



Validation of models for predicting milk urea nitrogen concentrations, estimating dry matter intake by the NRC (2001)¹

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ABSTRACT - The objective of this study was to validate three different models for predicting milk urea nitrogen using field conditions, attempting to evaluate the nutritional adequacy diets for dairy cows and prediction of nitrogen excreted to the environment. Observations (4,749) from 855 cows were used. Milk yield, body weight (BW), days in milk and parity were recorded on the milk sampling days. Milk was sampled monthly, for analysis of milk urea nitrogen (MUN), fat, protein, lactose and total solids concentration and somatic cells count. Individual dry matter intake was estimated using the NRC (2001). The three models studied were derived from a first one to predict urinary nitrogen (UN). Model 1 was $MUN = UN/12.54$, model 2 was $MUN = UN/17.6$ and model 3 was $MUN = UN/(0.0259 \times BW)$, adjusted by body weight effect. To evaluate models, they were tested for accuracy, precision and robustness. Despite being more accurate (mean bias = 0.94 mg/dL), model 2 was less precise (residual error = 4.50 mg/dL) than model 3 (mean bias = 1.41 and residual error = 4.11 mg/dL), while model 1 was the least accurate (mean bias = 6.94 mg/dL) and the least precise (residual error = 5.40 mg/dL). They were not robust, because they were influenced by almost all the variables studied. The three models for predicting milk urea nitrogen were different with respect to accuracy, precision and robustness.

Key Words: dairy cow, milk urea nitrogen, nitrogen excretion, protein utilization

Introduction

Milk urea nitrogen concentration has been used as a management tool to evaluate if a herd (or even the cows individually) has been fed with optimum quantities of protein, if the relation between rumen degraded and undegraded protein is proper and if the balance between protein and energy intake is adequate (Carlsson & Pehrson, 1994).

For this reason, mathematical models have been developed attempting to predict milk urea nitrogen target concentration and thus facilitate its use in the evaluation of nutritional values of diets and in the prediction of nitrogen excreted to the environment (Jonker et al., 1998). These models consider almost all the factors known by affecting milk urea nitrogen concentrations, including nitrogen intake, milk yield and milk protein content. Deviations from this target can identify overfeeding or underfeeding of protein or other issues related to feeding and management. Currently, there are three models which were derived from a model proposed by Jonker et al. (1998) to predict urinary nitrogen. Model 1 for predicting milk

urea nitrogen was developed by Jonker et al. (1998), while the other two are more recent (Kauffman & St-Pierre, 2001). In addition, one of the models was adjusted for body weight effect.

After the first model had been developed, Kohn et al. (2002) reported that, in September of 1998, a hardware defect was found on the milk urea nitrogen analyzer that was being used by the DHIA laboratories (Dairy Herd Improvement Association) across the USA to run milk urea nitrogen samples for standard curves. When this defect was corrected, milk urea nitrogen standards changed so that DHIA laboratories reported lower milk urea nitrogen values.

Another important fact is that those models were developed based on experimental databases, from trials carried out in the USA, using their feeds and environmental conditions. So, in order to use them properly, they should be validated under field conditions from different situations and countries. In the case of this study, by using data from a commercial herd, the attempt was to simulate real field conditions. This way, they may provide accurate and reliable results for predicting milk urea nitrogen, which was the objective of the present study.

Material and Methods

In the study, 4,749 observations from 855 Holstein cows of a commercial herd (Brazil) were used. Cows were confined in free-stall barn, with fans and sprinklers, turned on automatically when room temperature reached 23 °C. After birth, cows were milked in a 2 × 12 herringbone parlor, with AFIMILK[®] - SAE AFIKIM data recording system, where each animal was identified and had its milk yield recorded daily.

Cows were fed 7 times/day; the first meal offered at 5 h and the last at 21 h. They received a total mixed ration (48% roughage on a dry matter basis), composed of corn silage, grass haylage, soybean meal, corn germ, high moisture corn grain silage, corn gluten feed, citrus pulp and mineral mixture. Diet was balanced using NRC (1989). The average dry matter intake of the herd was calculated daily by the difference between total feed offered and orts (Table 1).

