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MILK UREA NITROGEN: QUALITY CONTROL OF TESTING, INDIVIDUAL COW FACTORS, AND CORRELATIONS WITH BULK TANK TESTING

A Thesis

Submitted to the Graduate Faculty

in Partial Fulfilment of the Requirements

for the Degree of

Master of Science

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Pipat Arunvipas

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ABSTRACT

Milk urea nitrogen (MUN) has been used to assess the nutritional status of dairy cows. However, in any program using MUN values to monitor nutrition in a dairy herd, other factors need to be evaluated and considered. These factors include the reliability of method of determination of MUN, the role of a variety of cow level factors and seasonal variation.

The first objective of this study was to evaluate the repeatability, precision and accuracy of the testing technology for measurement of milk urea nitrogen levels. For the repeatability evaluation, 200 composite milk samples from individual cows were collected by random sampling from routine work at the Prince Edward Island Milk Quality Lab (PEIMQL). Each milk sample was tested twice using the Fossomatic 4000 MilkoScan analyzer (Foss 4000) at the PEIMQL. For precision and accuracy testing, 161 cow milk samples were randomly selected and then divided into paired duplicate samples with one sample being analyzed for MUN using the infrared test (Foss 4000) at the PEIMQL, and the other using an enzymatic method (Eurochem CL 10) at the laboratory of the Ontario Dairy Herd Improvement Corporation (ODHIC). Repeatability and agreement were assessed using two methods: calculation of the concordance correlation coefficient (Pc) and a graphical procedure based on limits of agreement proposed by Bland and Altman (1995).

The MUN concentrations from the infrared method had a slightly higher mean (13.78), but significantly lower standard deviation (4.63) than by the enzymatic method (mean = 13.73 and SD = 4.84)($P < 0.05$). The concordance correlation coefficients from the comparison of the two tests and testing of repeatability were 0.972 and 0.983, respectively. The mean difference between the two tests and repeated tests were 0.05 mg/dl (SD. = 1.18) and 0.29 mg/dl (SD = 0.49), respectively, The 95% lower and upper limits of agreement were -2.287 & 2.185 mg/dl, and -0.681 & 1.269 mg/dl, respectively. Therefore, the repeatability, precision and accuracy of the Foss 4000 at the PEIMQL were excellent.

The second objective of this study was to evaluate the effect of cow factors on MUN. The dataset consisted of 68,158 observations for 10,688 lactating Holstein cows in 177 herds that participated in the Atlantic Dairy Livestock Improvement Corporation (ADLIC) production recording service. The dataset was used to calculate the effect on MUN of parity, days in milk, milk yield, milk fat%, milk protein%, and SCC.

The milk urea concentration was lower during the first month of lactation than later in lactation. A positive relationship existed between MUN concentration and milk yield. A negative relationship existed between MUN and %milk protein and linear score. A quadratic relationship was found between MUN and days in milk and % milk fat. These relationships are important to consider when interpreting MUN values, but because only 9% of the variation in MUN values was explained by the combination of studied factors, other factors, particularly nutritional factors, influence MUN values much more than the studied factors.

The third objective of this study was to determine seasonal variation in bulk tank milk urea nitrogen (BTMUN) concentration in PEI dairy herds, and to evaluate the relationship between BTMUN and the weighted herd average of individual cow MUN levels (WHMUN). The data for assessing seasonal variation in BTMUN were obtained from the PEIMQL for July, 1999 to February, 2001, totalling 11,223 test results. The WHMUN was calculated and matched (by closeness in time) with the BTMUN values for the period of July, 1999 to June 2000, totalling 1,772 matched test results.

The highest BTMUN levels were found in mid and late pasture season 2001 when above average rainfall produced excellent pasture quality. The concordance correlation coefficient between the WHMUN and BTMUN was lower than expected (Pc = 0.81). Adjustment for pasture use, various milk sampling protocols or herd size did not improve the correlation significantly. BTMUN is only a moderate indicator of WHMUN in a herd.

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TABLE OF ABBREVIATIONS

ADLIC	Atlantic Dairy Livestock Improvement Corporation
BTMUN	Bulk tank milk urea nitrogen
BUN	Blood urea nitrogen
CP	Crude protein
DIM	Days in milk
DIP	Degradable intake protein
DMI	Dry matter intake
GEE	Generalized estimating equations
MTP	Milk true protein
MNPN	Milk non protein nitrogen
MUN	Milk urea nitrogen
ODHIC	Ontario Dairy Herd Improvement Corporation
OFF-1X	official-sampled by a technician and milk collected in the morning one month and evening the next month
OFF-2X	official-sampled by a technician and milk collected 2 times 12 hours a part
PADHIA	Pennsylvania Dairy Herd Improvement Association
PEIDPA	Prince Edward Island Dairy Producer Association
PEIMQL	Prince Edward Island Milk Quality Laboratory
PUN	Plasma urea nitrogen
SCC	Somatic cell count
SUN	Serum urea nitrogen
UIP	Undegradable intake protein
UNOFF-1X	nonofficial-sampled by the farmer and milk collected in the morning one month and evening the next month
UNOFF-2X	nonofficial-sampled by the farmer and milk collected 2 times 12 hours a part
UNWHMUN	Unweighted herd milk urea nitrogen
WHMUN	Weighted herd milk urea nitrogen

Chapter 1

INTRODUCTION

In recent years, there has been growing interest in using the concentration of urea in milk as an indicator of the efficiency with which protein is utilized by dairy cows, and to assess how diets are balanced for energy and protein in their diet. However, there is a need to develop a better understanding of factors which influence milk urea nitrogen (MUN) concentrations before this information can be used as a tool to monitor the efficiency of protein utilization in dairy herds in the Maritime provinces. The following 2 sections briefly introduce what is known about the nutritional and non-nutritional factors which affect MUN, and describe why future research on MUN is necessary.

1.1 Protein and Urea in milk

The protein composition of milk has received heightened interest in recent years because an increasing proportion of the milk supply is directed towards cheese production (1). In addition, human dietary concerns about animal fats has resulted in the development of milk pricing systems that place less economic emphasis on milk fat and more on milk protein (2). Typically, the total crude protein percentage of milk is calculated as 6.38 times the percent nitrogen (%N) of the milk, with %N as the level of N determined from a Kjeldahl analysis (3-5).

The nitrogen content of milk can be divided into three broad fractions: casein nitrogen, whey nitrogen, and non- protein nitrogen (NPN), including urea. These constitute approximately 79.5%, 15.0%, and 5.5% of the total N in cow's milk, respectively (6). However, there is a wide variation in proportions of these components. The factors affecting variation in casein and whey components of milk protein have been more extensively studied than the NPN fraction. For example, the variation in casein content of milk is affected by environmental temperature, mammary disease, parity, stage of lactation, breed and nutrition (2;4;5;7-10). Novello and DeHaast (11) showed a wide variation in values of NPN between 12.70 and 38.90 mg N/100 ml, with an overall average of 26.01 ± 0.544 mg N/100ml. Cerbulis (4) reported that the NPN can range from 2.8 to 10.6% of the total N.

Urea accounts for the greatest proportion of the milk non-protein nitrogen (MNPN) (7). Kaufmann (12) reported that urea may constitute from 20 to 75% of the MNPN fraction, with the average proportion being approximately 48%. Urea is synthesized in the liver as a metabolic end product resulting from degraded ruminal protein, protein digested in the small intestine and gluconeogenesis from amino acids (7). Urea equilibrates in body water, and kinetic analysis suggests that there is passive transfer of urea from plasma to milk along with water (13). It has been shown that the concentration of NPN in milk typically increases from 29 to 40 mg/dl, and MUN, expressed as a percentage of MNPN, increases from 20 to 45%, as the concentration of dietary crude protein (CP) increases from 12.2 to 17.6 % of dry matter (DM) (14).

1.2 Milk Urea Nitrogen as a management tool

Efficiency of protein feeding is maximized when N supplied by the diet matches the N requirement of the rumen microbes and ruminant tissues. This optimum balance is reflected in a baseline concentration of urea in plasma and milk. Excess N supplied to the rumen or to postruminal tissues increases the concentration of urea in plasma and milk above baseline values, and suggests N wastage and inefficiency of protein feeding (15).

Blood urea nitrogen (BUN) has long been known to reflect inefficient utilization of dietary crude protein by ruminants (16). However, taking blood samples to assess BUN is not as cost effective as MUN because obtaining blood samples takes more time and expense. Also, milk is a highly suitable media for routine monitoring of urea because a sample of milk is taken by truck drivers (who work for milk processing companies) every time they pick up a load of milk from the farm, and by technicians (who work for milk production recording agencies) on a monthly basis. Measuring urea levels in milk is an easier way to closely monitor protein metabolism and nitrogen utilization in dairy herds. Urea concentrations in milk are highly correlated to those in plasma (14;17-22). To this end, it has been suggested that the optimum MUN concentration for an individual cow ranges from 8 to 25 mg/dl, while the optimum MUN concentration for a herd (based on a bulk tank sample or a weighted herd average MUN value) ranges from 12 to 16 mg/dl (14).

Interest in the analysis of MUN concentration has increased dramatically during the last few years as automated systems for measuring MUN have become available (23). A

rapid and efficient analytical instrument based on an analytical infrared (IR) technique has been developed (24). Infrared spectrophotometry can now be performed economically on large numbers of samples, making it possible to use MUN as a diagnostic tool in monitoring feeding programs on dairy farms. This provides another potential tool to dairy farmers for minimizing feed costs while maximizing milk production (19).

