Cow and herd variation in milk urea nitrogen concentrations in lactating dairy cattle¹

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ABSTRACT

Milk urea nitrogen (MUN) is correlated with N balance, N intake, and dietary N content, and thus is a good indicator of proper feeding management with respect to protein. It is commonly used to monitor feeding programs to achieve environmental goals; however, genetic diversity also exists among cows. It was hypothesized that phenotypic diversity among cows could bias feed management decisions when monitoring tools do not consider genetic diversity associated with MUN. The objective of the work was to evaluate the effect of cow and herd variation on MUN. Data from 2 previously published research trials and a field trial were subjected to multivariate regression analyses using a mixed model. Analyses of the research trial data showed that MUN concentrations could be predicted equally well from diet composition, milk yield, and milk components regardless of whether dry matter intake was included in the regression model. This indicated that cow and herd variation could be accurately estimated from field trial data when feed intake was not known. Milk urea N was correlated with dietary protein and neutral detergent fiber content, milk yield, milk protein content, and days in milk for both data sets. Cow was a highly significant determinant of MUN regardless of the data set used, and herd trended to significance for the field trial data. When all other variables were held constant, a percentage unit change in dietary protein concentration resulted in a 1.1 mg/dL change in MUN. Least squares means estimates of MUN concentrations across herds ranged from a low of 13.6 mg/dL to a high of 17.3 mg/dL. If the observed MUN for the high

herd were caused solely by high crude protein feeding, then the herd would have to reduce dietary protein to a concentration of 12.8% of dry matter to achieve a MUN concentration of 12 mg/dL, likely resulting in lost milk production. If the observed phenotypic variation is due to genetic differences among cows, genetic choices could result in herds that exceed target values for MUN when adhering to best management practices, which is consistent with the trend for differences in MUN among herds.

Key words: milk urea nitrogen, feeding management, dairy cattle

INTRODUCTION

Nitrogen emissions from agriculture cause air and water quality impairment (Tamminga, 1992; Van Horn et al., 1996). Animal agriculture has been identified as a major contributor of N pollution to water resources in the Chesapeake Bay watershed (Fisher and Openheimer, 1991; Boesch et al., 2001), and research resources have focused on identifying management practices to reduce the environmental impact (NRC, 2003).

Dairy cattle are commonly fed diets with protein levels exceeding 16% to ensure maximum milk output (NRC, 2001). This practice contributes to the relatively low N efficiency of lactating dairy cattle (Bequette et al., 2003; Huhtanen and Hristov, 2009) while adding dietary cost and potentially decreasing profit margins (Godden et al., 2001). Milk urea nitrogen is highly correlated with urinary N excretion (Jonker et al., 1998; Kauffman and St-Pierre, 2001) and is a good indicator of NH₃ emissions from dairy manure (Burgos et al., 2007).

Dietary protein available for microbial use in the rumen can be degraded to AA and peptides. These are utilized by microbes for microbial protein synthesis, or are deaminated and used to support energy needs (see Tamminga, 1979). Provision of ruminally available N in excess of microbial needs for protein synthesis results in the generation of NH₃ that is absorbed and converted

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to urea in the liver (Parker et al., 1995). Absorbed AA and peptides provided in excess of animal requirements are also deaminated and converted to urea. Thus, urea synthesis is proportional to the balance of dietary N and the use of N for productive purposes.

Synthesized urea is released into the blood and equilibrates with body fluids, including milk (Broderick and Clayton, 1997; Archibeque, 2000). This results in high correlations of blood urea N and MUN with dietary N (Nousiainen et al., 2004; Preston et al., 1965). Because the kidney urea clearance is concentration dependent, a high correlation also exists between MUN and urinary N excretion (Jonker et al., 1998). These relationships and routine measurement of MUN by milk processors and DHIA testing laboratories have led to the use of MUN as a tool for monitoring feeding programs and feed management practices (NRCS, 2011).