Data of individual cows - milk yield (kg/day), days in milk (DIM), body weight (BW) and parity and of the herd (dry matter intake) - were recorded on the milk sampling days, which occurred once a month. Milk was sampled, always in the morning milking, and transferred directly to plastic vials (60 mL), each one containing two broad-spectrum microtabs of bronopol as preservative, and homogenized for, at least, 15 seconds. They were sent to laboratory for analyses of fat, protein, lactose and total solids, milk urea nitrogen and somatic cells count (SCC). Concentrations (%) of fat, protein, lactose and total solids were determined by infrared absorption (Bentley Instruments, 1995a), SCC (x 10³ cells/mL) was done by flow

citometry (Bentley Instruments, 1995b) and milk urea nitrogen (mg/dL) by an enzymatic and colorimetric methodology (Bentley Instruments, 1998).

Seasons of the year and calving seasons were divided in summer (Nov. – Apr.) and winter (May – Oct.). Fat/protein ratio was obtained by the division of milk fat by protein percentage. Somatic cells count was analyzed as natural log transformation by the equation $\ln(\text{SCC}+1)$, because it has no normal distribution (Godden et al., 2001).

The 4% fat-corrected milk was calculated by the equation: (0.4*kg of milk produced) + (15*kg of fat produced), according to the NRC (1989). Then, dry matter intake was estimated by the equation of the NRC (2001), as follows:

$$\int DMI = ((0.0968 * BW^{0.75}) + (0.372 * 4\%FCM) - 0.293) * (-e^{(-0.192*(LW+3.67))})$$

where: DMI = dry matter intake (kg/animal/day); 4%FCM = 4% fat-corrected milk (kg/animal/day); BW = body weight (kg); LW = lactation week. The 4% fat-corrected milk was used to calculate DMI (NRC, 2001) and the latter was used indirectly in the calculation of the models for predicting milk urea nitrogen, when calculating the nitrogen intake.

The models, evaluated in this study, were derived from a first model proposed by Jonker et al. (1998) to predict urinary nitrogen (UN), as follows:

$$UN = (NI \times 0.83) - MN - 97;$$

where: UN = excretion of urinary nitrogen (g/animal/day); NI = nitrogen intake (g/ animal/day) and MN = nitrogen secretion in milk (g/animal/day).

To calculate urea nitrogen (UN), dry matter intake (estimated by the NRC, 2001) was used specifically in the equation to calculate nitrogen intake. Then, UN was used

Table 1 - Composition of diets and average dry matter intake of the herd

Months	Diet composition							
	DM	CP	EE	NDF	ADF	NFC	Ash	DMI
Sep./2000	472	171.0	31.5	340.4	196.9	387.7	63.7	20.9
Oct./2000	463	173.1	31.2	332.2	193.0	398.3	60.3	20.5
Nov./2000	468	176.7	30.9	327.2	194.0	396.9	62.8	20.3
Dec./2001	483	164.9	38.8	330.3	195.9	399.9	61.3	22.9
Jan./2001	498	159.1	42.0	310.4	185.2	422.7	60.8	19.3
Feb./2001	488	164.7	41.2	311.6	184.6	415.8	61.8	16.9
Apr./2001	492	164.8	40.6	298.3	178.9	430.2	61.2	16.8
May/2001	496	166.9	43.0	309.5	179.9	413.4	61.9	18.4
Jun./2001	501	168.0	53.4	328.8	192.4	379.7	63.7	19.1
Jul./2001	504	168.2	53.7	329.7	193.1	378.1	63.8	20.0
Aug./2001	504	169.0	42.6	334.8	196.6	385.5	62.2	23.2
Sept./2001	504	169.0	42.6	334.8	196.6	385.5	62.2	22.6
Oct./2001	497	171.1	41.9	330.2	196.3	377.0	72.1	23.3
Nov./2001	497	171.1	41.9	330.2	196.3	377.0	72.1	21.9
Dec./2001	501	171.1	41.9	330.2	196.3	377.0	72.1	22.9
Jan./2002	501	170.0	46.8	328.1	195.1	375.2	72.3	23.8