In order to use MUN as a measure of efficiency of protein feeding, factors that influence MUN need to be more clearly described. A number of factors have been reported to influence MUN concentration, including: breed, body weight, stage of lactation, parity, milk yield, and season (25;26). Some of the reported effects of these factors on MUN are not consistent among various studies, and the magnitude of the effects under conditions found in Atlantic Canada are not known. In addition, the test characteristics of the infra-red method and the quality of MUN data being produced by this method require further study. Further research is needed to better understand the various factors that influence MUN levels, and to develop specific guidelines to accurately interpret MUN measurement under commercial conditions in Atlantic Canada.

1.3 Dairy Industry in PEI

Although Prince Edward Island (PEI) is the smallest province of Canada, half of its landmass is used for agriculture. The dairy industry is one significant component of the agricultural industry in PEI, accounting for 13% of the gross value of agricultural production in 1997 (27). In 1999, there were approximately 400 dairy producers holding quota, and 199

farms used the service of the Atlantic Dairy Livestock Improvement Corporation (ADLIC) to record various production and health parameters, and to provide management recommendations. PEI dairy herds on ADLIC range in size between 10 and 180 cows, with the average herd being 45 cows. Average milk production per lactation is 8,352 liters (28).

The interest in the use of pasture for dairy production has increased in recent years. Many dairy farms in PEI use pasture in the spring, summer, and fall to reduce feeding and operating costs at that time of the year. However, Hovingh (29) showed that production in PEI declined over the summer-fall period, with test day milk production per cow averaging 25% less in the fall than in the spring and early summer months. How pasture utilization influences MUN levels, particularly in relation to the summer-fall slumps, has not been described and is of great interest to dairy producers in Atlantic Canada and elsewhere where pastures are used.

1.4 Milk Urea Nitrogen Project

Many questions remain about MUN concentrations and the value of their measurement. The relationships among MUN values, protein nutrition and the seasonal variation in milk production in dairy cattle in the Maritime provinces needs to be assessed. The milk urea nitrogen project has been a joint effort of individuals, institutions and organizations: the Atlantic Veterinary College (AVC), the Atlantic Dairy Livestock Improvement Corporation (ADLIC), the Nova Scotia Agricultural College (NSAC), the PEI Dairy Producers Association (PEIDPA), the PEI Milk Quality Laboratory (PEIMQL), and,

of course, the participating dairy producers. The main project goal was to evaluate the predictive ability of MUN to indicate nutritional imbalances in dairy herds under a range of feeding management systems in Atlantic Canada. Specific objectives included the following:

1. to validate infrared-based cow level tests through a comparison with a gold standard test;
2. to validate herd level tests through the comparison of bulk tank MUN with herd average MUN values;
3. to determine the effects of cow factors on MUN;
4. to evaluate the effect of MUN on reproductive performance;
5. to determine if MUN levels from individual cows or pooled bulk tank milk are indicative of protein-energy imbalance in the offered ration;
6. to evaluate MUN as a cost effective feeding management tool that evaluates protein and energy interactions in dairy cows; and
7. to determine specific guidelines for interpretation of MUN values on Maritime dairy herds.

Objective numbers 1, 2, 3 and 4 were evaluated in 199 herds, while objective 5, 6 and 7 utilized a subset of 90 herds. The 199 participating herds were all clients of the Atlantic Dairy Livestock Improvement Corporation (ADLIC), and therefore, compositional analyses of milk were already performed monthly on individual cows and bulk tank milk from these herds. The compositional analyses included fat, protein, solids and somatic cell counts, and were conducted at the P.E.I. Milk Quality Laboratory (PEIMQL) on a Fossomatic 4000 MilkoScan Analyzer (Foss North America, Brampton, Ontario). The participating herds benefitted from the MUN data for the two year duration of the project, giving them insight on its utility without its associated cost.

A subset of 90 herds that represented a range of feeding management systems, including total mixed ration (TMR), component based feeding, extensive pasture management, intensive pasture management, and total confinement management, underwent enhanced nutritional assessment and monitoring. These herds increased the contextual information of the MUN data for the 2 year duration of the project. This work will not be described in this thesis, but rather in future publications.

1.5 Specific Study Objectives

This thesis addresses the first 3 objectives of the MUN project. This includes a thorough literature review of factors related to MUN, an evaluation of the procedures for measuring MUN in PEI, and a study of some of the non-nutritional cow factors suspected to be related to MUN levels, such as: breed, stage of lactation, parity, milk composition, and milk quality. Bulk tank milk urea nitrogen is also assessed to determine whether it is a reliable guide to the average urea concentrations of the herds.

Chapter 2 provides a comprehensive literature review of MUN, and includes non-nutritional factors related to MUN levels such as cow factors: breed, stage of lactation, parity, milk composition and milk quality.

Chapter 3 includes an evaluation of the validity of the MUN measurements, including

1. comparison of the infrared technique with the enzymatic method, and
2. evaluation of intra-sample repeatability.

Both of these analyses contribute to an ongoing quality control program at the PEIMQL.

Chapter 4 includes the effects of cow factors, such as milk production and quality, breed, parity, stage of lactation and season of the year, on MUN values.

Chapter 5 includes a comparison of bulk tank MUN with herd average MUNs based on biweekly herd test results.

Chapter 6 is a summary of the thesis and a presentation of overall conclusions.

MUN analysis may provide the producer, veterinarian, and nutritionist with a direct, "real time" window into the energy - protein interactions occurring in dairy cattle. MUN use to assess protein and energy nutrition may be deemed as an effective feeding management evaluation tool. This thesis is one step toward achieving this goal.

1.6 References

1 Gustafsson AH, Palmquist DL. Diurnal variation of rumen ammonia, serum urea and milk urea in dairy cows at high and low yields. J Dairy Sci 1993; 76(2):475-484.

2 DePeters EJ, Cant JP. Nutritional factors influencing the nitrogen composition of bovine milk: A review. J Dairy Sci 1992; 75(8):2043-2070.

3 Barbano DM, Lynch JM. Direct and indirect determination of true protein content of milk by Kjeldahl analysis: Collaborative study. J Assoc Off Anal Chem 1991; 74(2):281-288.

4 Cerbulis J, Farrell HM. Composition of milks of dairy cattle. 1.Protein, lactose, and fat contents and distribution of protein fraction. J Dairy Sci 1975; 58(6):817-827.

5 Ng-Kwai-Hang KF, Hayes JF, Moxley JE, Monardes HG. Percentages of protein and nonprotein nitrogen with varying fat and somatic cells in bovine milk. J Dairy Sci 1985; 68(5):1257-1262.

6 DePeters EJ, Ferguson JD. Protein and nonprotein nitrogen distribution in milk and factors that influence it. J Dairy Sci 1991; 74[suppl.1], 118.

7 DePeters EJ, Ferguson JD. Nonprotein nitrogen and protein distribution in the milk of cows. J Dairy Sci 1992; 75(11):3192-3209.

8 Robertson NH, Westhuizen EVD. Non-protein nitrogen content of milk as influenced by various factors. S Afr J Dairy Sci 1990; 22:1-8.

9 Szijarto L, Biggs DA, Irvine DM. Variability of casein, serum protein and nonprotein nitrogen in plant milk supplies in Ontario. J Dairy Sci 1973; 56(1):45-51.

10 Blake RW, Nmai IB, Richter RL. Relationships between distribution of major milk proteins and milk yield. J Dairy Sci 1980; 63(1):141-147.

11 Novello JC, DeHaast J. Nitrogen distribution in non-mastitic milk. S Afr J Dairy Sci 1984; 16(3):109-116.

12 Kaufmann W. Variation in composition of raw milk with special regard to the urea content. Milchwissenschaft 1982; 37[1], 6-9.

13 Baker LD, Ferguson JD, Ramberg CF. Kinetic analysis of urea transport from plasma to milk in dairy cows. J Dairy Sci 1992; 75[suppl.1], 181.

14 Roseler DK, Ferguson JD, Sniffen CJ, Herrema J. Dietary protein degradability effects

on plasma and milk urea nitrogen and milk non-protein nitrogen in Holstein cows. J Dairy Sci 1993; 76(2):525-534.

15 Rodriguez LA, Stallings CC, Herbein JH, McGilliard ML. Effect of degradability of dietary protein and fat on ruminal, blood and milk components of Jersey and Holstein cows. J Dairy Sci 1997; 80(2):353-363.

16 Lewis D. Blood urea concentration in relation to protein utilization in the ruminant. J Agric Sci Camb 1957; 48:438-446.

17 Baker LD, Ferguson JD. Response in urea and true protein of milk to different protein feeding schemes for dairy cows. J Dairy Sci 1995; 78(11):2424-2434.

18 Broderick GA, Clayton MK. A statistical evaluation of animal and nutritional factors influencing concentrations of milk urea nitrogen. J Dairy Sci 1997; 80(11):2964-2971.

19 Lyatuu ET, Eastridge ML. Nutritional factors affecting milk production, milk composition, milk urea nitrogen, and plasma urea nitrogen. Special-Circular -Ohio-Agricultural-Research-and-Development-Center 1998; 163:65-71.

20 Oltner R, Wiktorsson H. Urea concentrations in milk and blood as influenced by feeding varying amounts of protein and energy to dairy cows. Livest Prod Sci 1983; 10:457-467.

21 Oltner R, Wiktorsson H, Emanuelson M. Urea concentrations in milk in relation to milk yield, live weight, lactation number and amount and composition of feed given to dairy cows. Livest Prod Sci 1985; 12:47-57.

22 Ropstad E, Vik-Mo L, Refsdal AO. Levels of milk urea, plasma constituents and rumen liquid ammonia in relation to the feeding of dairy cows during early lactation. Acta vet scand 1989; 30:199-208.

23 Moore DA, Varga GA. BUN and MUN: Urea nitrogen testing in dairy cattle. Comp Cont Ed Pract Vet 1996; 18(6):712-720.

24 Gustafsson AH. Current status concerning the use of milk urea concentration: analytical methods, use in different countries and how to utilise the results. Proceeding of the 30th Biennial Session of ICAR, Velhoven, The Netherlands, 1996; 223-228.

