

FACTORS AFFECTING THE APPLICATION OF ON-LINE MILK UREA SENSING

D. M. Jenkins, M. J. Delwiche, E. J. DePeters, R. H. BonDurant

ABSTRACT. Estimation of urea in the milk of dairy cows is routinely done to determine how efficiently the animals are using dietary nitrogen. The observed values are used to adjust the herd ration to minimize feeds costs, improve the health of the herd, and reduce nitrogenous waste from the farm. Real-time data supplied by a recently developed on-line urea sensor may improve the quality of nutritional management by allowing more frequent sampling and automated analysis of milk. For effective application and interpretation of data from the device, we undertook an analysis of several factors affecting the dynamics of milk urea in dairy cows. Analysis of variations among cows indicated that interpretation of milk urea should be based on averages of 10 or more cows in a group.

Based on observed changes in milk urea during milking, milk sampled as soon as possible after ejection of the foremilk would be representative of the entire milking. Consistent diurnal patterns in milk urea were observed, stressing the importance of uniform sampling time when evaluating milk urea. The nitrogen content of the ration was shown to have a strong positive effect on milk urea and no significant effect on yield, demonstrating the importance of balancing the diet. Evidence of heat stress was observed, with a positive effect on milk urea and a negative effect on yield. High nitrogen in the diet and periods of possible heat stress were both associated with an increase in the variation of milk urea in a group of cows, indicating that monitoring variance as well as mean milk urea may improve the ability to identify abnormal conditions at the dairy. Finally, changes in milk urea were observed within 3 days of changes in experimental conditions, suggesting that the ability to track milk urea every day may lead to faster and less ambiguous diagnosis of nutritional problems.

Keywords. Dairy nutrition, Waste nitrogen, Heat stress.

Measurement of systemic levels of urea has long been proposed as a method of ensuring optimal protein nutrition in ruminants (Lewis, 1957). Because it has also been shown that urea equilibrates rapidly between milk and blood in dairy cows (Peskest, 1934), measurement of urea in milk allows a minimally invasive means of monitoring systemic urea levels. Urea is a small molecule (molecular weight 60 Da) synthesized in the liver from ammonia generated by microbial action in the rumen or from the metabolism of amino acids and other organic nitrogen sources in the body. This urea, which is less toxic than ammonia, enters the bloodstream and is concentrated by the kidneys into the urine. Because urea is small and water soluble, it also readily diffuses across mammary tissue into milk.

It has been shown that low urea values are associated with a deficiency in dietary protein for animal growth and milk protein synthesis, that high urea values occur when protein in the diet exceeds levels required for these processes for a given dietary energy level, and that measurement of milk urea may be a useful tool for evaluating the nutritional program of lactating dairy cows (Ide et al., 1966). Research corroborating these observations has been voluminous (Baker et al., 1995; Broderick and Clayton, 1997; DePeters and Ferguson, 1992; Hof et al., 1997; Kaufmann, 1982; Kirchgessner et al., 1986; Kirchgessner and Kaufmann, 1987; Kirchgessner et al., 1988; Kirchgessner and Windisch, 1989; Schepers and Meijer, 1998).

Milk urea is also well correlated with total excretion of urinary nitrogen (Ciszuk and Gebregziabher, 1994; Jonker et al., 1998; Kauffmann and St-Pierre, 1999; Kröber et al., 2000), which has become a serious environmental concern in many areas. Urinary nitrogen may be volatilized as ammonia, or it may be oxidized to various nitrates and contaminate ground and surface water (Loehr, 1974; James et al., 1999). Researchers have reported dramatic reductions in nitrogen emissions from dairy farms by reducing crude protein in the diet (James et al., 1999; Kröber et al., 2000; Paul et al., 1998) and by supplementing feed with limiting essential amino acids (Kröber et al., 2000). Others have suggested that nitrogen excretion be controlled by adjusting the diet according to the observed milk urea values (Jonker et al., 1998). Manipulation of dietary nitrogen may also be beneficial because high systemic urea levels may impair the rate of conception of inseminated dairy cows (Butler et al., 1996; Larson et al., 1997), and at extremely high levels urea

Article was submitted for review in March 2001; approved for publication by the Biological Engineering Division of ASAE in January 2002.

The authors are **Daniel M. Jenkins, ASAE Member**, Assistant Professor, Molecular Biosciences and Bioengineering, University of Hawaii, Honolulu, Hawaii; **Michael J. Delwiche, ASAE Member Engineer**, Professor, Biological and Agricultural Engineering; **Edward J. DePeters**, Professor, Animal Science; and **Robert H. BonDurant**, Chairperson, Population Health and Reproduction, Veterinary Medicine, University of California at Davis, Davis, California. **Corresponding author:** Michael J. Delwiche, 3048 Bainer Hall, Biological and Agricultural Engineering, University of California at Davis, Davis, CA 95616; phone: 530-752-7023; fax: 530-752-2640; e-mail: mjdelwiche@ucdavis.edu.

has been linked to sickness, internal hemorrhaging, and even death (Hoque and Dey, 1998; Hwang et al., 1996).

As these concerns become more prominent in dairying, testing of milk urea has become more routine. Typically, samples are taken weekly or monthly from the dairy for urea analysis, and the results are used to adjust the herd diet to conserve feed costs and prevent adverse effects of excess nitrogen on herd health and the environment. Levels of milk urea nitrogen (MUN), measured as nitrogen in urea, usually range from 10 to 16 mg/dl (Jonker et al., 1998; Kohn, 2000), and values in excess of 20 mg/dl are considered abnormal.

Because milk urea is dependent on a number of factors and is subject to change over short periods of time, it was thought that the implementation of an on-line urea sensor in the dairy might improve the quality of information provided to the dairy nutritionist. Real-time analysis of milk urea on demand may improve the ability to discriminate the causes of change in milk urea. Furthermore, automated analysis may eliminate the expense and error associated with sampling, storing, transport, and remote analysis of milk. An on-line sensor for urea was developed for use in the dairy parlor (Jenkins, 2001). For successful application of such a device in the field, however, typical sources of variation in milk urea and the typical transient response to changes in external conditions must be understood.

LITERATURE REVIEW

In practice, some controversy exists with regard to the interpretation of observed MUN values. Variations over time and among individual cows can be large, so there is often confusion over the meaning of observed MUN. In general, higher yielding cows have been shown to have slightly higher levels of MUN, possibly because they are more likely to metabolize body proteins due to an energy deficit (Broderick and Clayton, 1997; Carlsson et al., 1995; Godden et al., 2001). MUN levels have been observed to change during a lactation period (Carlsson et al., 1995; Godden et al., 2001) and over subsequent lactations (Broderick and Clayton, 1997; Godden et al., 2001). Values of MUN are generally higher for grazing animals than for those fed a balanced mixed ration (Carlsson et al., 1995), usually due to a higher proportion of crude protein in the forage. Reports are inconsistent with respect to the effects of age and breed on MUN (Carlsson et al., 1995), as are reports on the effects of microbial infection in the udder (DePeters and Ferguson, 1992; Gustafsson and Palmquist, 1993).