DM - dry matter (g kg⁻¹); CP - crude protein (g kg⁻¹ DM); EE - ether extract (g kg⁻¹ DM); NDF - neutral detergent fiber (g kg⁻¹ DM); ADF - acid detergent fiber (g kg⁻¹ DM); NFC - non-fiber carbohydrates (g kg⁻¹ DM); ash (g kg⁻¹ DM); DMI - dry matter intake of the herd (kg/animal/ day).

to predict milk urea nitrogen (MUN). This way, the equation of the models for predicting milk urea nitrogen included nitrogen intake, milk yield and milk protein content. Additionally, model 3 was adjusted by body weight effect. Predicted milk urea nitrogen concentrations depended on the model used and were obtained by the following equations:

- model 1: $MUN = UN/12.54$ (Jonker et al., 1998);
- model 2: $MUN = UN/17.6$ (Kauffman & St-Pierre, 2001);
- model 3: $MUN = UN/(0.0259 \times BW)$ (Kauffman & St-Pierre, 2001).

After the first model had been developed, Kohn et al. (2002) reported that, on September 28, 1998, a hardware defect was found on the milk urea nitrogen analyzer that was being used by the DHIA (Dairy Herd Improvement Association) laboratories across the USA to run milk urea nitrogen samples for standard curves. When this defect was corrected, milk urea nitrogen standards changed so that DHIA laboratories reported lower milk urea nitrogen values. Thus, it has been exceedingly difficult to interpret milk urea nitrogen values based on models that were developed previously. Currently, DHIA laboratories use the average milk urea nitrogen reported by several different analyzers to develop standards for indirect methods.

However, more recent studies have suggested that there is a potential bias in this predictor. Kauffman and St.-Pierre (2001) reported milk urea nitrogen equal to $UN/17.64$ for Holstein cows (model 2). One of the differences between studies that may explain the different coefficients is that laboratory methods used to determine milk urea nitrogen have changed. Another consideration is that the appropriate coefficient depends on BW and so Kauffman & St.-Pierre (2001) developed the model 3.

The purpose of these models is to identify when observed milk urea nitrogen deviates from an expected value, so indicating a potential management problem. By using the 3 different models, expected milk urea nitrogen was predicted from diet and production parameters and these values were then compared with the observed milk urea nitrogen values.

To evaluate the models, they were tested for accuracy, precision and robustness. Accuracy and precision of models were determined by comparison of predicted with observed values. Robustness was determined by comparison of predicted values minus observed values (biases) with other studied factors (Kohn et al., 1998).

According to Kohn et al. (1998), accuracy is evaluated through the mean bias. Bias is the difference between predicted value by the model and observed value and represents the mean inaccuracy of model predictions. Therefore, the most accurate model is the one that presents mean bias as close to zero as possible. Accuracy is measured by the following equation:

$$\text{Mean bias} = \frac{\sum(\text{predicted} - \text{observed})}{\text{observations}}$$

Precision is a measurement of dispersion between predicted and observed values, i.e., it is the mean variability of the distance between predicted and observed value. It can be evaluated by the root mean square prediction error (RMSPE) or by the residual error. The RMSPE (Bibby & Toutenburg, 1977) is a measure of how much the predictions are well-adequate to the observed values and was calculated by the following equation:

$$\text{RMSPE} = \sqrt{\frac{\sum(\text{predicted} - \text{observed})^2}{\text{observations}}}$$

However, according to Rodrigues (2002)¹, every time the mean bias is high (lack of accuracy), the lack of precision will be overestimated, i.e., an overestimate of RMSPE, once the mean distance between predicted and observed value also leads to increase in variability between predicted and observed values. Thus, precision is well-evaluated when RMSPE is corrected for lack of accuracy, this way creating residual error, which is defined by remaining error in the prediction model, excluding the error due to mean bias. Residual error is also referred to as the prediction error, excluding the mean bias, obtained by the following equation:

$$\text{Residual error} = \sqrt{[\text{RMSPE}^2 - (\text{meanbias})^2]}$$

Mean bias for milk urea nitrogen was estimated by regressing bias (predicted milk urea nitrogen – observed milk urea nitrogen) *versus* observed milk urea nitrogen and could be used to identify if the magnitude of bias increases, decreases or does not exist with the magnitude of milk urea nitrogen observed values (Bibby & Toutenburg, 1977).