25 Butler BR. Milk urea nitrogen analysis: Field trial results on conception rate and dietary inputs. Proceeding Cornell Nutrition Conference for Feed Manufacture. Cornell University, Ithaca, 1995;89-95.

26 Gustafsson AH, Carlsson J. Effects of silage quality, protein evaluation systems and milk urea content on milk yield and reproduction in dairy cows. Livest Prod Sci 1993; 37:91-105.

27 Department of Agriculture and Forestry of PEI 1999. 1998 Agricultural Statistics. Hogan S, editor. 32. 1999. The Department of Agriculture and Forestry, Charlottetown, Prince Edward Island, Canada.

28 Atlantic Dairy Livestock Improvement Corporation. 1999 Annual Production Report. 1-53. 2000.

29 Hovingh EP. The summer-fall slump:seasonal variation in average dairy milk production in PEI, Canada. [PhD dissertation]. Charlottetown, Prince Edward Island: University of Prince Edward Island, 1998.

Chapter 2

A Literature Review of Milk Urea Nitrogen

2.1 Milk Urea Nitrogen

2.1.1 What is Milk Urea Nitrogen

Urea, $(NH_2)_2 CO$, a small organic molecule, is a common constituent of blood and other body fluids. Blood (serum) urea is derived from the conversion of ammonia (NH_3) to urea in the liver or from tissue catabolism of amino acids. Urea diffuses freely from serum into the milk within the alveoli of the mammary gland. As a result, milk urea nitrogen (MUN) concentrations are dependant on blood plasma concentrations (1-4). Milk non protein nitrogen (MNPN) constitutes 5 to 6 % of the total milk nitrogen. Urea N is the largest component of MNPN, accounting for approximately 50% of the MNPN. Other nitrogenous compounds contributing to the MNPN include orotic acid, creatinine, creatine, ammonia, hippuric acid, uric acid, peptides, amino acids and a small fraction of other unknown compounds (3;5).

There are a number of sources of urea in the ruminant. In ruminants, a variable amount of the protein and non-protein nitrogen in the diet is normally metabolized to ammonia (NH_3) by ruminal microorganisms. These microbes utilize ammonia along with energy from fermentable carbohydrates and organic acids to synthesize amino acids, which are then incorporated into microbial protein (Figure 2.1). When ruminal ammonia

concentrations exceed the synthetic ability of ruminal microbes (due to insufficient microbes, energy, or organic acids), ammonia is absorbed, predominantly through the ruminal wall (6). The rate of ruminal absorption depends on the rumen pH. Absorption is rapid at pH 6.5 and higher, and declines to nearly zero at pH 4.5 (7). This is because, at a low rumen pH, the ionized form, ammonium, predominates, resulting in decreased diffusion across the rumen wall (8). Ammonia is very toxic in the systemic circulation, so under normal physiological conditions ammonia is constantly converted in the liver to the less toxic metabolite, urea, which is eventually excreted in urine. This process ensures low circulatory ammonia concentrations (6;9).

A second source of urea in the body is the oxidation of amino acid in tissues (2). Amino acid catabolism is a significant contributor to the blood urea nitrogen (BUN) pool, with approximately one-third of the urea N production in lactating cows arising from oxidation of amino acid (usually glutamine, alanine or glycine) by tissues (10). In contrast, Veen and Bakker (11) suggested that blood urea concentration is more dependent on protein catabolism than ammonia production in the rumen. DePeters and Cant (2) argued that although arginine catabolism contributed to the BUN pool, the urea nitrogen contribution from tissue catabolism was less significant than that reported by Bruckental (9).

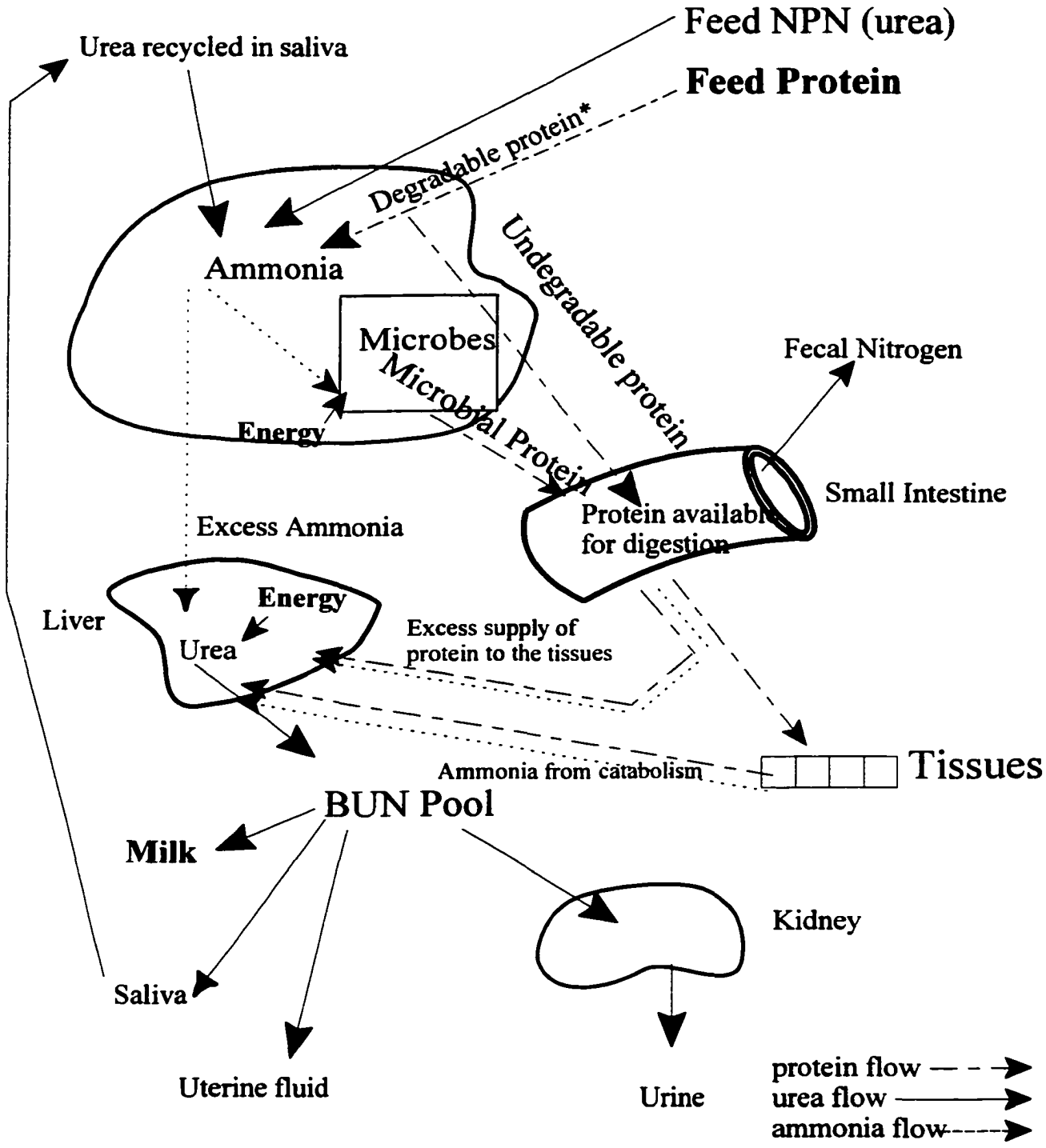


Figure 2.1. Protein and Nitrogen metabolism in the dairy cow.

* degradation to NH_4 by cellulytic bacteria in rumen.

Broderick and Clayton (12) described a third contributor to the urea nitrogen pool. This third source is from an absorption of excess dietary protein. Some absorbed amino acids are catabolized for energy and contribute directly to the BUN. Cannas *et al.* (13) explained the mechanism whereby absorbed proteins contribute to the urea pool. In their study, MUN levels were the same regardless of energy intake. They proposed that the observed elevated MUN levels were due to high feed intake and increased rate of passage. These circumstances resulted in a large amount of protein escaping from the rumen and reaching the intestine. The concentration of MUN therefore was more closely related to the total amount of protein absorbed from the intestine as opposed to the amount of protein fermented in the rumen.

2.1.2 Urea diffusion in the body

Urea is a small water soluble molecule that readily diffuses out of the blood and across cell membranes. The amount of urea liberated into saliva, uterine fluid and milk rises as a linear function of the amount present in blood. Changes in urea concentrations in the blood rapidly translate into changes in urea concentrations in other body fluids due to a urea equilibrium between blood and various body fluids (4). Urea kinetic analysis suggests that passive transfer is responsible for the transport of urea from plasma to milk. Passive transfer is possibly due to urea water solubility (1)(14).

Gustafsson and Palmquist (4) concluded that urea levels in a total milk sample tended to be more closely correlated to serum than levels obtained from samples taken from the

gland cistern, but deviations were minor. Equilibration in the cistern may be explained by the diffusion of urea along the mammary ducts and through the mucosa of the alveoli.

2.1.3 MUN and BUN relationship

BUN is one of the metabolic profile tests that can be performed in dairy cattle to monitor metabolic conditions as they relate to health and disease (15). These profiles can also be applied to evaluate nutritional status in dairy herds. Of all the parameters measured in a metabolic profile, BUN is the most closely related to protein status (16;17). Changes in BUN were directly proportional to the level of ingested N (18). Therefore, blood urea is a sensitive indicator of dietary intake of digestible crude protein and it may act as a guide for measuring efficiency of protein utilization in ruminants (19-21). Kinetic analysis has suggested that MUN concentration is a reasonable indicator of mean plasma urea concentration (22;23). Since milk samples are routinely collected on dairy farms, as part of production monitoring schemes, MUN testing is more practical than BUN for routine monitoring (24). High correlations (0.77-0.98 - see Table 2.1) between urea in blood and urea in milk have been reported in many studies (3;12;23-35) The high correlation between MUN and BUN found in these studies suggested that blood is the major source of urea nitrogen in milk.