Although MUN concentration is clearly related to N balance within a cow, several factors can cause deviations from expected values. These include time of sampling, season of the year, BW, breed, and nutritional factors (DePeters and Cant, 1992; Broderick and Clayton, 1997; Kauffman and St-Pierre, 2001). Significant cow effects (Wattiaux et al., 2005) are also at least partially explained by genetic variance (Wood et al., 2003; Mitchell et al., 2005; Miglior et al., 2007; Stoop et al., 2007). Given the genetic effects on MUN, it is possible that sire selection decisions within a herd may result in herd concentrations of MUN differing from the expected values based on feed management. When the model of Kauffman and St-Pierre (2001) was used to predict MUN concentrations for individual cows in trials performed by Cyriac et al. (2008) and Rius et al. (2010), the variance in residual MUN associated with the cow was 4.1 ± 1.1 mg/dL ($P < 0.001$), indicating that the cow itself was an important determinant of MUN. Because differences in DMI were accommodated in the model, DMI could be ruled out as a contributor to the observed cow variance. The model did not exhibit mean bias, although the range in residual errors (mean residual = -0.1 ± 6.5 mg/dL) was large. If herd-level deviations are as large, it could result in poor feed management decisions when using MUN to guide feeding choices.

Studies examining genetic parameters for cow and herd variation in MUN have not used dietary information (Wood et al., 2003; Mitchell et al., 2005; Miglior et al., 2007; Stoop et al., 2007). Part of the cow and herd variance, therefore, may be associated with feeding multiple diets within a herd and differing diets across herds (Jonker et al., 1998; Kauffman and St-Pierre, 2001). Conversely, studies examining the relationships among dietary nutrients and MUN have not reported cow and herd variance. The objective of this study was

to determine variation associated with animal and herd MUN levels while accounting for differences in dietary nutrient content, level of production, and stage of lactation. It was hypothesized that cow variation in MUN could affect overall herd MUN and bias feed management decisions.

MATERIALS AND METHODS

Intake, dietary nutrients, and production data from 2 previously published trials (Cyriac et al., 2008; Rius et al., 2010) were used to assess whether MUN could be predicted as well from dietary nutrient concentrations as from nutrient intakes. A total of 68 multiparous Holstein and 12 multiparous Jersey $\times$ Holstein crossbreds were included in the data set.

The first trial included observations from 40 midlactation cows randomly assigned to 1 of 4 diets that contained 11.3, 10.1, 8.8, or 7.6% RDP (DM basis) with corresponding reductions in dietary CP (Cyriac et al., 2008). The second trial included observations from 40 midlactation cows assigned to 1 of 4 diets that contained high (1.55 Mcal of NE_L/kg) or low dietary energy (LE, 1.44 Mcal of NE_L/kg) and high (6.6% RUP) or low ruminally undegraded protein (4.6% RUP) arranged in a 2×2 factorial design (Rius et al., 2010). Feed samples were analyzed for total N (Perkin-Elmer 2410 Series II, Perkin-Elmer, Norwalk, CT), ether extract (AOAC International, 1996; method 920.39), ash (AOAC International, 1996; method 942.05), ADF (AOAC International, 1997), NDF (Van Soest et al., 1991), lignin (AOAC International, 1997), soluble CP (Licitra et al., 1996), neutral detergent-insoluble CP (Licitra et al., 1996), acid detergent-insoluble CP (Licitra et al., 1996), starch (2700 Select Biochemistry Analyzer, YSI, Yellow Springs, OH), minerals (AOAC International, 1997), and gross energy (bomb calorimetry, model 1271, Parr Instruments, Moline, IL). A summary of the data are provided in Table 1.

A second analysis was performed using data from a field trial with 5 herds (predominantly Holstein) that were being intensively monitored for P feeding plus the Virginia Tech herd (Holstein, Jersey, and various crossbreeds of Holstein, Jersey, Brown Swiss, and Swedish Red). All herds used DHIA testing services, and each herd was feeding a single lactating cow ration. On 2 consecutive test dates, milk production was recorded and milk samples were analyzed for true protein, fat, and lactose by infrared analyses (Fossomatic 4000 Combi infrared analyzer; Foss, Eden Prairie, MN) and for MUN using a modification of the Berthelot procedure (ChemSpec 150 Analyzer; Bentley Instruments, Chaska, MN; DHIA, Blacksburg, VA). In total, data were collected from 741 cows.