Robustness is the characterization of the model that is less influenced by other selected factors. In order to fit in this concept, the model must have, in relation to the regression straight lines of bias *versus* variables, low slope coefficients and coefficient of determination (R^2) and also lack of slope, indicated by high statistical probabilities (Rodrigues, 2002)¹.

In order to compare the models with respect to accuracy, mean bias was submitted to analysis of variance (F test) and

¹ RODRIGUES, P.H.M. (Faculdade de Medicina Veterinária e Zootecnia da Universidade de São Paulo, Pirassununga/SP). Personal communication, 2002.

the comparison of means was accomplished using the Tukey test (5%). For determination of mean bias significance, i.e., if it differed significantly from 0, the T-test was used for mean=0, using PROC UNIVARIATE (SAS, 1985). To compare the models for precision, residual errors were submitted to test of homogeneity of variance (the test of Hartley) and compared pairwise, using PROC TTEST (Statistical Analysis System, version 5), according to Ott (1993).

Linear bias for milk urea nitrogen was estimated by regression of bias (predicted milk urea nitrogen – observed milk urea nitrogen) *versus* observed milk urea nitrogen, using PROC REG of the software SAS (Statistical Analysis System, version 5). Robustness was obtained by regression of bias *versus* selected factors (parity, days in milk, body weight, milk yield, milk fat, protein, lactose and total solid concentrations, natural logarithm of somatic cells count, calving season and season of the year), by PROC REG of SAS (Statistical Analysis System, version 5). Comparison of slope coefficients was accomplished by methods of comparing two straight lines, with the objective to evaluate the interaction between selected variable and models, using analysis of variance (F test) and pairwise comparison by PROC GLM of SAS (1985).

Results and Discussion

Average milk urea nitrogen concentration was 13.15 mg/dL. Cows presented, on average, 662 kg of body weight, 2.3 lactations, 197 days in milk and 34.2 kg/day of milk yield.

According to estimated mean biases (Table 2), model 1 overestimated observed milk urea nitrogen by approximately 50%, i.e., by 6.94 mg/dL. Model 2 overestimated milk urea nitrogen by approximately 7% (0.94 mg/dL) and model 3, by

approximately 10% (1.41 mg/dL). Thus, all models lacked accuracy, because their mean biases differed from 0 ($P < 0.01$). However, the accuracy of models, represented by mean biases, differed between themselves ($P < 0.05$), showing that model 2 is the most accurate, because it had the lowest mean bias (0.94 mg/dL), while model 1 is the least accurate (6.94 mg/dL) (Table 2).

The models differed in precision (residual errors); model 3 was the most precise (4.11 mg/dL) and model 1 was the least precise (5.40 mg/dL). The RMSPE were 8.79, 4.60 and 4.35 mg/dL for models 1, 2 and 3, respectively. Thus, despite being the most accurate, model 2 was intermediate precise with residual error of 4.50 mg/dL (Table 2). On the other hand, model 1 was the least accurate and the least precise.

By regressing bias (predicted milk urea nitrogen – observed milk urea nitrogen) *versus* observed milk urea nitrogen, negative linear biases (slope coefficients) of -0.9540, -0.9673 and -0.9320 mg/dL were found ($P < 0.0001$) for models 1, 2 and 3, respectively (Table 2). This means that bias was the lowest when observed milk urea nitrogen was the highest. However, the linear bias of model 3 differed from model 2 ($P = 0.0390$), but not from model 1 ($P = 0.3039$), the same way linear bias of model 2 did not differ from model 1 ($P = 0.5541$) (Figure 1).

The 3 models were influenced by almost all studied variables ($P < 0.01$). However, model 3 was not affected by logarithm of somatic cells count ($P = 0.4204$), or by calving season ($P = 0.2194$) (Table 3). By analyzing R^2 , fat and total solids concentration and fat/protein ratio showed the highest values and may explain variations in the models. In addition, they also showed the highest slope coefficients, yet including lactose concentration and season of the year, despite their low R^2 .