Gustafsson and Palmquist (4) studied the variation in MUN and BUN concentrations in relation to feeding time and milk production. BUN was significantly higher than MUN in the high yielding group ($P<.01$) but only slightly higher for the low yielding group ($P>.01$)

Table 2.1. The correlation between blood urea nitrogen and milk urea nitrogen.

Author	Correlation
Oltner and Wiktorsson, 1983	0.98
Refsdal, 1983	0.77
Oltner et al, 1985	0.91
Ropstad et al, 1989	0.88
Martinez et al, 1991	0.91
DePeters and Ferguson, 1992	0.89
Roseler et al, 1993	0.89
Kolver and MacMillan, 1993	0.98
Gonda and Lindberg, 1994	0.85
Wattiaux et al, 1994	0.85
Baker and Ferguson, 1995	0.96
Bulter et al, 1996	0.82
Broderick and Clayton, 1997	0.92
Lyatuu and Eastridge, 1998	0.86
Wittwer et al, 1999	0.95

when measured at 3 hours after feeding. In the high producing group, post-feeding BUN values peaked about 1 hour before MUN values. This 1 hour delay resulted in MUN levels being higher than BUN levels once BUN levels had passed their peak and were decreasing. As a result, the MUN concentration was somewhat greater than that of BUN when milk and blood samples were collected 4 to 5 hours after feeding (25).

2.1.4 Scale of measurement

There are two scales of measurement to express urea concentrations: mg/dl and mmol/l used in North America and Europe, respectively. Other units of measure include g/L. Formulae to convert between the various units are presented in Appendix A.

To summarize this section, urea is a small organic molecule and is a byproduct of protein breakdown in cattle. Microorganisms in the rumen convert ammonia to microbial protein by using energy from a carbohydrate source. Excess ammonia is absorbed through the rumen wall, and then converted to urea in the liver. The urea acts as a carrier of excess nitrogen out of the body primarily through urine, thus preventing ammonia toxicity. Blood urea concentration is influenced by protein catabolism and energy intake. Blood urea freely diffuses into milk and is part of the normal nitrogen constituents in milk. A strong relationship has been found between urea in blood and urea in milk in several studies. Since milk is an easy fluid to collect and is collected at least twice a day on most farms, measuring milk urea is an practical way to estimate blood urea levels.

2.2 Factors affecting Milk Urea Nitrogen

2.2.1 Sample processing

2.2.1.1 Sample type

Gustafsson and Palmquist (4) found that the MUN concentration measured from a quarter sample taken every 2 h (left front) was similar to MUN concentration taken from a quarter sampled every hour (right front). The result was similar to what Carlsson *et al.* and Eicher *et al.* (14;36) found, that the front and hind or left and right quarters did not significantly influence urea concentration. Eicher *et al.* (36) also found no difference in MUN value between a pooled sample and one from individual quarters.

Butler *et al.* (25) compared pre- and post-milking samples with composite milk samples using wet chemistry. They demonstrated that there was no significant variation between these samples. They also found little variation in the MUN concentration in different milk fractions collected during milking. Carlsson and Bergstrom (14) found that five of six cows had the lowest urea concentration in the residual milk when normal milking had finished and five had the highest concentration in the pre-milking stripped milk. While these differences were statistically significant ($P < 0.05$), when the concentration of urea was calculated as the concentration in the water portion, no significant differences remained. In disagreement with Butler *et al.*, Godden *et al.* (37) found that the effect of abnormal fat levels in a pre-stripping or a post-stripping sample led to inaccurate estimates of the MUN

concentration. Urea is soluble in water but not in fat, and therefore spectrophotometry can yield misleading results for milk samples with a high fat content. Hand stripping at the end of milking yields a sample with higher fat content. Consequently, it is not recommended to obtain pre or post milking strip samples for MUN testing. When automated lab techniques do not use spectrophotometry, fat is not a consideration (38).

2.2.1.2 Sample storage

2.2.1.2.1 Ambient temperature

Miettinen and Juvonen (39) found urea levels gradually became lower with time in nonpreserved milk kept at room temperature. After 2 days, levels decreased by 50% with the degradation rate depending on the types of microorganism (eg. bacteria that utilize urease enzymes) present in the milk sample. Therefore, milk samples kept at room temperature for more than a few hours would not produce a representative MUN value. A milk sample that has turned sour should be discarded (14).

2.2.1.2.2 Preservative

A preservative is routinely used in all milk samples collected by recording agencies. The preservative currently used by Ontario Dairy Herd Improvement Corporation (Ont.DHIC), Pennsylvania Dairy Herd Improvement Association (PA DHIA), Atlantic Dairy Livestock Improvement Corporation (ADLIC) is bronopol (2-Bromo-2Nitropropane-1,3

Diol: 6 mg/tablet)(D&F Control System, San Ramon, CA). Urea concentrations remain unchanged for 17 days in refrigerated milk when bronopol is added to the milk sample, showing that is no augmentation or degradation of MUN with bronopol (14). Unrefrigerated milk samples containing bronopol preservative had significantly higher MUN levels than did paired non-preserved milk samples analyzed on day 1 (mean difference = 0.25 mg/dl, S.E. = 0.10, $P = 0.02$), using infrared technology (40), confirming the beneficial preservative impact of bronopol.

Results from Butler *et al.* (25) disagree with those from Godden *et al.*, concluding that a preservative had no significant effect on MUN results. Their study utilized an automated diacetylmonoxamine method and a manual urease method to measure MUN levels, stating that the preservative that was used did not interfere with these chemical analyses. The differences in these studies may come from different methods of analyses. Miettinen and Juvonen (39) used sodium azide as a preservative, and they found that azide-preserved milk resulted in unchanged MUN levels for one week.

2.2.1.2.3 Refrigeration

The effect of duration of refrigeration was examined in 5 studies. In three of the studies, MUN values from milk samples stored at 4 °C remained unchanged for one week (39), up to 10 days (14) and up to 11 days (40). Two studies had conflicting results. Eicher *et al.* (36) found that after 1 wk of refrigeration at 4 °C, the mean MUN concentration increased by 0.41 ± 0.24 mmol/l ($P = 0.0001$). Similarly, Oltner and Sjaunja (41) showed

that milk urea levels increased by 0.18 mmol ($P < 0.001$) after storage in a refrigerator for 14 days. It is unclear why these increases took place in these latter two studies.

2.2.1.2.4 Freezing

The effects of freezing milk samples on MUN concentration are not clear. Carlsson *et al.* and Oltner *et al.* (14;41) reported no effect of freezing while Eicher *et al.* (36) showed that the urea concentrations were higher after being frozen for one month (mean urea change = 1.52 ± 1.25 mmol/L ($P = 0.001$).

2.2.1.3 Time of sampling

Coggins and Field (42) described a diurnal pattern for urea levels in lactating beef cows. In their study, they evaluated various energy levels in three different diets. In low, medium and high energy diets, plasma urea nitrogen peaked 5.5, 3.5 and 5.5 hours (h) after feeding respectively. Gustafsson and Palmquist (4) found similar results. They observed that urea in blood serum peaked about 3 h after feeding. They studied the ruminal ammonia , SUN and MUN diurnal variations in four dairy cows when cows were fed a total mixed ration ad libitum once a day. The ruminal ammonia level peaked within an hour after feeding. The SUN peaked 1.5 to 2 h after the rumen ammonia peak. The MUN peak occurred about 1.0 h after the SUN peak. Carlsson and Bergstrom (14) reported a diurnal variation in MUN in six dairy cows fed a ration of hay, grass, silage and concentrate twice a day. The highest values were found 3-5 h after feeding and lowest values were found late at night

when the cows were not feeding. The lowest value equaled 60% of the maximum value.

Miettinen and Juvonen (39) stated that the effect of diurnal variation and the time of day of sample collection could be important in the interpretation of MUN values. He made this comment since the milk urea level was lower in the morning than in the afternoon. The increase from morning to afternoon was more marked in hay plus urea fed cows (3.9 to 5.6 mmol/l, $P < 0.001$) than in silage fed cows (3.8 to 4.3 mmol/l, $P < 0.05$). These results are similar to those from other studies (4;12;34;39;43).

Ferguson (44) reported results from a 5 month study involving 37 cows. From December through to April, 166 samples were collected from one dairy herd being fed a total mixed ration at 11.00 a.m. The total mixed rations (TMR) were balanced for two groups. The high production group was balanced for 40.8 kg. of milk, while the low production group was balanced for 24.9 kg. of milk. Milking occurred twice a day from 5:00 a.m. to 7:00 a.m. and from 4:00 p.m. to 6:00 p.m.. Blood samples were collected around 2:00 p.m. The morning milk urea level was approximately 2 mg/dl lower than the evening milk urea level. The author attributed the difference due to the rise in urea levels seen after the feeding (11:00 a.m.).

Ciszuk and Gebregziabher (42) suggested that a diet with more protein of high ruminal degradability showed a more pronounced difference between the concentration of milk urea in afternoon milkings compared to morning milkings. The pattern of lower urea levels in the morning was also supported by studies on BUN (4;42;45;46). There were a few

studies (25;26;30;47) that reported similar concentrations of MUN in the morning and evening milkings, with small daily variations.

2.2.1.4 Summary of sample collection and handling factors.

In summary, milk samples routinely collected by milk testing laboratories should be suitable for MUN analysis. Composite milk samples that are preserved with bronopol and refrigerated should be suitable for MUN testing for 10-14 days after collection. Longer storage will require freezing, but this may elevate MUN levels slightly. MUN values were not significantly different among udder quarters. Similarly, no differences in the pre-stripping and post-stripping urea concentration were found when using wet chemistry methods, but differences were found when using infrared methods due to the extra fat content of these strippings. Therefore, the method of MUN determination will dictate the type of sample that is acceptable for testing, as explained in more detail in the next section.