Table 1. A summary of intake, dietary nutrients, and production values for cows from the Cyriac et al. (2008) and Rius et al. (2010) experiments

Variable	Mean	SD	Minimum	Maximum
Intake (kg/d, DM basis)				
DMI	24.8	3.39	13.1	34.9
N	0.68	0.14	0.29	1.03
CP	4.23	0.89	1.78	6.45
NDF	9.52	2.05	4.65	17.7
ADF	6.11	1.41	2.99	12.3
Starch	5.97	1.12	3.32	8.31
Ash	1.49	0.28	0.91	2.23
DIM	175	71.3	35	413
Milk (kg/d)	36.9	8.53	12.6	60.7
Milk fat (%)	3.48	0.61	2.15	5.72
Milk protein (%)	3.08	0.27	2.54	4.03
Milk lactose (%)	4.89	0.21	4.06	5.26
MUN (mg/dL)	17.9	4.06	9.68	29.15
Dietary nutrient (% of DM)				
DM	51.4	2.15	47.9	53.9
CP	16.9	2.31	13.6	19.9
NDF	38.3	5.69	31.4	50.7
ADF	24.5	4.09	18.6	35.2
Starch	24.1	3.39	14.8	28.8
Ash	6.05	0.88	4.92	7.20

Samples of the TMR were collected for the 5 herds on the P project, and ingredients used in the ration were sampled at the Virginia Tech herd. All samples were submitted to Cumberland Valley Analytical Services (Hagerstown, MD) for analyses of total N, NDF, ADF, and ash. Nitrogen was analyzed according to method 990.03 (AOAC International, 2000), whereas NDF, ADF, and ash were analyzed using methods noted previously. Dietary NE_L was predicted from the other nutrients. Dietary nutrient concentrations were calculated for the Virginia Tech herd based on ingredient analyses and dietary inclusion rates. A summary of the data are provided in Table 2.

Regression analyses were performed using the Mixed procedure of SAS (version 9.2; SAS Institute Inc., Cary, NC). Two models were used for analyses of the research trial data. The first model regressed MUN concentra-

tions on intakes of CP, NDF, ADF, and starch (DM basis), DIM, milk yield, milk composition, milk component yield, SCC, period, experiment, and BW, and all 2-way interactions of these terms. The second model regressed MUN concentrations on dietary concentrations of CP, NDF, ADF, and starch, DIM, milk yield, milk composition, milk component yield, SCC, period, experiment, and BW, and all 2-way interactions. Variables with *P*-values greater than 0.1 were sequentially excluded from models by using a backward elimination procedure. Cow was included as a random variable, and the effect of cow was tested using a Covtest statement.

Field trial data were analyzed in the same manner using the Mixed procedure of SAS (version 9.2; SAS Institute Inc.). Milk urea nitrogen was regressed on dietary concentrations of CP, ADF, and NDF (% of DM), dietary NE_L (Mcal/kg of DM), DIM, milk yield (kg/d),

Table 2. A summary of production and dietary factors for field trial cows

Variable	Mean	SD	Minimum	Maximum
DIM	198	157	2	977
Milk (kg/d)	37.8	10.0	7.4	78.6
Milk fat (%)	3.76	0.88	1.5	8.5
Milk protein (%)	3.04	0.34	2.2	5.6
MUN (mg/dL)	15.5	3.9	4	35
Dietary nutrient (% of DM)				
DM	48.9	4.7	43.4	57.8
CP	17.6	1.6	14.4	23.4
NDF	35.3	3.4	30.5	41.5
ADF	21.2	2.4	17.6	25.5
Ash	7.1	0.6	6.0	9.7
NE_L (Mcal/kg)	1.68	0.06	1.54	1.75

Table 3. Parameter estimates for a mixed model relating MUN (mg/dL) to nutrient intake, production, and cow factors for the trials summarized in Table 1