Table 2 - Mean bias (accuracy), residual error (precision), root mean square prediction error (RMSPE) and coefficient of determination (R^2) for models 1, 2 and 3, and regression of bias *versus* observed milk urea nitrogen (MUN)

	Model 1	Model 2	Model 3
Observed MUN	13.81	13.81	13.81
Predicted MUN	20.75	14.75	15.22
Mean bias ^{1,2}	6.94A*	0.94C*	1.41B*
Residual error ³	5.40A	4.50B	4.11C
RMSPE	8.79	4.60	4.35
R^2	0.001327	0.001327	0.007868
	Regression of bias <i>versus</i> observed MUN ⁴		
Linear bias ^{5,6}	-0.9540AB	-0.9673B	-0.9320A
R^2 (Probability) ⁷	0.3639 (<0.0001)	0.5378 (<0.0001)	0.5985 (<0.0001)

¹ Means within the row, followed by different letters, differ ($P < 0.05$) by the Tukey Test.

² Probability of the T-test for mean = 0. * Mean different from 0 ($P < 0.01$).

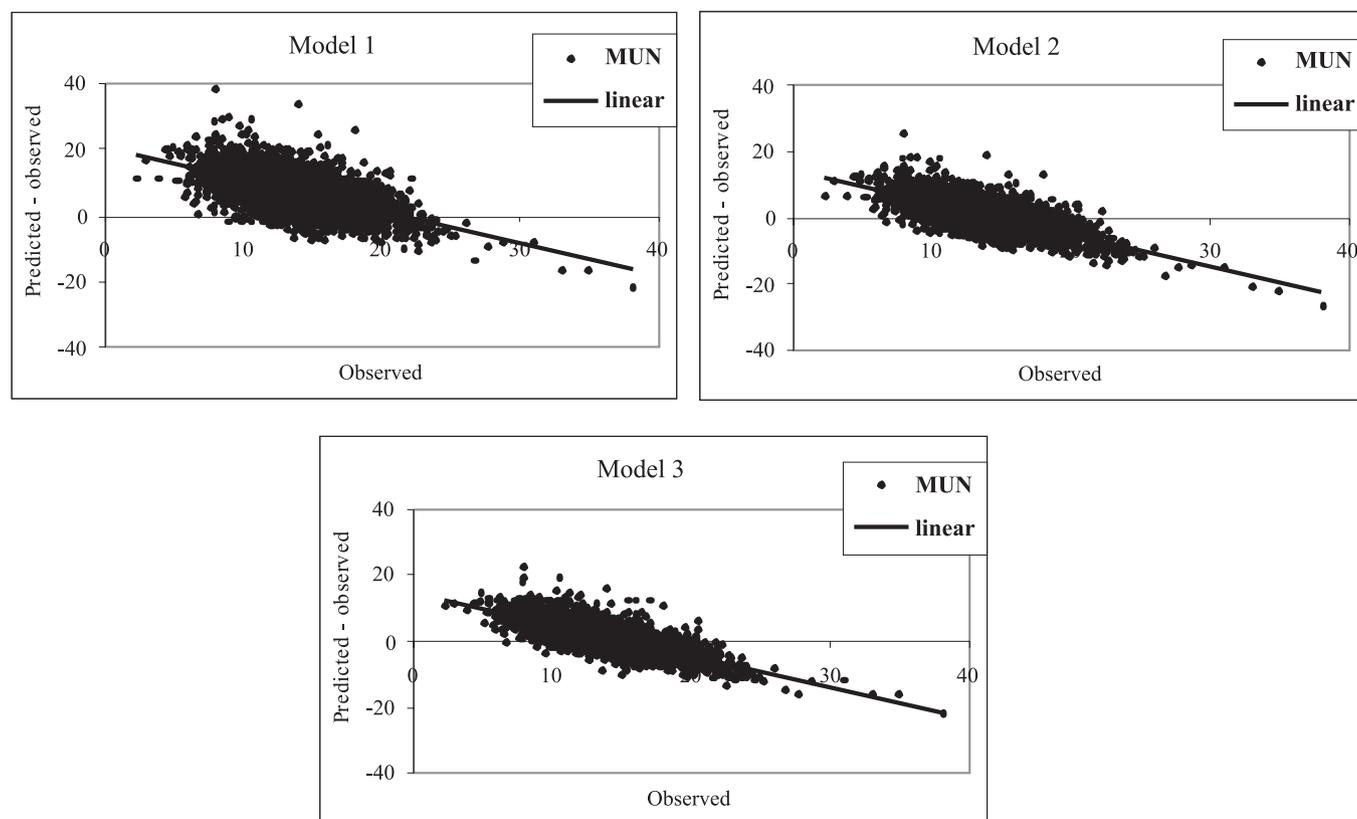
³ Residual errors within the row, followed by different letters, differ by the Hartley test ($P < 0.05$).