Temporal variations in milk urea are more pronounced and consistent than those due to age, breed, parity, and yield. Cows fed at discrete intervals through the day show consistent peaks in ruminal ammonia within hours of feeding, followed by peaks in blood urea and then MUN (Broderick and Clayton, 1997; Carlsson and Bergström, 1994; Carlsson et al., 1995; Gustafsson and Palmquist, 1993; Rodriguez et al., 1997). These findings demonstrate the importance of standardizing the time of milk sampling relative to feeding for MUN analysis. Variation in MUN over the course of a single milking has been shown to be primarily due to displacement of water and water-soluble components by the higher fat content in later fractions (Carlsson and

Bergström, 1994; Peskett, 1934). This suggests that the most consistent results should be obtained when milk is sampled early in the milking, when the milk fat content is the lowest. Seasonal changes in MUN have also been recorded, especially when cows are grazed for part of the year (Wolfschoon-Pombo and Klostermeyer, 1982). Consequently, seasonal changes in conditions and herd management should be taken into consideration when evaluating MUN data.

For interpretation of data from an on-line sensor in the field, it is important to understand how quickly MUN changes when the diet is altered. Changes in MUN in response to a high-protein diet were characterized as immediate when milk was sampled for analysis twice weekly (Kirchgesner et al., 1988). When the diets were returned to normal four weeks later, MUN returned to normal levels just as quickly, with no residual effects. Oltner and Wiktorsson (1983) showed the transient response of MUN in cows experiencing a number of transitions in diets composed of varying protein and energy levels. They took composite samples at each milking (twice daily), and equilibrium in MUN was reached within about two or three days after the transition in all cases. Cows undergoing a short-term fast (24 h) showed elevated MUN levels, presumably as body protein was mobilized to meet the energy requirements of the animals (Dehareng et al., 1996). These experiments show that MUN response to abnormal dietary conditions is quick, and diagnosis of these problems should be unambiguous in a well-managed herd if MUN is analyzed on a daily basis.

A rational strategy for MUN analysis must take into consideration all of the sources of variation in the herd. Sampling time should be consistent and should take into account the feeding schedule of the animals. The sample should be taken during milking at a time when the MUN is representative of the whole milking and interfering components are minimized. Because much variation in MUN among individuals remains unexplained, dairy nutritionists recommend that MUN values for a given production string be estimated by averaging the individual values from at least 10 cows (Broderick and Clayton, 1997; Ferguson, 1996; Kohn, 2000). The frequency of MUN analysis should ideally be high enough to recognize the first sign of significant changes in the diet; current practices of weekly and monthly spot-checking may be insufficient for today's proactive farmer who is constantly adjusting the management of the herd, including its diet. Finally, the system should be intelligent enough to distinguish random fluctuations in MUN from those due to a change in the conditions and management of the dairy.

OBJECTIVES

The objective of this research was to analyze the dynamics of MUN in a herd of dairy cows in order to be able to intelligently apply and interpret data from a real-time urea sensor. Several factors were identified as being critical for this purpose. Determination of typical variations in MUN among cows, during a single milking, and over the course of a day was important in deciding how to apply and interpret results from an on-line sensor. Determination of the duration of a typical transient response to a change in the diet was undertaken in order to be able to interpret observed changes in MUN.

EXPERIMENTAL METHODS

DETERMINATION OF MUN VARIATIONS AMONG COWS AND AMONG MILK FRACTIONS

To find MUN variations among cows and during a single milking of individual cows, samples of foremilk, post-milking strippings, and milk composited from the entire milking were taken once daily from the morning milking of eight randomly selected mid-production Holstein cows over four consecutive days. These milk samples were then tested for MUN using the on-line sensor (Jenkins, 2001) and an autoanalyzer (Technicon Corp. Series 2 Autoanalyzer, method N-1C, Tarrytown, N.Y.; Marsh et al., 1957). Two-way analysis of variance (ANOVA) with interactions (Neter et al., 1996, pp. 849–865), using individual cows and milk fraction collected as factors, was performed on the data to determine the significance of variations among cows and variations due to the milk fraction collected.

Different fractions of milk were collected and analyzed in order to identify a time during milking when milk urea is most representative of that in the composite milk. Milk was collected during the morning milkings of three randomly selected mid-production Holstein cows over a period of two days. These were collected manually by a sampling device (Westfalia Separator, Inc., WS 7161–2457–020, Oelde, Germany) during machine milking, noting the start and end weight of the milk fraction from which the sample was taken. For purposes of comparison, observed MUN for each fraction was scaled to the average MUN for the milking, and each fraction was normalized to a value between 0, corresponding to foremilk, and 1.0, corresponding to post-milking strippings.

Because a good correlation existed for the MUN evaluated by the two different sensors in these experiments (fig. 1), these data were analyzed using the results from the on-line sensor in order to show its ability to discriminate significant differences in MUN.

TRANSIENT RESPONSE TO CHANGE IN DIET

To determine how quickly MUN changes in response to a change in dietary nitrogen concentration, a crossover experiment was performed on two groups of cows with two diets. Each test group was composed of six multiparous Holstein

cows in mid to late lactation, with an average milk yield between 40 and 45 kg/day. All cows were fed a balanced diet for the duration of the trial (table 1), and granular urea was alternately supplemented into the diets of the two groups. The bulk of the mixed ration was fed during three equal meals throughout the day, once immediately after the morning milking, once at about noon, and once after the evening milking. An allotment of unchopped alfalfa hay was fed during the night to provide roughage for cows experiencing digestive trouble with the concentrate fed during the main meals (table 1). Urea was mixed in the ration fed to the pen containing the cows receiving the supplement to give about 38 g urea per cow in each of the three main meals. All of the cows in the first test group were housed in a pen with a total of 48 cows (pen 1), and the cows in the second test group were housed in a pen with a total of 24 cows (pen 2). Pen 2 was partitioned to be half of the space of a pen that was otherwise identical to pen 1.

Initially, both groups of cows started off on the baseline diet with no urea supplement. The trial lasted for 14 days, which were enumerated from 0 (20 August 2000) to 13 (2 September 2000). Pen 2 was fed the urea supplement starting on the noon feeding of day 1 through the morning feeding of day 5, and then from the evening feeding of day 9 through the evening feeding of day 12 (period 2). Pen 1 received the urea supplement starting on the noon feeding of day 5 through the noon feeding of day 9 (period 1).