Item	Estimate	SE	P-value
Effect			
Intercept	6.89	6.76	<0.31
ADF intake (kg/d)	−6.38	1.47	<0.0001
CP intake (kg/d)	20.3	2.50	<0.0001
Starch intake (kg/d)	−4.73	1.89	<0.02
Milk yield (kg/d)	−0.33	0.14	<0.03
Milk protein yield (kg/d)	5.35	3.16	<0.09
SCC (×1,000)	−0.002	0.0009	<0.01
ADF intake (kg/d) × milk lactose yield (kg/d)	1.54	0.69	<0.03
ADF intake (kg/d) × milk fat yield (kg/d)	14.7	4.25	<0.001
ADF intake (kg/d) × DIM	0.04	0.02	<0.02
CP intake (kg/d) × starch intake (kg/d)	−1.33	0.36	<0.0005
CP intake (kg/d) × milk lactose yield (kg/d)	−2.93	1.08	<0.009
CP intake (kg/d) × DIM	−0.01	0.005	<0.04
NDF intake (kg/d) × starch intake (kg/d)	2.02	0.71	<0.006
NDF intake (kg/d) × milk fat yield (kg/d)	−10.5	2.99	<0.0008
NDF intake (kg/d) × DIM	−0.03	0.01	<0.03
Starch intake (kg/d) × ADF intake (kg/d)	−2.62	1.04	<0.01
Starch intake (kg/d) × milk lactose yield (kg/d)	1.25	0.72	<0.09
Starch intake (kg/d) × milk fat yield (kg/d)	2.12	0.72	<0.004
Starch intake (kg/d) × DIM	0.01	0.004	<0.0007
Random effect			
Cow			<0.0004
Model precision ¹			Value
AIC			750
RMSE			1.77

¹AIC = Akaike information criterion; RMSE = root mean square error.

milk protein (%), and all 2-way interactions. Herd and cow nested within herd were included as random variables in the model. The effects of cow and herd were tested using a Covtest statement.

RESULTS

Results from analyses of the research trial data using models 1 and 2 are presented in Tables 3 and 4, respectively. When nutrient intakes were included in the regression model, MUN was correlated with intakes of ADF, CP, and starch; interactions of ADF, CP, NDF, and starch intakes; milk yield, milk protein yield, SCC; interactions of ADF intake with milk lactose yield, milk fat yield, and DIM; interactions of CP intake with milk lactose yield and DIM; the interaction of NDF intake and milk fat yield; and interactions of starch intake and milk lactose yield, milk fat yield, and DIM (Table 3). When dietary nutrient concentrations, but not intakes, were included in the model, MUN was highly correlated with milk protein yield, milk lactose yield, milk fat yield, SCC, and the interactions of CP, NDF, starch, and ADF (% of DM), with milk protein yield, milk lactose yield, milk fat yield, and DIM (Table 4). The effect of BW was not significant in either model. Inclusion of nutrient intakes in the model explained slightly

more variation, but the Akaike information criterion (version 9.2; SAS Institute Inc.) increased only from 750 to 758, suggesting that either approach resulted in similar precision. This is likely due to the high correlation between milk yield and energy intake (Brown et al., 1977; Buttazzoni and Mao, 1989; NRC, 2001). Cow was highly significant in both models.

Having established that MUN can be predicted with comparable precision when nutrient intakes are not known, we used model 2 to analyze the larger field data set (Table 5). As previously observed (Nousiainen et al., 2004) and consistent with the research trial analyses, MUN was highly correlated with dietary CP content. An increase of 1 percentage unit in dietary CP resulted in an increase in MUN of 1.04 and 1.24 mg/dL for milk yields of 40 and 30 kg/d, when other parameters in the model were held constant at the mean observed values listed in Table 2. This slope was less than the 1.7 mg/dL observed by Nousiainen et al. (2004). Milk urea N was also associated with dietary NDF, milk yield, and milk protein content and the interactions of dietary CP with milk yield and milk protein, dietary NDF with DIM and milk protein, and milk yield and milk protein. Cow was highly significant as observed for the research data, and a trend was found for a herd effect ($P < 0.08$).

Table 4. Parameter estimates for a mixed model relating MUN (mg/dL) to dietary nutrient concentrations, production, and cow factors for the trials summarized in Table 1

