relationship holds:

$$\text{Predicted} - \text{MUN}_{new} = 1.6 \quad [8]$$

Subtracting equation 8 from equation 7 yields the following relationship:

$$\text{MUN}_{new} - \text{MUN}_{old} = -0.17 \times \text{MUN}_{old} - 1.84 \quad [9]$$

or

$$\text{MUN}_{new} = 0.83 \times \text{MUN}_{old} - 1.84 \quad [10]$$

DISCUSSION

Predicting UN and Target MUN

The model of Jonker et al. (1998) was developed and evaluated extensively, and these previous evaluations confirmed the accuracy of the model. However, the present study and other recent evaluations show that the model now has a mean bias. It is apparent from the work summarized above that the model fits the data collected before the changing of standards by DHIA laboratories. Comparison of the two studies described in this study suggests that the MUN reported by DHIA laboratories decreased by a mean of 4.0 mg/dl across the values in the study. These conclusions do not rest solely on the data presented in this study, but these data confirm other work conducted both before September 1998 and reviewed by Jonker et al. (1998), and subsequent to this time (Kauffman and St.-Pierre, 2001; Sannes et al., 2000).

Rearranging equation 10 to solve for the previous MUN yields the following:

$$\text{MUN}_{old} = (\text{MUN}_{new} + 1.84)/0.83 \quad [11]$$

Table 3. Predicted milk urea nitrogen (MUN) for different models compared with observed MUN by using the second data set (Kalscheur et al., 2000; n = 12).

Model[1]	Mean	Mean bias[2]	Residual error[3]	RMSPE
Observed	9.5	(SD = 3.1)		
$_{p}$UN/12.54	14.3	4.8**	3.8	6.1
$_{p}$UN/17.64	10.2	0.7	2.8	2.9
$_{p}$UN/(0.0259 × BW)	11.1	1.6*	2.1	2.6

[1] $_{p}$UN = NI (0.83) – MN – 97, BW = body weight (kg).

[2]Mean bias = Mean of residuals (predicted – observed).

[3]Residual error was determined as standard deviation of residuals (predicted – observed) times √ {(n – 1)/n}.

[4]RMSPE = Root mean square prediction error = √ {(mean bias)^2 + (residual error)^2}.

*Bias differed from 0 at $P < 0.05$, or **$P < 0.01$.

Substituting MUN_{new} into the equation of Jonker et al. (1998), ${}_{p}UN_2 = MUN_{old} \times 12.54$, yields the relationship

$$ {}_{p}UN_2 = 12.54\ (MUN_{new} + 1.84)/0.83 \quad [12] $$

which simplifies to the following:

$$ {}_{p}UN_2 = 15.1 \times MUN_{new} + 27.8 \quad [13] $$

In the range of most MUN data, this equation is similar to that of Kauffman and St.-Pierre (2001) (equation 3). For example, if the observed MUN by a current direct chemistry method is 8, the predicted UN would be 149 g/d by using the modified Jonker et al. (1998) model (equation 13), and 141 g/d by using the equation 3 of Kauffman and St. Pierre (2001). If the observed MUN is 14 mg/dl, the predicted UN would be 239 g/d versus 247 g/d for equations 13 and 3, respectively. Sannes et al. (2000) reported that the model of Jonker et al. (1998) underestimated urinary N excretion by 55 g/d and the model of Kauffman and St.-Pierre (2001) overestimated N excretion by 25 g/d. Adjusting the model of Jonker et al. (1998) for the new standards (equation 13) would have reduced the bias to 13 g/d.

Jonker et al. (1999) calculated target urinary N for cows fed according to NRC (1989) recommendations to range from approximately 150 to 200 g/d. The corresponding MUN, by using their model (equation 2), was 12 to 16 mg/dl, but by using the model of Kauffman and St.-Pierre (equation 3) would be 8.5 to 11.4 mg/dl. By using the modified (equation 13) model of Jonker et al. (1999), this target range would be 8.1 to 11.5 mg/dl.

For recent data where BW is known or can be estimated, equation 4 is preferable for prediction of UN, and as a component of the model of Jonker et al. (1999) for prediction of target MUN. The values for target MUN reported previously (Jonker et al., 1999) should be adjusted for current data by adapting them to the new model (equation 4) as follows:

$$ \text{Target } MUN_{new} = \text{Target } MUN_{old} \times 12.54/(0.0259 \times BW) \quad [15] $$

where the new subscript refers to the adjusted value and the old subscript refers to the value reported by Jonker et al. (1999) and BW refers to body weight in kilograms (kg). For the same range in urinary N (150 to 200 g/d) as described previously, the more robust equation of Kauffman and St.-Pierre (2001) (equation 4), would predict the target range for MUN from 9.6 to 12.9 mg/dl for a 600-kg cow or 8.3 to 11.0 mg/dl for a 700-kg cow. Except for the effect of BW on MUN, the factors reported by Jonker et al. (1999) to affect target MUN would remain, and the magnitude of the effect can be calculated by using equation 14. Jonker et al. (1999) reported that a 100-kg increase in BW would increase target MUN by 0.9 mg/dl because of increasing protein requirements. Use of equation 14 to adjust the response shows that the expected MUN should decrease with increasing BW (Table 4). For the same level of production, a Jersey herd that averages 400 kg in BW would expect to have an MUN of 3 mg/dl higher than a Holstein herd that averages 600 kg in BW with the same production parameters. Nonetheless, smaller cows are likely to produce less milk, which would decrease the gap between breeds.

Table 4. Effect of BW on expected milk urea nitrogen (MUN) concentrations calculated as described by Jonker et al. (1999) after modification proposed by Kauffman and St.-Pierre (2001).[1]

	MUN	
BW, kg	Mean[2]	Maximum[3]
	mg/dl	
400	13.7	16.7
500	11.8	13.6
600	10.6	11.6
700	9.7	10.4
800	9.0	9.8

[1]Predicted for cows fed according to NRC (1989) (without safety or lead factors) for milk = 10,000 kg per 305-d lactation, milk fat = 3.55%, milk protein = 3.0%, and parity = 2.

[2]Mean MUN weighted by milk production for the entire 305-d lactation.

[3]Maximum MUN would occur near peak lactation.

CONCLUSIONS

Urinary N should currently be estimated as 0.026 × BW (kg) × MUN (mg/dl). The target range for MUN of Holstein cows when using current DHIA labs appears to have decreased to 8 to 12 for most herds compared with the target range of 12 to 16 that was previously determined from older data gathered with different calibration standards.

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