⁴ Regression of bias (predicted MUN – observed MUN) *versus* observed MUN.

⁵ Slope coefficient of regression of bias (predicted MUN – observed MUN) *versus* observed MUN.

⁶ Means within row, followed by different letters, are different ($P < 0.05$).

⁷ Coefficient of determination and statistical probability of existence of slope coefficient (F test).



MUN - milk urea nitrogen.

Figure 1 - Linear biases for MUN, by regressing bias (predicted MUN – observed MUN) versus observed MUN for models 1, 2 and 3.

Table 3 - Evaluation of robustness (slope, R^2 and probability) for models 1, 2 and 3, including other variables

Variable		Model 1	Model 2	Model 3
Parity	Slope ^{2,3}	0.9551A	0.6073B	-0.2384C
	R^2 (Prob.) ⁴	0.0626 (<0.0001)	0.0364 (<0.0001)	0.0067 (<0.0001)
Days in milk	Slope	0.0091A	0.0078 A	0.0018B
	R^2 (Prob.)	0.0448 (<0.0001)	0.0477 (<0.0001)	0.0031 (<0.0001)
Body weight (kg)	Slope	0.0271A	0.0198B	-0.0018C
	R^2 (Prob.)	0.2362 (<0.0001)	0.1806 (<0.0001)	0.0018 (0.0034)
Milk yield (kg/dia)	Slope	0.0948A	0.0464B	0.0610B
	R^2 (Prob.)	0.0256 (<0.0001)	0.0088 (<0.0001)	0.0182 (<0.0001)
Milk fat (%)	Slope	3.2825A	2.5417B	2.3549C
	R^2 (Prob.)	0.2502 (<0.0001)	0.2157 (<0.0001)	0.2219 (<0.0001)
Milk protein (%)	Slope	0.7619A	1.0037A	-0.5942B
	R^2 (Prob.)	0.0024 (0.0007)	0.0061 (<0.0001)	0.0026 (0.0005)
Fat/protein ratio	Slope	10.4857A	7.8573B	8.2603B
	R^2 (Prob.)	0.2463 (<0.0001)	0.1989 (<0.0001)	0.2635 (<0.0001)
Lactose (%)	Slope	-2.2982C	-1.5482B	0.9965A
	R^2 (Prob.)	0.01885 (<0.0001)	0.0123 (<0.0001)	0.0061 (<0.0001)
Milk total solids (%)	Slope	1.8378A	1.5048B	1.4729B
	R^2 (Prob.)	0.1312 (<0.0001)	0.1265 (<0.0001)	0.1453 (<0.0001)
LSCC ¹	Slope	0.3850A	0.3574A	0.0319B
	R^2 (Prob.)	0.0115 (<0.0001)	0.0143 (<0.0001)	0.0001 (0.4204)
Calving season	Slope	0.8181A	0.7417A	0.1485B
	R^2 (Prob.)	0.0056 (<0.0001)	0.0066 (<0.0001)	0.0003 (0.2194)
Year season	Slope	-2.7077B	-2.2231A	-2.1561A
	R^2 (Prob.)	0.0601 (<0.0001)	0.0583 (<0.0001)	0.0657 (<0.0001)

¹ Natural logarithm of (SCC+1).

² Slope coefficient of a straight line $y = a + bX$, by regressing bias (predicted milk urea nitrogen – observed milk urea nitrogen) versus variables.