The effect of diurnal variation and time of day of sample collection is an important factor to consider in the interpretation and use of MUN results. Morning and afternoon samples of milk will have urea concentrations which reflect the time of feeding relative to milking. Therefore, it has been suggested that sampling should be at a fixed time in relation to feeding as this scheduling will minimize errors due to post-prandial variations in MUN concentrations (27;42;45;46). Another suggestion for smoothing the effect of diurnal variation may be to submit pooled samples, combining AM and PM samples (48).

2.2.2 Method of MUN determination

Manual methods using wet chemistry for analyzing urea concentrations in the body fluids have been used for many decades. The commonly available methods for analyzing MUN can be classified in three groups (49):

1.) indirect methods, which measure a complex between a degraded urea metabolite (ammonia) and a reagent;

2.) direct methods, which measure a complex between urea and a reagent; and

3.) physical methods, which use infra-red spectrophotometry to estimate the amount of urea present, based on the amount of light absorbed at a wavelength specific for urea.

The most common indirect test employs spectrophotometric analysis. Urease is added to the milk in order to break urea down to ammonia. A reagent which reacts with ammonia to form a blue color is then added to the sample. The intensity of the blue color correlates with the concentration of urea in the sample (44).

The most common direct method involves a dye agent, diacethylmonoxine, which reacts with the urea molecule to form a pink color. The intensity of pink, measured by spectrophotometry, correlates with the urea concentration in the sample (50).

The two techniques described above require fat extraction from the milk for two reasons. First, fat extraction prolongs the functional life span of the osmotic membrane,

which is integral to the laboratory equipment. The second reason is that urea is soluble in water and not fat. As a result, spectrophotometry can yield misleading results for milk samples with high fat content. Butler *et al.* (25) compared an automated diacetylmonoxamine method with a manual urease method. The defatted MUN results from both methods were highly correlated. These methods are, however, costly and time consuming.

Another direct method is the dipstick urease/pH method which has practical advantages but numerous disadvantages. This technique uses a dipstick that reacts with urea and turns an orange to deep green color. The intensity of green color reflects urea concentration. This test is semiquantitative, accurate only to within 5 mg/dl, and is subject to operator interpretation. As a result, it may be unable to differentiate moderate from unacceptably high MUN levels (38). Roseler *et al.* (33) evaluated the sensitivity of the MUN test strips, and reported that timing of the process was apparently critical to the interpretation. Strips left in the sample too long tended to be darker, while excessive rinsing of the strip can reduce the intensity of the color. The test strips may work best as a qualitative rather than quantitative tool. Rodriguez *et al.* (51) reported that Azotest strips were correlated with MUN ($r = 0.46$). However, prediction of elevated MUN concentrations by Azotest strips was very poor. In their study, Azotest strips predicted that 85% of samples were equal to or above 25 mg/dl, while spectrophotometric analysis showed that only 23% were above 25 mg/dl.

Recently, a physical method of measuring MUN, using infrared technology, has become available. Infrared technology has long been used to measure fat and protein in milk. Heated organic molecules give off an infrared reflectance spectrum. The reflectance

spectrum is consistent with the type of molecule present. The reflectance spectrum is correlated with the concentration of molecules present in a sample (44). The Foss 4000 Milkoscan infrared analyzer uses mid-infrared (IR) specific absorption spectrophotometry for milk compositional analysis. Addition of MUN capacity to a current Foss 4000 Milkoscan testing system requires the attachment of a MUN-specific IR filter and a small amount of technical training (Foss Electric). Automated systems of MUN determination occasionally yield a result of 0 or a negative number. These values are uninterpretable and should not be included in the average (38). Godden (48) showed that MUN levels using both the Foss 4000 Milkoscan analyzer and a selected reference test (indirect enzymatic method) had good agreement. The concordance correlation was 0.86. The Foss 4000 Milkoscan estimate appears to be accurate compared to the reference test used, and is inexpensive, rapid and simple to conduct.

2.2.2.1 Summary of Methods of MUN determination.

Laboratory methods for the analysis of urea involve direct or indirect (enzymatic conversion of urea to ammonia) measurement using colorimetric determination (52). Recently infrared technology has become available and practical and many DHIA centers have begun to employ this technology to measure milk urea. There is some indication that this new method has a good agreement and high correlation with a reference test. This new method affords a very rapid, easy way to measure MUN in a large number of samples.

2.2.3 Cow factors

2.2.3.1 Breed

Distinctive breed differences exist in the N composition of milk. Milk from Holstein cows has the lowest content of crude protein, true protein, and casein, while milk from Jersey cows has the highest. The NPN content of milk is less variable among breeds, but the range within a given breed is considerable (53). Data from Ontario Dairy Herd Improvement (Ont DHI) showed that of the major breeds, Holstein had the lowest MUN average at 14.0 mg/dl and Brown Swiss had the highest at 16.5 mg/dl, while Jersey had the second highest at 16 mg/dl. Other breeds averaged between 14.7 - 15.4 mg/dl (54). Ferguson *et al.* (55) found MUN values of 15.8 mg/dl in Jersey cows and 14.0 mg/dl in Holstein cows. Only descriptive statistics were given in these two reports, and therefore, it is not known whether these differences are statistically significant. Others (39;56) observed no significant correlation of MUN among breeds. Breed differences in DHI records may reflect true physiological or genetic differences among breeds, or they may be due to differences in feeding management programs among breeds (eg. some breeds may be pastured more frequently) .

2.2.3.2 Body Weight

Oltner *et al.* (31) found that MUN concentration was negatively related to live weight. A live weight increase of 100 kg resulted in a 1.68 mg/dl decrease in MUN when the

cows were fed in accordance with the Swedish standard recommendations. This relationship might be explained by dilution. In a large cow, the compartment for urea distribution is naturally larger than in a small cow. If we assume the same amount of urea is formed in the liver regardless of animal size, the urea concentration in blood and milk will be lower (due to dilution) in larger animals compared to smaller ones. The effect of body weight on MUN depends on the total amount of urea produced and distributed in the body, which depends on the intake of degradable protein and metabolic body size. Jonker *et al.* and Diab *et al.* (57;58) reported data which are consistent with Oltner *et al.*. One study by Ropstad *et al.* (32) showed that body weight had no significant effect on milk urea.

2.2.3.3 Parity

Oltner *et al.* (31) showed that cows in their first lactation had, on average, 2.13 mg/dl lower milk urea concentration than older animals. This might be due to the fact that first-lactation animals are still growing and may therefore utilize amino acids more effectively. The consequence of this would be reduced deamination and urea formation in the liver. However, in their study, first lactation animals had a lower average days in milk than the multiparous cows. First lactation animals should not experience energy deficits of the same magnitude that multiparous animals experience early in lactation because they are not producing as much milk. Carlsson *et al.* (56) did a preliminary analysis suggesting that during the winter months, multiparous cows had slightly higher milk urea concentrations than primiparous cows. However, different studies (32;55;59;60) have shown that parity had no significant effect on the MUN diurnal changes. Data from Ontario DHI (54) and data

from 62 Quebec Holstein herds (61) showed that MUN values varied only slightly when lactation number was considered. First lactation cows may have lower MUN levels than older cows, but differences are likely to be small.

2.2.3.4 Stage of lactation

At the onset of lactation, crude protein, true protein, and NPN in milk have been reported to be high, with means of 3.83%, 3.57% and 0.26%, respectively. These fall to minimums of 3.11%, 2.97%, and 0.14% during the first 2 months of lactation followed by a gradual increase to approximately the original concentrations at 12 months in lactation (61). Milk urea concentration was much lower in early lactation when compared to late lactation (62) regardless of parity (2;56).

There are many factors that may relate to this low MUN at peak lactation. One factor may be the inability of cows to ingest sufficient feed early in lactation, resulting in preferential intake of the grain portion of the ration and/or reduced protein consumption relative to the increased milk production (38). Another factor may be the rumen environment. Ruminal papillae are in a growth phase and the ruminal microflora are adapting to the change in diet after calving. These factors result in sub-optimal function of the rumen (63).

However, there are other researchers with conflicting reports on the effect of days in milk on MUN values. Ubertalle *et al.* (64) reported that Italian Friesian cows fed a total mixed ration had average milk urea values that did not change during the lactation. In a

different study, Schepers and Meijer (65) reported that stage of lactation did not significantly influence milk urea concentration. It is unclear whether there truly is a days in milk effect on MUN values.

2.2.3.5 Milk yield

Hewett (66) showed that milk urea values may vary with milk yield as well. MUN levels tended to be higher in high producing herds compared to low producing herds (31;67). Carlsson and Pehrson (68) showed that the mean annual milk yield of the herds with low milk urea nitrogen was less than the yield of herds with normal MUN concentration. In contrast, Gustafsson *et al.*; Ropstad *et al.* and Eicher *et al.* (4;32;36) found no significant correlation between milk urea and milk yield.

2.2.3.6 Mastitis

Mastitis can influence the urea concentration in a quarter sample. Using wet chemistry, milk from quarters with a positive California mastitis test was 2.7 mg/dl lower in MUN when compared to MUN from healthy quarters (69). Sampling from infected quarters should be avoided, as a changed permeability of cell membranes may affect the milk urea concentration (14). It is unknown whether this difference would be observed using the infrared method of determination, based on changes to the spectrophotometric appearance of the mastitic milk.