Composite milk samples from all cows were collected for each milking starting on the evening milking of day 0 through the morning milking of day 2 to ensure that pen 2 cows were responding as expected to the urea supplement. Composite milk samples were then taken for all cows at each milking from the evening of day 4 through the morning of day 13 in order to observe the responses in MUN to each transition in diet. Milk yields were recorded for each sampled milking, and the samples collected were analyzed for MUN in the lab by the on-line sensor and by the autoanalyzer. All samples collected on the evening milkings of days 7 and 11 and on the morning milkings of days 8 and 12 were also analyzed by infrared spectroscopy (DairyLab I, Foss Electric, Hillerød, Denmark) for fat, protein, lactose, and solids-non-fat (SNF) in order to determine if the changes in diet were associated with changes in the main milk components. Feed samples from each pen were taken for chemical analysis on the

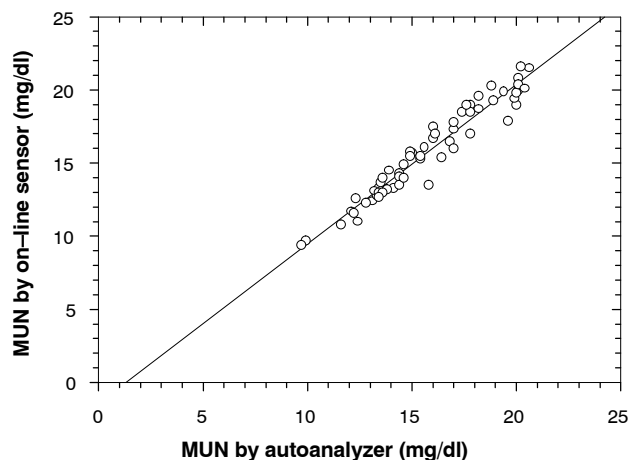


Figure 1. Correlation of milk urea nitrogen (MUN) data obtained from two sensors during the experiments to determine effects of cow and milk fraction on MUN ($Y = 1.09 X - 1.41$; $R^2 = 0.940$; standard error = 0.72 mg/dl).

Table 1. Composition of daily ration fed to cows in transient MUN response experiment.

Ration	Quantity (kg/day per cow)
Concentrate (total)	12.3
Corn, steam flaked	5.9
Barley, rolled	2.9
Soybean meal	1.7
Beet pulp	1.45
Tallow	0.31
Alfalfa hay (1.2 unchopped, fed at night)	9.1
Whole cotton seed	3.2
Almond hulls	2.3
Mineral/buffer	0.45
Energy II (Ca salts of long-chain fatty acids)	0.23
Water	10.3

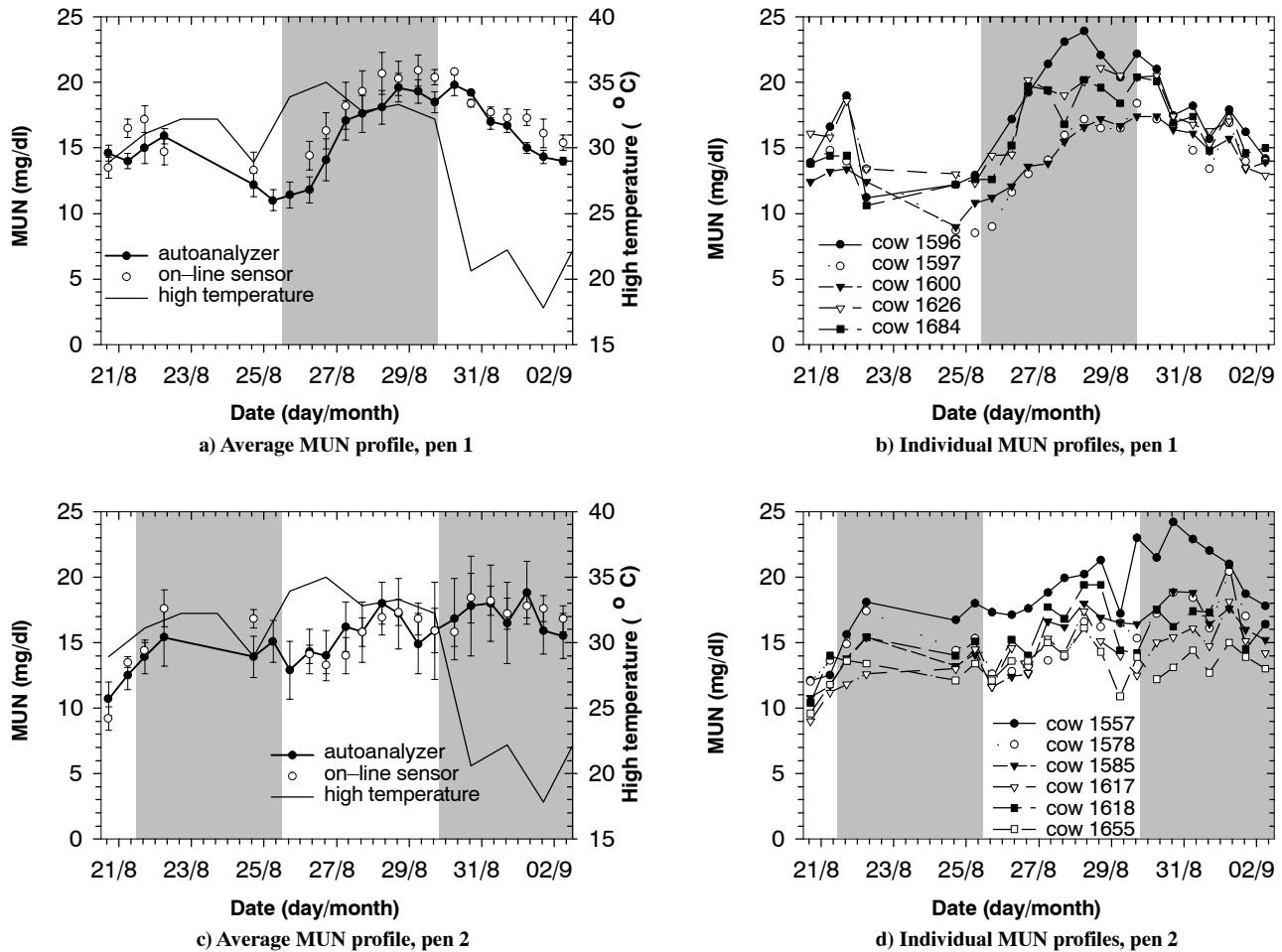


Figure 2. Profiles of milk urea nitrogen (MUN) observed during MUN transient experiments. Shaded areas represent periods of urea supplementation in the diet. Data in (b) and (d) were recorded by autoanalyzer.

evening feedings of days 7 and 11 and on the morning feedings of days 8 and 12.