³ Comparison between slope coefficients of straight lines, where different letters within row differ by the methodology of straight lines comparison (analysis of variance) ($P < 0.05$).

⁴ Coefficient of determination and statistical probability of the analysis of variance to indicate existence of slope coefficient (F test).

For model 1, body weight presented relatively high R^2 (0.2362), but, for model 3, R^2 (0.0018) and slope coefficient were low ($P < 0.05$) (Table 3). Model 3 had already been previously corrected for body weight in its equation. In addition, for estimating dry matter intake from the NRC (2001), body weight was also included in its original equation. Dunlap et al. (2000) validated model 1 and observed a linear effect of BW on predicted milk urea nitrogen concentrations, so that predicted values were higher than observed values for high body weight cows and lower for low body weight cows.

Model 1, proposed by Jonker et al. (1998), estimates a renal clearance rate of 1,254 L/day for all cows. But the authors affirmed that heavier cows have more blood than the smaller ones and, with the same protein intake, are more likely to have a higher clearance rate, while smaller cows are more likely to have lower rates (Jonker et al., 1998). Both effects would lead to reduction in predicted milk urea nitrogen for heavier cows and increase for smaller cows.

Although these effects had been noted by Jonker et al. (1999), they explained that less than 3% of the model variation were attributed to body weight. For this reason, body weight was not included in model 1, because the data used in its development were not robust enough to be considered. Yet, they reported that the variation in the body weight, caused by gastrointestinal filling, milk volume of the mammary gland, and measurements methods, seemed to add so much variation in the prediction of their original model (Jonker et al., 1998) that its inclusion was not recommended.

Regarding the regression of biases *versus* milk yield, although the slope coefficients of the 3 models differ from 0 ($P < 0.0001$), their values were low, as well as the coefficients of determination (Table 3). This lack of milk yield effect was probably due to the previous correction of the models when dry matter intake was estimated by the NRC (2001), once milk yield was included in its equation. Also, milk yield and protein content were previously used in the model for predicting urinary nitrogen, which was later used for predicting milk urea nitrogen. Thus, the lack of milk yield effect demonstrates that the model accurately considers its influence.

According to Jonker et al. (1999), milk yield drives the nitrogen requirements in lactating dairy cows fed diets balanced by the NRC (1989). As milk yield increases, predicted milk urea nitrogen concentrations increase linearly because of the higher nitrogen intake and excretion. Subsequently, milk urea nitrogen target concentrations are extremely sensitive to changes in milk yield. Jonker et al. (1999) reported that the average milk urea nitrogen

concentration of their model was more sensitive to feeding requirements and to milk yield and less to body weight and parity. In addition, Nousiainen et al. (2004), after testing the effects of nutritional and non-nutritional factors on milk urea nitrogen concentration, observed that crude protein content in the diet was the best factor to predict milk urea nitrogen.

More recently, Burgos et al. (2007) found that the relationship between urinary urea nitrogen excretion and milk urea nitrogen concentration was different according to lactation stage and diverged from linearity for early and late lactation over a wide range of milk urea nitrogen values. However, these differences were restricted to very high milk urea nitrogen concentrations. Thus, the prediction of urinary urea nitrogen excretion based on milk urea nitrogen concentration depends on the range of milk urea nitrogen concentration. They concluded that milk urea nitrogen can be used to predict urinary urea nitrogen excretion and may be extended to estimate NH_3 emissions from dairy cattle manure, because there is a strong relationship between urinary urea nitrogen excretion and NH_3 emissions.

Meyer et al. (2006) evaluated these models to predict milk urea nitrogen, using average herd intake to estimate the individual dry matter intake, and concluded that the models differed between themselves regarding accuracy, precision and robustness, presenting questionable use when the objective is to predict milk urea nitrogen for dairy cows or the urinary nitrogen excretion in the field conditions used.

Conclusions

The three models for predicting milk urea nitrogen are different with respect to accuracy, precision and robustness. Therefore, they are of limited value when the objective is to predict milk urea nitrogen or urinary nitrogen excretion in field conditions, when the dry matter intake is estimated from the NRC (2001).

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