Item	Estimate	SE	P-value
Effect			
Intercept	16.3	1.61	<0.0001
Milk protein yield (kg/d)	-1,201	324	<0.0004
Milk lactose yield (kg/d)	682	199	<0.001
Milk fat yield (kg/d)	93.9	40.1	<0.02
SCC ($\times 1,000$)	-0.003	0.0009	<0.008
Dietary CP (% of DM) $\times$ milk protein yield (kg/d)	15.4	4.23	<0.0006
Dietary CP (% of DM) $\times$ milk lactose yield (kg/d)	-9.07	2.59	<0.0009
Dietary CP (% of DM) $\times$ DIM	0.002	0.0009	<0.03
Dietary NDF (% of DM) $\times$ milk protein yield (kg/d)	-45.7	13.3	<0.001
Dietary NDF (% of DM) $\times$ milk lactose yield (kg/d)	25.9	8.22	<0.002
Dietary NDF (% of DM) $\times$ milk fat yield (kg/d)	2.72	1.58	<0.09
Dietary NDF (% of DM) $\times$ DIM	-0.001	0.0004	<0.01
Dietary starch (% of DM) $\times$ milk protein yield (kg/d)	28.6	8.03	<0.0007
Dietary starch (% of DM) $\times$ milk lactose yield (kg/d)	-16.2	4.94	<0.002
Dietary starch (% of DM) $\times$ milk fat yield (kg/d)	-2.40	1.24	<0.06
Dietary ADF (% of DM) $\times$ milk protein yield (kg/d)	81.4	23.2	<0.0008
Dietary ADF (% of DM) $\times$ milk lactose yield (kg/d)	-46.0	14.3	<0.002
Dietary ADF (% of DM) $\times$ milk fat yield (kg/d)	-5.72	2.73	<0.04
Random effect			
Cow			<0.008
Model precision ¹			Value
AIC			758
RMSE			2.16

¹AIC = Akaike information criterion; RMSE = root mean square error.

DISCUSSION

Urea excretion is ultimately determined by the balance of N intake and N deposited in body and milk protein. Nitrogen intake in excess of deposition is converted to urea by the liver and released into blood. Because BUN concentrations are reflective of urea production by the liver, BUN and MUN are indicative of protein balance and thus useful for dietary protein management (Oltner and Wiktorsson, 1983; Broderick and Clayton, 1997). A variety of factors are known to contribute to excess urea N production, a key being excessive protein or N consumption. Excessive protein consumption can be caused by greater than expected DMI, deviations in forage protein, an improperly balanced ration, and improper mixing of the ration. Inadequate fermentable or total energy in the diet could fail to support the energy needs of the cow, causing lower than expected milk production. Dehydration will also result in increased BUN and MUN (Weeth and Lesperance, 1965; Steiger Burgos et al., 2001). Other factors that affect MUN but likely have little effect on urea N excretion include DIM and breed (Broderick and Clayton, 1997; Kauffman and St-Pierre, 2001). Although BW has previously been observed to affect MUN, we did not observe a similar relationship herein (Oltner et al., 1985; Broderick and Clayton, 1997).

Milk urea nitrogen levels in the research trial data were highly correlated with dietary nutrients, production characteristics, and stage of lactation regardless

Table 5. Parameter estimates for a mixed model relating MUN concentration (mg/dL) to dietary nutrients, cow, and herd factors for the data summarized in Table 2

Item	Estimate	SE	P-value
Effect			
Intercept	-166	26	<0.002
Dietary CP (% of DM)	5.4	1.1	<0.0001
Dietary NDF (% of DM)	2.84	0.45	<0.0001
Milk yield (kg/d)	0.66	0.12	<0.0001
Milk protein (%)	37.7	7.3	<0.0001
CP $\times$ NDF	-0.038	0.018	<0.03
CP $\times$ milk yield	-0.0194	0.0057	<0.001
CP $\times$ milk protein	-0.73	0.24	<0.003
NDF $\times$ DIM	-0.00005	0.00002	<0.009
NDF $\times$ milk protein	-0.65	0.11	<0.0001
Milk $\times$ milk protein	-0.073	0.023	<0.002
Random effect			
Herd			<0.08
Cow(herd)			<0.0001
Model precision ¹			Value
AIC			6,959
RMSE			2.42

¹AIC = Akaike information criterion; RMSE = root mean square error.

of whether DMI was considered in the model. Precision analysis of both models indicates that inclusion of nutrient intake explains only slightly more variation in MUN than simply considering dietary nutrient concentrations, supporting the management practice of adjusting dietary protein concentrations to achieve changes in MUN. It also supports the use of data from herds with unknown DMI to assess herd variation in MUN.