2.2.4 Herd management factors

2.2.4.1 Protein and energy levels in ration

High levels of urea in serum likely indicate a protein/energy imbalance in a dairy herd (3;32;33;38;70). The level of SUN and MUN depend largely on the efficiency and the adequacy of protein utilization in ruminants. Increased levels of protein intake were associated with increases in the level of urea nitrogen in plasma (22;32;71-73) and milk (12;22;26;30;32). The increased urea levels are due primarily to the production of excess ammonia in the rumen and conversion of the ammonia to urea by the liver. Varying the percentages and the proportions of degradable and undegradable protein in the diet significantly affects MUN (22;33).

The source of dietary N also influences the urea levels (74). In a hay-urea fed group of cows, MUN and SUN were significantly higher than in a silage fed group due to a rapid absorption of urea from the hay-urea diet. The prolonged feeding period in this group provided a continuous supply which elevated serum and milk urea levels (39). Ropstad *et al.* (32) observed that the quantity of undegradable protein versus degradable protein, and the intake of digestible crude protein significantly affected the MUN concentration.

Milk urea nitrogen is also affected by the balance between dietary energy and protein in the ration (4;12;30-32;47;67;75-78). There exists a relationship between the levels of carbohydrate and protein in the diet and the amount of rumen ammonia produced. Microbial

growth in the rumen depends on a source of carbohydrate to supply ATP and C skeletons for biosynthesis of cell material. Rate of microbial growth depends on the rates of ATP production (carbohydrate fermentation) and ammonium production (79). Microbial protein synthesis decreases when ruminal carbohydrate is limited (80). If dietary energy is deficient, the effect of feeding excess rumen degradable protein will result in increased ruminal ammonia. Nocek and Russel (81) found that the level of fermentable carbohydrate affected the ammonia utilization by the microbial population in the rumen. They also found that degradation rate of dietary protein in the rumen affects the rumen ammonia pool. The urea content of milk decreases when the energy to nitrogen ratio of the diet increases (30;31;39). Therefore, rumen fermentable carbohydrates play an important role in determining MUN concentrations by supplying energy and carbon skeletons for microbial protein synthesis. To ensure high rumen microbial protein synthesis, nonstructural carbohydrate should constitute 35 to 45 % of dietary dry matter (7).

2.2.4.2 Feeding management system

Various feeding management systems are used successfully in Canadian dairies. Total mixed rations (TMR) and component based rations with or without summer pasture are examples of the various feeding management systems. Each management system has its own economic, production and health-related advantages and disadvantages. While TMR feeding management provides a more easily controlled ration program for the herd, a pasture system offers reduced feeding and operating costs at certain times of the year (82).

2.2.4.2.1 Effect of pasture

Several studies have reported elevated BUN levels (15;83;84) and MUN levels (14;35;67) when cows were put on pasture. Hammond (52) showed that plasma urea nitrogen varies with protein solubility as well as dietary protein level and time after feeding in diets with the same energy content. Ubertalle *et al.* (64) reported that Valdostana Red Pied cows had urea values that correlated with grass quality and composition. High urea levels were observed when cows grazed alpine pastures rich in protein and the concentrate that was fed did not meet the energy requirement. Under these conditions, supplementation of the diet with easily degradable nitrogen-containing substances is obviously contraindicated. If economically and practically feasible, supplementation with degradable energy-yielding feedstuffs could result in a more efficient microbial utilization of the ammonia (31).

2.2.4.2.2 Total Mixed Rations (TMR)

Carroll *et al.* (71) studied the impact of dietary CP concentration (13 vs.20%) and feeding strategy (total mixed ration vs component feeding) on urea concentrations and reproductive performance of 57 early lactation dairy cows. Cows fed a TMR had lower ruminal ammonia (11.4 vs 13.6 mg/100 ml) and PUN (16.8 vs 17.6 mg/ 100 ml) when compared to cows fed component rations. Cows fed a 20% CP ration had higher CP intake (kg/d), higher ruminal ammonia (18.1 vs 7.0 mg/100 ml), higher PUN (24.5 vs 10.0 mg/100 ml) and higher vaginal mucus urea levels (20.9 vs 8.2 mg/100 ml) compared to cows fed a 13% CP ration. These high vaginal urea levels can be toxic to sperm, ovum and the

developing embryo. Ubertalle *et al.* (62) reported that cows fed a total mixed ration had average milk urea nitrogen values that did not change during lactation.

In summary, feeding a total mixed ration versus component feeding will influence the milk urea concentration due to differences in feeding schedule and frequency of feeding. Component feeding tended to increase urea concentration more than TMR feeding following consumption (44).

2.2.5 Season

Seasonal variation in MUN levels is closely linked to the exposure of cows to pasture. Urea nitrogen levels are normally higher in the summer when animals are grazing, compared to the winter when animals are housed (84;85). Concentrations of blood urea (15) and MUN (56;68) were lower during the housed winter period compared to those of the summer grazing period. Ferguson *et al* (55) reported summary MUN data by season in Pennsylvania: winter, 14.01 mg/dl; spring, 14.98 mg/dl; summer, 16.31 mg/dl; and fall, 14.19 mg/dl. Hof *et al.* (47) found that during the summer, grazing cows ingested mainly grass with a high N content which resulted in an increase in the NPN content of milk. Refsdal *et al.* (78) found that the season had an effect on bulk tank MUN levels with urea levels being highest in the summer and early fall. Their data showed three MUN peaks: beginning of June, mid July and end of September. There was a significant difference ($P < 0.005$) between the highest mean value obtained in June (5.4 $\pm$ 1.9 mmol/l) and the lowest mean in November (4.5 $\pm$ 0.9 mmol/l). The peaks reflected feed changes and use of N

fertilizer commonly practiced in the area. Ontario DHI data indicate two peaks, June and September, were 14.8 mg/dl and 15.6 mg/dl, respectively. The rest of the months fell into a narrow range of 13.5 mg/dl to 14.5 mg/dl (54).

2.2.6 Region

Studies that have looked at geographic variation in NPN levels in England and Wales (15), Norway (78) and four different regions in California (86), have concluded that any variation was a result of variation in dairy management practices, especially in the feeding systems used. Dairy farms from the same area also had similar MUN values. During the winter, grass silage was the main roughage fed in the eastern part of Norway. Some farmers fed roots, hay, and ammonia treated straw etc. along with grass silage in the winter. In these herds, individual herd bulk milk urea often reflected the changes in diet composition (78). MUN was independent of geographical region. Therefore, differences in breed and management practices, and especially in feeding regime, contributed to difference in MUN values.

2.3. Effect of elevated MUN

2.3.1 Reproductive system

Surplus protein feeding can have a detrimental effect on reproductive performance. Fertilization failure or early degeneration of embryos may occur in cows fed excess rumen

degradable protein (87). Sonderegger and Schurch (88) showed that a surplus of rumen degradable protein extended the interval between calving and first service. Protecting protein from ruminal degradation can improve conception rates, decrease the number of services per conception and decrease days open (89).

Researchers have reported that cows fed a high crude protein diet; 19% (90), 20% (60), 21% (91) had lower conception rates than those of cows fed a low protein diet (15% CP) regardless of parity. Barton *et al.* (92) showed that cows fed the 20% CP diet had numerically more days open than did cows fed the 13% CP diet (71.4 vs 80.7, $P = 0.1072$). Butler *et al.* (25) found that urea nitrogen concentrations greater than 19 mg/dl in plasma and milk were associated with decreased conception rates in dairy cattle. Kim *et al.* (93) also showed that cows fed high protein diets had elevated BUN level (21.5 mg/dl) and a first service conception rate of 7.1% when compared to cows fed a normal protein level which had a BUN of 16.6 mg/dl and first service conception rate of 56.2%. The relationship between PUN and conception rate from the first AI was evaluated in 160 lactating dairy cows (3). The study showed that conception rate was reduced ($P < 0.02$) in cows with PUN >19 mg/dl.

Three general theories have been proposed to explain how high dietary protein levels suppress fertility (90), including (details provided below):

1) direct toxic effects of either urea or ammonia on the uterine environment;

2) alteration of gonadotropin or progesterone secretion by urea or ammonia; or

3) an imbalance in the protein:energy relationship leading to a negative energy

balance that reduces fertility.

Jordan *et al.* (73) found that the ammonia concentration increased in uterine secretions and blood of cows fed high protein diets. He suggested that enzymes of the urea cycle did not convert all of the excess ruminal ammonia into urea-N, possibly because uptake into hepatic cells was insufficient. Another possibility is that ammonia in the intestines, which is dependent on the dietary protein, may diffuse across the peritoneal cavity to the peripheral circulation without passing through the liver.

A significant relationship (r = 0.80) exists between concentrations of urea in plasma and uterine secretions (73). This relationship suggests urea readily diffuses into the uterine lumen where it might impair fertility. Duby *et al.* (94) showed that urea concentrations in the reproductive tract fluids and blood were altered by crude protein level of the diet. High ammonia and urea levels in uterine fluid were toxic to spermatozoa in rats and humans (95), ova and embryos in cows (9;87;96) and in ewes (97). Breau *et al.* (98) found that the ability of the spermatozoa to migrate through synthetic cervical mucus decreased when exposed to both 100 and 50 mg/ 100 ml urea solutions. Larson *et al.* (99) found that increased MUN was statistically associated with decreased fertility. A high concentration of urea in body fluids at breeding may cause fertility failure or very early embryonic loss prior to maternal recognition of pregnancy.

A second mechanism, proposed by Jordan *et al.* (73), is that high urea N or ammonia concentrations in body fluids may reduce leutenizing hormone (LH) binding to ovarian

receptors. Decreased LH binding leads to a decreased serum progesterone and decreased fertility.

Elrod and Butler (100) found that in the high protein group (21% CP diet), luteal phase uterine pH was significantly lower than in the normal protein group (15% CP diet) in nulliparous heifers. The uterine pH in the high protein group was similar to the uterine pH observed at estrous. Increased blood urea nitrogen decreased uterine pH several days after breeding, which could make the uterine environment more hostile to the early embryo. This effect of excess dietary protein on uterine pH was also observed by Elrod *et al* (101) in lactating cows. In that study, uterine pH on d 7 was 7.13 in cows fed a diet with balanced undegradable intake protein (UIP) and degradable intake protein (DIP), 6.95 in cows fed high UIP diet and 6.85 in cows fed high DIP diet. This result indicated that both UIP and DIP, when in excess, can have similar effects on uterine pH.