ANALYSIS OF FEED COMPOSITION

Feed samples collected were immediately stored in a freezer until analysis. Samples were analyzed for % dry matter, % ash, % nitrogen, % ether extract, % acid detergent fiber (ADF), and % neutral detergent fiber (NDF). Dry matter was the mass of material left after oven drying; ash was the material (minerals and other solids) left after combustion of the organic matter; nitrogen was the total nitrogen as determined by the Kjeldahl method; ether extract was the fat-soluble material in the sample; ADF was the material not soluble in acid detergent; NDF was the material not soluble in neutral detergent. Ash, nitrogen, and ether extract were determined as prescribed by the Association of Official Analytical Chemists (AOAC, 1990), NDF was determined as prescribed by Van Soest et al. (1991), and ADF was determined as prescribed by Robertson and Van Soest (1981, pp. 123–130).

STATISTICAL EVALUATION OF TRANSIENT EVENTS

Before the experiment it was assumed that, at equilibrium, MUN would vary by cow, milking time, and diet. Observation of the collected data (fig. 2) suggested an effect of the trial periods (period 1 from noon on 25 August, or day 5,

through the evening milking of 29 August, or day 9, and period 2 from the end of period 1 through the morning milking of 2 September, or day 13). For the purposes of statistical analysis, the equilibrium level of MUN was modeled as:

$$\bar{U}_{ijkl} = \mu + \alpha_i + \beta_j + \gamma_k + \delta_l \quad (1)$$

where

- μ = overall mean
- α_i = effect of cow i
- β_j = effect of milking time j
- γ_k = effect of diet k
- δ_l = effect of trial period l

\bar{U}_{ijkl} = mean level of urea for the given effects.

For a different combination of trial period (l') and diet (k'), the model could be represented as:

$$\bar{U}_{ijk'l'} = \mu + \alpha_i + \beta_j + \gamma_{k'} + \delta_{l'} \quad (2)$$

Assuming a first-order rate of change, a transition from the second mean level to the first mean level could be mathematically described by:

$$\bar{U}_{ijklm} = \mu + \alpha_i + \beta_j + \gamma_k + \delta_l + [(\gamma_{k'} - \gamma_k) + (\delta_{l'} - \delta_l)](e^{-t/\tau})_m \quad (3)$$

where

- τ = time constant of the transition

\bar{U}_{ijklm} = mean level of urea at time t after the transition.

In the format in which the experiment was conducted, the values of k' and l' (levels of diet and period before the transition) were dependent on the values of k and l (levels of diet and period after the transition). Therefore, the term in equation 3 in square brackets could be represented as an interaction term between the effects γ_k and δ_l . Assuming an independent, normally distributed error ε_{ijklm} of the observations U_{ijklm} about \bar{U}_{ijklm} , the full model of the system could be represented as:

$$U_{ijklm} = \mu + \alpha_i + \beta_j + \gamma_k + \delta_l + \gamma\delta_{kl}(e^{-t/\tau})_m + \varepsilon_{ijklm} \quad (4)$$

Analyses of covariance (ANCOVA; Neter et al., 1996, pp. 1010–1094) using this model were performed on the observed data to determine the effects of individual cows, milking time (morning or evening), diet (high or normal nitrogen content), and trial period (1 or 2) on MUN and milk yield. Because no simple analytical method was available to fit a value of τ to the data, the value of τ was assumed to be 36 hrs, based on inspection of graphical data from these experiments (fig. 2) and from Oltner and Wiktorsson (1983). Iterative methods to determine a better fitting τ were not used because the small range of possible τ from the graphical estimates (about 30 to 42 hrs) was less than the typical interval between sampling (1 day).

Additional tests were performed on the collected data in order to determine other effects of the changing experimental conditions. To test experimental effects on individual cow variation, standard deviations at the group level for each pen were calculated for MUN and yield at each milking, and two-way ANOVA with interactions were performed on both of these data sets using diet (high or normal nitrogen) and period (1 or 2) as factors. Observed milk fat, protein, lactose, and SNF were grouped by trial period and averaged for each cow. Paired t -tests (paired by cow; Ryan and Joiner, 1994, pp. 233–234) were then performed on these data comparing the effect of trial period (1 or 2). Similar t -tests were performed grouping by diet instead of period to compare the effects of diet (high or normal nitrogen).

All statistical tests shown for the MUN transient response experiment were evaluated using data from the autoanalyzer due to malfunctioning of the on-line sensor during portions of that experiment (fig. 3). Statistical significance for all tests was evaluated at the 5% level.

RESULTS AND DISCUSSION

MUN VARIATIONS BY COW AND MILK FRACTION

The ANOVA for effects of individual cow and milk fraction collected showed that both factors had a significant effect on MUN value (table 2). The same statistical conclusions could be drawn by performing the analysis with data from the on-line sensor or with data from the autoanalyzer. While individual cows showed reasonably stable MUN values, variations among cows suggest that decisions regarding herd nutrition should not be based on individual cow data. The lack of interaction between factors ($p = 0.232$) also suggested that the relative MUN changes during milking were consistent among cows. Given the observed standard deviation in composite milk MUN for individual cows (3.2 mg/dl), application of the convergence

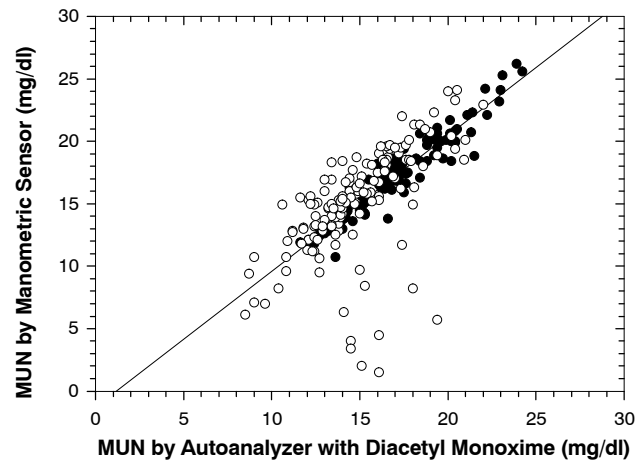


Figure 3. Correlation of milk urea nitrogen (MUN) data obtained from two sensors during the MUN transient response experiment. Open circles represent data taken during suspected malfunctioning of on-line sensor. Correlation of remaining data (solid circles) is similar to that of previous comparisons ($Y = 1.09 X - 1.26$; $R^2 = 0.911$; standard error = 0.99 mg/dl).