On the basis of the results from the field trial (Figure 1), the average herd with cows producing between 30 and 40 kg of milk/d would have MUN concentrations below 12 mg/dL only if diets contained less than 16% CP. However, the effect of herd trended to significance ($P < 0.08$). Least squares means for MUN by herd ranged from a low of 13.6 mg/dL to a high of 17.3 mg/dL. On the basis of an estimated slope of 1.1 mg/dL per unit of dietary CP (Figure 1), the herd with the highest MUN would have to reduce dietary CP by approximately 4.8 percentage units to achieve an MUN of 12 mg/dL if all other factors are held constant. The diet would need to be 12.8% CP based on the average observed dietary CP for that herd. It seems unlikely that such a low level of CP could be fed without compromising milk production (NRC, 2001). Of course, other management factors not considered in the regression analyses may also explain such a deviation from the norm; thus, one should assess all aspects of the operation before resorting to reduced protein feeding to achieve an MUN goal.

The significant effect of cow in the research and field trials is clear evidence of phenotypic differences in MUN concentrations among cows that are not explained by N intake, milk yield, BW, or other production-related factors. Mitchell et al. (2005) observed that MUN concentrations were heritable; thus, a genetic component to the observed variation exists. Urea transporters exist in several tissues, including the kidney (Yang et al., 2002) and rumen wall (Stewart et al., 2005), and more than 1 allele for the gene exists (You et al., 1993; Marini and Van Amburgh, 2003; Marini et al., 2004). Genetic variation in these transporters could result in variable transport activity that may explain the observed variation in MUN. If an allele codes for a transporter with reduced activity, this would result in elevated concentrations, and the reverse is true for alleles that code for increased transport activity.

Phenotypic effects on MUN concentrations are potentially problematic from a regulatory standpoint. Environmental or genetic variance may predispose a herd to abnormally high MUN levels even when the herd is following protein feeding guidelines. Attempting to reduce MUN levels to achieve a static regulatory MUN target in such a herd may result in lost production. If MUN is elevated because of low blood urea transport to urine, these cows will be excreting no more urinary N than cows with more active urea transporters when fed the same diet. Such variation would not be accommodated in the current excretion models (Jonker et al., 1998;

Figure 1. Least squares means estimates for MUN versus dietary CP predicted from the model summarized in Table 5 with varying milk yield, and the observed mean inputs for milk protein, dietary NDF, and DIM set to values listed in Table 2: 30 kg of milk/d (◆), 32 kg of milk/d (■), 34 kg of milk/d (▲), 36 kg of milk/d (×), 38 kg of milk/d (*), 40 kg of milk/d (●), 40 kg of milk/d regression (solid line; $y = 1.04 \text{ CP} - 3.0$), 30 kg of milk/d regression (dashed line; $y = 1.23 \text{ CP} - 7.34$).

Kauffman and St-Pierre, 2001), and producers may be penalized for failing to meet target levels even though their cows are not excreting any more N than herds with lower MUN. In the absence of selection measures, a producer may have inadvertently selected for a herd of cows that are predisposed to elevated MUN concentrations. Therefore, target values for MUN should not be used across herds without calibration for each herd unless some accommodation for normal herd variation is considered. If a common target value is to be used across herds, a safety margin should be included to accommodate those herds with intrinsically high MUN. Variation in MUN for herds that participated in the field trial was 1.6 ± 1.1 mg/dL. On the basis of this estimate, the target MUN level should be set 1.6 units above the population mean from the prediction models to accommodate intrinsic MUN variation. This should ensure that 83% of well-managed herds can achieve the goal if well managed regardless of their genetic selections. However, the estimate of herd variance was calculated from only 6 herds, which limits the precision of the value, as evidenced by the large standard deviation.

Herd calibration could be achieved through an assessment of the herd feeding program, taking into account all possible factors that may affect the observed variation in MUN. If the herd is well managed and feeding a balanced diet that does not exceed NRC (2001) requirements for protein and has adequate energy, the prevailing MUN could serve as a calibrated target value for that herd. If the herd is overfeeding protein relative to energy supply and milk production, the ration would have to be rebalanced and fed for a period of 2 or 3 weeks before reassessing MUN. The MUN value achieved after this period of feeding to requirements should reflect the calibrated target for the herd. Having determined a herd target value, deviations in MUN above the target would be indicative of overfeeding protein, whereas deviations below the target MUN may indicate that cows are being underfed protein. In the absence of such calibration, some accommodation for variation associated with the cow should be considered when setting guidelines for acceptable MUN values.

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