A relative energy deficit may also explain some of the detrimental effects of high protein diets on reproductive performance (60;90;102). High urea concentrations may indicate the presence of a low dietary energy:protein ratio. In this case inadequate dietary energy may be the primary cause of infertility. Alternatively, if there is insufficient available energy to the rumen flora when a high amount of degradable protein is broken down into ammonia, the ammonia is absorbed through the rumen lining into the blood, and passes to the liver where it is converted into urea. This conversion requires energy, and it has been suggested that the added energy cost of liver urea synthesis may result in further exacerbation of the negative energy balance in a postpartum cow (48) .

2.3.2 Production

Clark and Davis (103) observed that an excess of both degradable and undegradable protein in the rumen reduced milk production and efficiency of feed utilization. Sklan and Tinsky (104) observed that lack of degradable intake protein and crude protein reduced milk production. A number of reasons could explain this relationship. Folman *et al.* (89) showed that the high rumen ammonia concentration impaired milk production by increasing energy utilization. Excess ammonia is also damaging to the ruminal papillae. This damage leads to poor absorption of nutrients and ruminal acidosis. These two conditions reduce feed intake and result in lower milk production. It has been suggested that milk urea nitrogen values over 20 mg/dl could decrease milk production by 3 kg/day due to the energy cost involved in converting ammonia to urea (38). Bazeley *et al.* and Manson *et al.* (105;106) found that herds fed excessively high levels of dietary protein had a high incidence of laminitis. The laminitis may be due to increased production stress in these herds, high concentrate:forage ratio in the diet, or an effect of the protein itself. Excess protein may cause laminitis by upsetting the protein balance in the rumen, resulting in excess ammonia in the blood, although it is unclear how high ammonia levels lead to laminitis. The second way is through the production of toxic metabolites (eg. histamine) that can cause ischemic damage to capillaries of the feet.

2.4 Interpretation of MUN

2.4.1 Individual cow values, group averages and bulk tank milk values

Evaluation of milk urea or MNPN and milk true protein (MTP) from individualcows may be beneficial in the evaluation of herd feeding programs. An advantage of using MUN values from individual cow samples is that the values can be adjusted for days in milk, feeding group, and age of the cow. In order to utilize individual milk sample values, normal ranges for MTP , MNPN and MUN under varying management systems and production levels are required (3). However, even when cows were fed similarly, there were prominent differences among individual cows (30). Oltner *et al.* (31) observed that MUN concentrations could differ as much as 2-3 mmol/L between cows under similar nutritional management. This amount of variation suggests that interpretation of MUN data should be based on group means rather than on individual observations. Cannas *et al.* (13) observed large individual variation in MUN concentrations among ewes fed the same rations. Concentrations of urea in milk are variable from herd to herd and within cows in the same herd. It is impossible for a single milk urea nitrogen value to estimate with certainty the nutritional status of an individual cow (31;38;65). When mean milk urea concentrations were determined for groups of animals or a herd, direct inferences regarding nutritional adequacy are possible (12;13;27;31;65;107). Ferguson (44) suggested that testing less than 8 cows will not result in a meaningful average value of milk urea and should be interpreted with caution.

Bulk tank MUN are commonly used to report MUN concentrations in Europe (49). At the herd level, bulk milk MUN levels yield information about the overall protein/energy ratio in the herd's ration (108). Based on these studies, bulk tank MUN can be used for evaluation of the urea status of the herd (23;56).

One common question asked is whether single bulk milk MUN might be used instead of individual cow MUN's. The cost advantage makes bulk tank samples an attractive alternative to sampling individual cows. Schepers and Meijer (65) pointed out that interpretation of bulk tank MUN will be difficult if multiple feeding groups exist within a herd. There is increasing evidence that suggests the bulk milk MUN is less useful than a herd profile based on individual cow values. The main reasons that single bulk milk MUN is not a good substitute for individual cow MUN values are (109):

1. Each cow contributes a different volume of milk to the bulk tank. Depending on cow to cow variations such as the age, stage of lactation and health of the cow, the amount of milk and MUN that are contributed to the bulk milk tank will vary. Consequently, individual bulk milk tank sample will not fairly represent the entire herd.
2. Collection of a proper bulk milk sample is difficult. Only licenced milk transporters are permitted to remove milk from the bulk tank. Transporters are trained to collect bulk milk samples that are representative of the tank. Improper collection of the bulk milk sample may produce incorrect bulk tank MUN values.

In summary, given the variation in MUN, values for individual cows should not be interpreted. They should not to be used to move individual cows between different ration groups. Mean values for groups of at least 8 cows should be computed and interpreted. In addition, only cows that have free access to feed and are healthy should be tested (44). Perhaps bulk tank MUN values should not be interpreted without group MUN averages.

2.4.2 Normal MUN level

Research has shown that there is a normal range for MUN concentration. This range allows urea concentration to serve as a monitoring tool that assesses the nutritional status in a herd (44). Normal levels for MUN vary somewhat depending on the procedure and the laboratory used. Normal urea levels were initially reported to be near 10 mg/dl of serum and milk (70). Ferguson *et al.* (110) reported that with automated milk testing, the normal MUN range was between 10 - 14 mg/dl, with standard deviation of 4 mg/dl. Roseler *et al.* (33) reported that mid- to late-lactation cows consuming a diet balanced for UIP and DIP should have MUN concentrations of 11.6 mg/dl. Carlsson and Pehrson (111) observed a mean MUN of 13.3 mg/dl when the diet was balanced in metabolizable energy and digestible crude protein. Nelson (112) found MUNs that ranged between 12-16 mg/dl. Broderick and Clayton (11) found that mean MUN concentration was 14.8 mg/dl.

Using a Foss 4000 milkoscan analyzer, Godden(113) reported an average of 13.7 mg/dl, with standard deviation 2.4 mg/dl (n = 4,756 samples from 60 herds). Ferguson (44) showed that Pennsylvania DHIA cows had an average MUN of 14 mg/dl, with standard deviation of 4.03 mg/dl (n = 312,005 from 1731 herds). Grexton (54) showed Ontario DHI data with an average MUN for the year of 14.2 mg/dl, varying between 12.9 mg/dl and 15.4 mg/dl, depending on the month with June to September tending to have the highest MUN values compared to the rest of the year. Eighty percent of the Ontario herds were in the range of 10-18 mg/dl.

In summary, a normal MUN range might reasonably be considered to be 10-16 mg/dl. A producer should be concerned when a group of cows, or the herd, has an average value below 10 mg/dl or above 16 mg/dl. The protein and carbohydrate availability in the diet should be evaluated. However, these results should be interpreted in light of all of the factors discussed above which influence MUN values.

2.4.3 High MUN level

The main reason for high MUN levels is inappropriate nutrition. A surplus of rumen degradable protein on its own or coupled with a shortage of rumen available energy in the diet results in excess ammonia and increased MUN (24;81;114;115). Poor carbohydrate to protein match leads to rumen nitrogen waste, even if the protein level in the diet is "normal". Nitrogen and energy must be available at the same time for ruminant microbes to be most efficient in the production of microbial protein (107).

Pathological and physiological abnormalities can also cause a high urea level, although likely only at the cow level. Prerenal azothemia (high BUN) may result from reduced renal perfusion, hypovolemia and dehydration(116)(116). Congestive heart failure and ammonia toxicity can also increase urea levels (117). Renal azothemia can arise from acute or chronic renal failure. Post-renal azothemia can result from urolithiasis or urinary bladder rupture. There are many others causes of renopathies that lead to azotemia: toxicity, heavy metals, non-steroid, anti-inflammatory drugs, aminoglycoside antibiotics and poisonous plants.

In summary, urea is mostly excreted by the kidney, and blood and milk urea is increased in renal failure. However, in healthy animals the serum and milk urea levels are primarily affected by feeding. Excess crude protein or an imbalance of UIP and DIP or lack of energy supply can elevate MUN.

2.4.4 Subnormal MUN level

Urea levels may be low if the ration is deficient in protein resulting in the formation of less ammonia. Also if there is an excess of fermentable energy from carbohydrates relative to protein availability in the diet (16;31;90;111;115;116), MUN will be lower than normal. Insufficient ruminal ammonia results in reduced microbial protein production which is possibly associated with sub-optimal production (38).

Liver failure, malabsorption, the use of anabolic steroids and over hydration can also reduce urea levels. Because urea is excreted from the body through urine, increasing water intake may increase urine production and decrease urea concentration (38).

Whitaker *et al.* (118) reported that urea nitrogen concentrations were significantly lower in bovine somatotropin treated cows. This suggests that bovine somatotropin treated cows utilize metabolizable protein more efficiently.

2.4.5 Uses of MUN

2.4.5.1 Feeding management

It may be possible to check inappropriate protein feeding by evaluating SUN and/or MUN levels (70;70). Efficiency of protein feeding is maximized when the N supplied in the diet is synchronized with the N required by ruminal microbes and essential amino acid required by ruminant tissue. This balance is associated with a baseline concentration of urea in plasma and milk (24). Therefore, MUN can be used to monitor feeding programs on a dairy farm. It can also be used to improve feeding management, nutrition programs and evaluate protein efficiency status.