Table 2. Analysis of variance (ANOVA) of milk urea nitrogen (MUN) by cow and milk fraction, using MUN data from on-line sensor (ANOVA using MUN data from autoanalyzer is similar).

Source	DF	SS	MS	F	p
Cow ID	7	757.4	108.2	35.5	<0.001
Fraction	2	148.0	74.0	24.3	<0.001
Interaction	14	55.3	4.0	1.3	0.232
Error	72	219.7	3.1		
Total	95	1180.3			

theorem to estimate the true population mean (Neter et. al., 1996, p. 1323) suggests that at least 10 cows should be sampled for the estimate to have an accuracy of ± 1 mg/dl. This is consistent with current practices, which suggest sampling from at least 10 cows or from the bulk tank (DePeters and Ferguson, 1992; Ferguson, 1996; Kohn, 2000).

Plotting normalized MUN value against normalized fraction collected (fig. 4) showed that MUN decreased by a slight but statistically significant amount as milking progressed. Poststripped milk showed a more dramatic decrease in MUN. These effects were probably due to higher milk fat later in the milking displacing water in which urea was dissolved, as was observed by Carlsson and Bergström (1994) and Peskett (1934). Foremilk samples, shown as fraction 0, also had slightly lower MUN values than the composite values. We suspect that this was due to changes in blood urea overnight as the milk already in the gland and teat cisterns at the beginning of milking was not in as close contact with the blood as was the milk ejected during full let down. Because of this, the relative value of MUN in the foremilk compared to MUN in the composite milk may change depending on the milking time, particularly in relation to the feeding schedule. This lag in foremilk MUN is also evident in the data of Gustafsson and Palmquist (1993). Considering these observed variations in MUN during milking, sampling for MUN analysis should be done early in the milking, but after removal of the foremilk. The resulting sample should be representative of the composite milk and relatively low in fat content. Sampling early in the milking also ensures the collection of a sample if there is a large variation in milk yield.

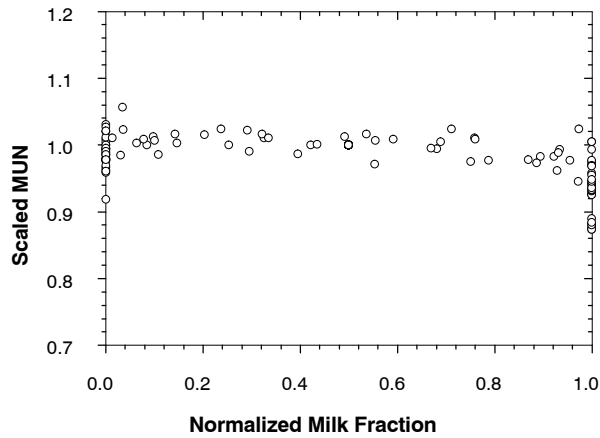


Figure 4. Observed changes in milk urea nitrogen (MUN) during milking, using data from autoanalyzer. MUN is scaled to the average MUN value for the milking. Slope of least squares regression line excluding fractions 0 and 1 ($Y = -0.0312X + 1.0148$; $R^2 = 0.267$) is slight but statistically significant at the 5% level.

Table 3. Analysis of covariance of milk urea nitrogen (MUN) recorded by autoanalyzer, modeled by equation 4.

Source	DF	SS	MS	F	p
Cow ID (α)	10	659.3	65.9	27.8	<0.0001
Milking time (β)	1	15.5	15.5	6.5	0.0114
Trial period (γ)	1	153.6	153.6	64.7	<0.0001
Diet (δ)	1	194.8	194.8	82.0	<0.0001
Time ($\gamma\delta$)	4	582.8	145.7	61.35	<0.0001
Error	179	425.2	2.4		
Total	196	1768.0			

TRANSIENT RESPONSE TO STEP CHANGES IN DIET

The observed profiles of MUN did not all show the expected effects of the changes in diet (fig. 2). As was discussed in the methods section, the different periods during the experiment also seemed to have an effect on MUN. The most readily apparent difference in conditions recorded during these two periods was daily temperature (also plotted in fig. 2), which was relatively high during the first period (highs ranging from 30° C to 35° C) and moderate during the second period (highs below 25° C).

Application of the model described in equation 4, using MUN data from the autoanalyzer, showed a significant effect on MUN of all the factors evaluated (table 3). With the exception of milking time, the same effects were determined to be significant using the data from the on-line sensor. The variation of MUN among cows is already well established, and a change in MUN with milking time is a normal consequence of diurnal variations. Likewise, detected differences in MUN due to diet were entirely expected. The significance of the time effects lends some credibility to the simple model of transition effects, and the unexpected significance of the effect of period suggests the presence of an uncontrolled experimental variable.

Application of the same model to the yield data (table 4) showed that all effects were significant except diet. This provides further evidence that increasing the nitrogen content of an otherwise balanced diet does not affect yield, although it increases waste nitrogen. As in MUN, variations in yield among cows are well documented and expected. Differences in yield between the morning and evening

Table 4. Analysis of covariance of milk yield, modeled by equation 4.

Source	DF	SS	MS	F	p
Cow ID (α)	10	1451.3	145.1	27.2	<0.0001
Milking time (β)	1	324.9	324.9	61.0	<0.0001
Trial period (γ)	1	22.1	22.1	4.1	0.0431
Diet (δ)	1	15.8	15.8	3.0	0.0868
Time ($\gamma\delta$)	4	76.1	19.0	3.6	0.0079
Error	180	958.6	5.3		
Total	197	2972.9			

Table 5. Selected estimates of parameters affecting milk urea nitrogen and milk yield, as determined by analysis of covariance.

Effect	Δ MUN ^[a] (mg/dl)	Δ Milk Yield ^[b] (kg/milking)
AM milking compared to PM	0.6	2.6
High-nitrogen (N) diet compared to normal	3.3	0.9 ^[c]
Period 1 (high temperatures) compared to 2	3.0	-1.1
(Period 1 \times high-N diet) $e^{-t/\tau}$	-9.0	1.7
(Period 1 \times normal-N diet) $e^{-t/\tau}$	-3.0	2.8
(Period 2 \times high-N diet) $e^{-t/\tau}$	-0.1 ^[c]	-0.8 ^[c]
(Period 2 \times normal-N diet) $e^{-t/\tau}$	6.4	-2.0

^[a] Average MUN during experiment was 16.4 mg/dl.

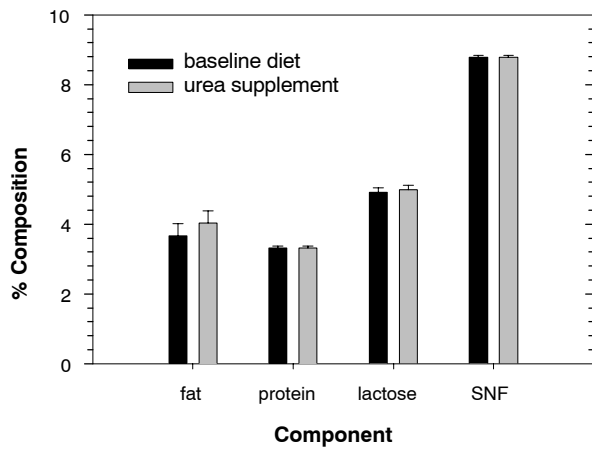
^[b] Average milk yield during experiment was 21.1 kg/milking.

^[c] Indicates no significance at the 5% level.

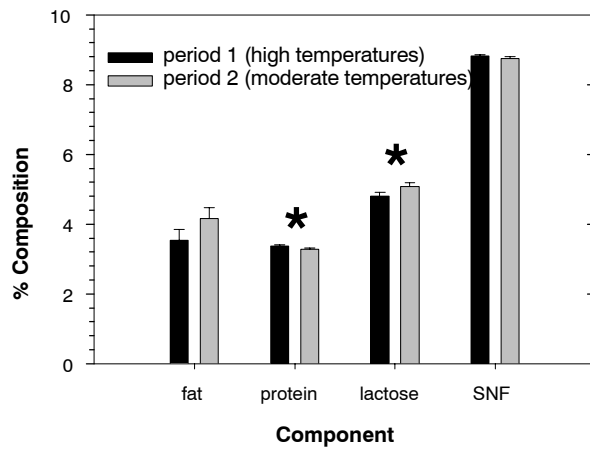
milking were also expected due to an asymmetry in the milking times (morning milkings were started at approximately 0400, and evening milkings were started at approximately 1500). The effect of the different periods again suggested an effect of an uncontrolled experimental variable.

Physiological and behavioral changes in cows due to heat stress are common phenomena known to dairy farmers. Many of these changes occur as the cows decrease their dry matter intake in order to maintain thermal equilibrium (Collier et al., 1982). The resulting caloric and nutrient deficits cause a drop in milk yield (Collier et al., 1982; Maltz et al., 2000) and may result in an increase in systemic urea levels if body protein is metabolized to maintain vital functions. The data observed in this experiment suggest an unintended effect of different levels of heat stress during the different trial periods of the experiment. The estimated effects of the different factors on MUN and yield (table 5) show that the average positive effects of the high-nitrogen diet and high-temperature period on MUN were both about 3 mg/dl, and that during the period of high temperature the milk yields diminished by about 1 kg per milking. Analysis of differences in other milk components due to changes in the diet or trial period (fig. 5) showed that diet had no detectable effect on any measured component, but the protein content was higher and lactose content lower in milk sampled during the period of higher temperatures. This may also have been an effect of heat stress.

The estimated interactions between the factors of trial period, diet, and time (table 5) lend further validity to the model of equation 4. The interaction terms with period 2 sum almost perfectly to the main effects terms from period 1 and the diets corresponding to the different groups during period 1, just as assumed in the mathematical relationship of equation 4 to equation 3. The interaction terms with period 1 do not sum so neatly to the main effects of the model because they depend on the main effects from the period preceding the experiment, which were not recorded. The mathematical implications of the interaction terms also suggest that



a) Milk composition by urea in the diet



b) Milk composition by test period

Figure 5. Comparisons of mean observed milk components (SNF = solids non-fat; statistical difference at the 5% level as determined by t-test paired by cow is denoted by “*”).

Table 6. Selected estimates of parameters affecting the group standard deviation (sd) of milk urea nitrogen and milk yield, as determined by two-way analysis of variance.

Effect	Δ sd _{MUN} ^[a] (mg/dl)	Δ sd _{yield} ^[b] (kg/milking)
High-nitrogen diet	1.63	0.31 ^[c]
Period 1 (high temperatures)	1.21	0.43 ^[c]

[a] Average standard deviation in group MUN at each milking during experiment was 2.20 mg/dl.

[b] Average standard deviation in group milk yield at each milking during experiment was 3.50 kg/milking.

[c] Indicates no significance at the 5% level.

Table 7. Chemical analysis (% dry matter basis) of ration fed to cows during the transient MUN response experiment.

Sample Description	Ash (%)	Nitrogen (%)	Ether Extract (%)	ADF (%)	NDF (%)
Pen 1, period 1, AM sample	6.55	3.05 ^[a]	5.81	22.90	33.90
Pen 2, period 1, AM sample	6.80	2.72	6.42	21.45	33.48
Pen 1, period 1, PM sample	6.94	3.19 ^[a]	6.29	19.63	30.08
Pen 2, period 1, PM sample	6.99	3.02	6.00	22.15	34.57
Pen 1, period 2, AM sample	6.62	3.05	5.99	19.76	31.27
Pen 2, period 2, AM sample	6.71	2.86 ^[a]	5.53	22.48	32.61
Pen 1, period 2, PM sample	6.48	2.96	5.93	20.13	32.09
Pen 2, period 2, PM sample	6.57	3.27 ^[a]	5.65	21.24	33.19

[a] Indicates ration supplemented with urea.

modeling the transition of MUN after a change in dietary or other conditions as a first-order process with a time constant of 36 hours reflects the true transition reasonably well. Under these assumptions, the time to reach 90% equilibrium is about 3 days.

The estimation of the effects of diet and trial period, as determined by two-way ANOVA, on group standard deviation of MUN and yield is summarized in table 6. Increasing urea in the diet and increasing temperatures were both associated with increases in the standard deviation of MUN observed at the group level, suggesting that changes in group standard deviation as well as group mean in MUN may be used to identify significant changes in the dairy. The observed changes in variation may have been caused by differences in physiological or behavioral responses to the different stimuli among cows. For example, some animals

may have been more selective about eating parts of the mixed ration supplemented with urea, particularly if the ration was not well mixed. Further evidence of non-uniform experimental conditions was apparent in the chemical analysis of sample feeds taken during the experiment (table 7). The nitrogen content of the diet appeared particularly susceptible to daily variation, and the diets supplemented with urea did not always show higher nitrogen content than those not supplemented. These factors and other inconsistent management practices may have contributed to the error terms observed in the ANCOVA model (tables 3 and 4).

Profiles of MUN for individual cows also suggested some unexpected effects. Cow 1667 was accidentally switched from pen 1 to pen 2 before the noon feeding on day 8 of the experiment, so that she experienced a unique dietary treatment. Because of this, data collected for her were not included in the preceding analyses. Even so, her MUN levels increased in response to the high-urea diet and period of high temperatures. However, her MUN values continued increasing after being put in a new pen, even after the temperatures cooled off. A coincident drop in milk yield (fig. 6) suggests that she may have experienced other stresses in the new pen. For example, her dry matter intake may have been suppressed if she was pushed away from the manger by other cows while adjusting to the new social hierarchy.

CONCLUSIONS

Some simple trials using the automated sensor and analysis of data from the literature suggest that real-time analysis of milk urea can improve the understanding of the nitrogen balance of dairy cows. Consideration of the typical variations in milk urea and the transient responses of milk urea to changes in the diet showed that nutritional management could be improved by on-line measurement of milk urea every day as opposed to every week or month. Because of the high variation in individual milk urea, current suggestions to base nutritional management decisions on the average of milk urea values from 10 or more individuals in a production string were shown to be prudent. Observed changes in milk urea during milking, partly due to the increase in fat content in later fractions and partly due to

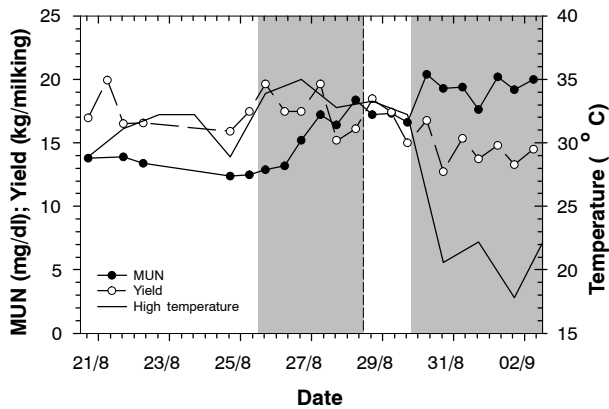


Figure 6. Milk urea nitrogen (MUN, by autoanalyzer) and milk yield profiles of cow 1667. Shaded areas represent periods of urea supplementation in the diet, and dashed vertical line represents time at which cow was switched from pen 1 to pen 2.

imperfect mass transfer of urea between milk secreted at different times during the day, suggested that milk for analysis of urea would best be sampled as soon as possible after removal of the foremilk.

A model including the transient effects of a change in experimental conditions fit observed data well, and showed that MUN was positively affected by nitrogen content in the diet and an uncontrolled effect that was presumed to be heat stress. The model also showed that equilibrium in MUN was effectively reached after a transition in experimental conditions within about 3 days. This suggests that there is considerable room for improvement in nutritional management by frequent, on-line analysis of milk urea. Dependence of observed group variation in MUN on diet and temperature suggested that variation in observed MUN data, as well as mean MUN data, can be used to identify abnormal conditions at the dairy.

FUTURE WORK

This research was carried out to determine the dynamics of how milk urea changes in response to several different factors important for dairy nutrition. In the future, this information could be useful for developing an intelligent system to make suggestions regarding herd nutrition based on observed MUN profiles and other conditions recorded at the dairy. A detailed economic analysis of the costs and possible benefits of this technology should be undertaken. The economic analysis should be sophisticated enough to determine the likely costs and benefits from management decisions regarding herd nutrition.

Failure of the on-line sensor during parts of this research illustrates other practical problems with on-line sensing. Failures in this case were mostly due to milk solids precipitating and obstructing the plumbing of the sensor. To improve the reliability of the sensor, modifications need to be made to prevent this and other possible failures from occurring. Other measures should also be taken to identify grossly inaccurate data recorded during sensor failure. These may include on-board diagnostic sensors to identify problems such as abnormal pressures during reagent pumping,

and regular comparison of milk urea values analyzed with the on-line sensor to those measured by other means.

ACKNOWLEDGEMENTS

Thanks to Scott Taylor and Jenni Pareas for performing many of the chemical analyses reported here. This work was partially funded by USDA/BARD Research Project US-2638-95.

REFERENCES

- AOAC. 1990. *Official Methods of Analysis*. 15th ed. Arlington, Va.: Association of Official Analytical Chemists, International.
- Baker, L. D., J. D. Ferguson, and W. Chalupa. 1995. Responses in urea and true protein of milk to different feeding schemes for dairy cows. *J. Dairy Science* 78(11): 2424-2434.
- Broderick, G. A., and M. K. Clayton. 1997. A statistical evaluation of animal and nutritional factors influencing concentrations of milk urea nitrogen. *J. Dairy Science* 80(11): 2964-2971.
- Butler, W. R., J. J. Calaman, and S. W. Beam. 1996. Plasma and milk urea nitrogen in relation to pregnancy rate in lactating dairy cows. *J. Dairy Science* 74(4): 858-865.
- Carlsson, J., and J. Bergström. 1994. The diurnal variation of urea in cow's milk and how milk fat content, storage, and preservation affects analysis by a flow injection technique. *Acta Veterinaria Scandinavica* 35(1): 67-77.
- Carlsson, J., J. Bergström, and B. Pehrson. 1995. Variations in breed, age, season, yield, stage of lactation, and herd in the concentration of urea in bulk milk and individual cows' milk. *Acta Veterinaria Scandinavica* 36(2): 245-254.
- Ciszek, P., and T. Gebregziabher. 1994. Milk urea as an estimate of urine nitrogen of dairy cows and goats. *Acta Agriculturae Scandinavica* (Section A, Animal Science) 44(2): 87-95.
- Collier, R. J., D. K. Beede, W. W. Thatcher, L. A. Israel, and C. J. Wilcox. 1982. Influences of environment and its modification on dairy animal health and production. *J. Dairy Science* 65(11): 2213-2227.
- Dehareng, D., B. B. Ndibualonji, and J. M. Godeau. 1996. Continuous profiles of ruminal ammonia and plasma urea in dry Friesian cows on hay-based rations. *J. Animal Physiology and Animal Nutrition* 75(2): 57-72.
- DePeters, E. J., and J. D. Ferguson. 1992. Non-protein nitrogen and protein distribution in the milk of cows. *J. Dairy Science* 75(11): 3192-3209.
- Ferguson, J. D. 1996. Milk urea nitrogen. *California Dairy Herd Improvement* 5(6): 18-19.
- Godden, S. M., K. D. Lissimore, D. F. Kelton, K. E. Leslie, J. S. Walton, and J. H. Lumsden. 2001. Factors associated with milk urea concentrations in Ontario dairy cows. *J. Dairy Science* 84(1): 107-114.
- Gustafsson, A. H., and D. L. Palmquist. 1993. Diurnal variation of rumen ammonia, serum urea, and milk urea in cows at high and low yields. *J. Dairy Science* 76(2): 475-484.
- Hof, G., M. D. Vervoorn, P. J. Lenaers, and S. Tamminga. 1997. Milk urea nitrogen as a tool to monitor the protein nutrition of dairy cows. *J. Dairy Science* 80(12): 3333-3340.
- Hoque, M., and S. Dey. 1998. Management of urea poisoning in a heifer. *Indian Veterinary J.* 75(3): 279-280.
- Hwang, E. K., G. S. Chung, J. H. Kim, Y. C. Bae, H. J. Sohn, and S. H. Choi. 1996. A case report of urea poisoning in dairy cattle in Korea. *RDA J. Agric. Science Veterinary* 38(1): 817-824.
- Ide, Y., K. Shimbayashi, and T. Yonemura. 1966. Effect of dietary conditions upon serum and milk-urea nitrogen in cows. *Japanese J. Veterinary Science* 28(6): 321-327.
- James, T., D. Meyer, E. Esparza, E. J. DePeters, and H. Pérez-Monti. 1999. Effects of dietary nitrogen manipulation on

- ammonia volatilization from manure from Holstein heifers. *J. Dairy Science* 82(11): 2430–2439.
- Jenkins, D. M. 2001. Design and use of a manometric sensor to measure urea in milk for improvement of dairy cow nutritional management. PhD diss. Davis, Calif.: University of California at Davis.
- Jonker, J. S., R. A. Kohn, and R. A. Erdman. 1998. Using milk urea nitrogen to predict nitrogen excretion and utilization in lactating dairy cows. *J. Dairy Science* 81(10): 2681–2692.
- Kauffman, A. J., and N. R. St-Pierre. 1999. Effect of breed and concentrations of dietary crude fiber on milk urea nitrogen. In *1999 Research and Reviews*, 60–65. Columbus, Ohio: Ohio State University, Dept. of Animal Science.
- Kaufmann, Von W. 1982. Variation in der Zusammensetzung des Rohstoffes Milch unter besonderer Berücksichtigung des Harnstoffgehaltes. *Milchwissenschaft* 37(1): 6–9.
- Kirchgessner, M., and T. E. G. Kaufmann. 1987. Harnstoff und Allantoin in der Milch von Kühen während und nach energetischer Überversorgung. *J. Animal Physiology and Animal Nutrition* 58(3): 147–156.
- Kirchgessner, M., and W. Windisch. 1989. Harnstoffgehalt der Milch und Allantoinausscheidung von Kühen während und nach Energie- und Proteinmangel. *J. Animal Physiology and Animal Nutrition* 62(3): 112–118.
- Kirchgessner, M., M. Kreuzer, and D. A. Roth-Maier. 1986. Milk urea and protein content to diagnose energy and protein malnutrition of dairy cows. *Archives of Animal Nutrition* 36(2–3): 192–197.
- Kirchgessner, M., B. R. Paulicks, and F. J. Schwarz. 1988. Veränderungen im Harnstoffgehalt der Kuhmilch bei unzureichender und überhöhter Proteinversorgung. *J. Animal Physiology and Animal Nutrition* 59(2): 79–84.
- Kohn, R. 2000. Caution needed when interpreting MUNs. *Hoard's Dairyman* 145(2): 58.
- Kröber, T. F., D. R. Külling, H. Menzi, F. Sutter, and M. Kreuzer. 2000. Quantitative effects of feed protein reduction and methionine on nitrogen use by cows and nitrogen emission from slurry. *J. Dairy Science* 83(12): 2941–2951.
- Larson, S. F., W. R. Butler, and W. B. Currie. 1997. Reduced fertility associated with low progesterone postbreeding and increased milk urea nitrogen in lactating dairy cows. *J. Dairy Science* 80(7): 1288–1295.
- Lewis, D. 1957. Blood-urea concentration in relation to protein utilization in the ruminant. *J. Agric. Science* 48(4): 438–446.
- Loehr, R. C. 1974. *Agricultural Waste Management: Problems, Processes, and Approaches*. New York, N.Y.: Academic Press.
- Maltz, E., O. Kroll, H. Barash, A. Shamy, and N. Silanikove. 2000. Lactation and body weight of dairy cows: Interrelationships among heat stress, calving season, and milk yield. *J. Animal and Feed Sciences* 9(1): 33–45.
- Neter, J., M. H. Kutner, C. J. Nachtsheim, and W. Wasserman. 1996. *Applied Linear Statistical Models*. 4th ed. Chicago, Ill.: Irwin.
- Oltner, R., and H. Wiktorsson. 1983. Urea concentrations in milk and blood as influenced by feeding varying amounts of protein and energy to dairy cows. *Livestock Production Science* 10(5): 457–467.
- Paul, J. W., N. E. Dinn, T. Kannangara, and L. J. Fisher. 1998. Protein content in dairy cattle diets affects ammonia losses and fertilizer nitrogen value. *J. Environmental Quality* 27(3): 528–534.
- Peskett, G. L. 1934. Milk secretion in relation to blood composition: The urea contents of blood and milk-serum. *Biochemistry J.* 28(5): 1657–1658.
- Robertson, J. B., and P. J. Van Soest. 1981. *The Analysis of Dietary Fiber in Food*. New York, N.Y.: Marcel Dekker.
- Rodriguez, L. A., C. C. Stallings, J. H. Herbein, and M. L. McGilliard. 1997. Diurnal variation in milk and plasma urea nitrogen in Holstein and Jersey cows in response to degradable dietary protein and added fat. *J. Dairy Science* 80(12): 3368–3376.
- Ryan, B. F., and B. L. Joiner. 1994. *Minitab Handbook*. 3rd ed. Belmont, Calif. Duxbury Press.
- Schepers, A. J., and R. G. M. Meijer. 1998. Evaluation of the utilization of dietary nitrogen by dairy cows based on urea concentration in milk. *J. Dairy Science* 81(2): 579–584.
- Van Soest, P. J., J. B. Robertson, and B. A. Lewis. 1991. Methods of dietary fiber, neutral detergent fiber, and nonstarch polysaccharides in relation to animal nutrition. *J. Dairy Science* 74(10): 3583–3597.
- Wolfschoon-Pombo, A., and H. Klostermeyer. 1982. Die NPN-Fraktion der Kuhmilch. *Milchwissenschaft* 37(1): 10–12.