Assessment of these production parameters can result in recommendations that may improve the efficiency of protein utilization and milk production (4;28;30;32;40;62;67;70;78;119;120). Another advantage of MUN evaluation is that it can help reduce excess protein feeding and hence reduce feeding costs. Overfeeding protein appears to be wasteful in that it is expensive and potentially impairs reproduction efficiency in dairy cattle without increasing milk production (3;24;76;89;90;121). Decreased feeding of protein was associated with saving in feeds purchased (113). Some of the protein requirements for milk production greater than 25 to 30 kg/day must come from undegradable intake protein (UIP) or body reserves (89). Balancing undegradable intake protein (UIP) to NRC requirement had a positive influence on milk protein yield. Supplying excess undegradable intake protein (UIP) did not increase milk protein yield (33;122).

There are two costs associated with high MUN. First, high MUN is an indication of inefficient protein utilization and potentially excess protein in the diet, resulting in increased feed cost. Second, there is an "energy cost" to transform ammonia to urea. Energy deficient diets generate excess ammonia and the energy cost of converting ammonia to urea renders the diet even more energy deficient (54).

2.4.5.2 Fertility management

Gustafsson (49) pointed out that MUN could be used in the fertility management program for high reproductive efficiency. Urea nitrogen levels affect the reproductive performance of cows (38). The number of days to first ovulation increases because the conversion of ammonia to urea requires energy, worsening the negative energy balance (71;123).

Gustafsson and Palmquist (4) reported that herds with high MUN (>15.4 mg/dl) had an increase in the calving to first service interval and a longer calving to conception interval. Days to first service was longer in herds with either low (<11.2 mg/dl) or high (>16.8 mg/dl) MUN concentrations in a study of 29 Swedish herds fed silage and concentrate (119). The first service conception rate was reduced in cows that had MUN values greater than 18 mg/dl (124), >19 mg/dl (25), >20.4 mg/dl (35). Larson *et al.* (99) found that cows with milk urea nitrogen concentrations > 21 mg/dl were more likely to be detected in estrus at 21 days following breeding compared to cows with MUN < 21 mg/dl. The calving to first service and the calving to conception intervals were significantly longer in the herds with low MUN

concentration (68).

Melendez *et al.* (125) found an interaction between breeding season (summer) and elevated MUN levels. Non pregnancy relative odds increase from 1.02 to 17.87 when elevated MUNs were present between May and October.

On the contrary, Godden *et al.* (113) found a different result. She found that MUN data produced by routine DHI can not be useful as a tool to improve the reproductive performance. She concluded that the lack of association between MUN and reproductive performance may have been affected from the other factors such as season, and herd management.

In conclusion, the correlation between MUN and reproduction is of great interest. Research has demonstrated that conception rate decreases at a MUN level above approximately 18-19 mg/dl. Therefore, it may be beneficial to dairy producers to monitor urea concentrations in bulk tank milk or body fluids of cows in efforts to improve reproductive efficiency.

2.4.5.3 Environmental pollution management

Nutritional management is important for preventing environmental pollution, by improving the nitrogen efficiency in dairy production and avoiding high losses from manure to air and water (49). Tamminga (126) stated that fecal and urinary losses of nitrogen can

be reduced by increasing feed quality through lowered endogenous protein losses. Appropriate protein energy synchronization is also important. Excess MUN represents dietary CP that is not utilized for productive purposes. Urea levels in milk appear to be a useful parameter to estimate the urinary N losses (26;58). Milk urea offers more information pertaining to urinary nitrogen losses than does the protein and energy balance in the ration (43).

Nutrition plays a paramount role in animal health and production, and hence represents a core element in the dairy farm. The intelligent use of metabolic profile tests allows assessment of nutritional management (127). Milk urea measurements seem to provide reliable information on the metabolic (protein/energy) balance in the cow (28;39). The sampling and analysis of milk is simple and inexpensive so it is the most economical way to determine the urea status of cows and herds (31-33;38). MUN provides an index of N utilization in the lactating dairy cow and identifies opportunities to improve nutritional management. This provides another tool to dairy farmers for minimizing feed cost and while maximizing production. However, it should not be used in isolation from evaluating other management procedures involving production and nutritional efficiency within dairy herds.

2.5 References

1 Baker LD, Ferguson JD, Ramberg CF. Kinetic analysis of urea transport from plasma to milk in dairy cows. J Dairy Sci 1992;75 [suppl.1], 181.

2 DePeters EJ, Cant JP. Nutritional factors influencing the nitrogen composition of bovine milk: A review. J Dairy Sci 1992; 75(8):2043-2070.

3 DePeters EJ, Ferguson JD. Nonprotein nitrogen and protein distribution in the milk of cows. J Dairy Sci 1992; 75(11):3192-3209.

4 Gustafsson AH, Palmquist DL. Diurnal variation of rumen ammonia, serum urea and milk urea in dairy cows at high and low yields. J Dairy Sci 1993; 76(2):475-484.

5 Karman AH, van Boekel MAJS. Evaluation of the Kjeldahl factor for conversion of the nitrogen content of milk and milk products to protein content. Neth Milk Dairy J 1986; 40:315-336.

6 Symonds HW, Mather DL, Collis KA. The maximum capacity of the liver of the adult dairy cow to metabolize ammonia. Br J Nutr 1981; 46:481-486.

7 Hoover WH, Miller TM. Rumen digestive physiology and microbial ecology. Vet Cli North Am:Food Animal Practice 1991; 7(2):311-325.

8 Wohlt JE, Clark JH, Blaisdell FS. Effect of sampling location, time, and method of concentration of ammonia nitrogen in rumen fluid. J Dairy Sci 1976; 59(3):459-464.

9 Visek WJ. Ammonia: its effects on biological systems, metabolic hormones, and reproduction. J Dairy Sci 1984; 67:481-498.

10 Bruckental I, Oldham JD, Sutton JD. Glucose and urea kinetics in cows in early lactation. Br J Nutr 1980; 44:33-45.

11 Veen WGA, Bakker YTJ. The influence of slowly and rapidly degradable concentrate protein on a number of blood parameters in dairy cattle. Netherlands Journal of Agricultural Science 1988; 36:51-62.

12 Broderick GA, Clayton MK. A statistical evaluation of animal and nutritional factors influencing concentrations of milk urea nitrogen. J Dairy Sci 1997; 80(11):2964-2971.

13 Cannas A, Pes A, Mancuso R, Vodret B, Nudda A. Effect of dietary energy and protein concentration on the concentration of milk urea nitrogen in dairy ewes. J Dairy Sci 1998; 81(2):499-508.

14 Carlsson J, Bergstrom J. 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 vet scand 1994; 35:67-77.

15 Payne JM, Rowlands GJ, Manston R, Dew SM. A statistical appraisal of the results of metabolic profile tests on 75 dairy herds. Br Vet J 1973; 129:370-381.

16 Hammond AC. The use of blood urea nitrogen concentration as an indicator of protein status in cattle. Bovine Pract 1983; 18(November):114-118.

17 Lewis D. Blood urea concentration in relation to protein utilization in the ruminant. J Agric Sci Camb 1957; 48:438-446.

18 Biddle GN, Evans JL. Nitrogen utilization in cattle using a nitrogen depletion-repletion technique. J Anim Sci 1973; 36(1):123-129.

19 Blowey RW. A practical application of metabolic profiles. Vet Rec 1975; 124(October):324-327.

20 Manston R, Russell AM, Payne JM, Dew SM. The influence of dietary protein upon blood composition in dairy cows. Vet Rec 1975; 96:497-502.

21 Prewitt LR, Kertz AF, Lane AG, Campbell JR, Weinman DE. Effects of dietary protein on blood, urine and milk composition. Am J Vet Res 1971; 32(3):393-397.

22 Baker LD, Ferguson JD. Milk urea nitrogen as a metabolic indicator of protein feeding efficiency on dairy farms. Proc Am Assoc Bov Prac 1993; 26(January):165-166.

23 Refsdal AO. Urea in bulk milk as compared to the herd mean of urea in blood. Acta vet scand 1983; 24:518-520.

24 Baker LD, Ferguson JD. Response in urea and true protein of milk to different protein feeding schemes for dairy cows. J Dairy Sci 1995; 78(11):2424-2434.

25 Butler WR, Calaman JJ, Beam SW. Plasma and milk urea nitrogen in relation to pregnancy rate in lactating dairy cattle. J Anim Sci 1996; 74:858-865.

26 Gonda HL, Lindberg JE. Evaluation of dietary nitrogen utilization in dairy cows based on urea concentrations in blood, urine, milk, and on urea concentration of purine derivatives. Acta Agric Scand Sect A Animal Sci 1994; 44:236-245.

27 Kolver ES, MacMillan KL. Short term changes in selected metabolites in pasture fed dairy cows during peak lactation. Proceedings of the New Zealand Society of Animal Production 1993; 53:77-81.

28 Lyatuu ET, Eastridge ML. Nutritional factors affecting milk production, milk composition, milk urea nitrogen, and plasma urea nitrogen. Special-Circular -Ohio-Agricultural-Research-and-Development-Center 1998; 163:65-71.

29 Martinez N, DePeters EJ, Bath DL. Supplement niacin and fat effects on milk composition of lactating Holstein cows. J Dairy Sci 1991; 74(1):202-210.

30 Oltner R, Wiktorsson H. Urea concentrations in milk and blood as influenced by feeding varying amounts of protein and energy to dairy cows. Livest Prod Sci 1983; 10:457-467.

31 Oltner R, Wiktorsson H, Emanuelson M. Urea concentrations in milk in relation to milk yield, live weight, lactation number and amount and composition of feed given to dairy cows. Livest Prod Sci 1985; 12:47-57.

32 Ropstad E, Vik-Mo L, Refsdal AO. Levels of milk urea, plasma constituents and rumen liquid ammonia in relation to the feeding of dairy cows during early lactation. Acta vet scand 1989; 30:199-208.

33 Roseler DK, Ferguson JD, Sniffen CJ, Herrema J. Dietary protein degradability effects on plasma and milk urea nitrogen and milk nonprotein nitrogen in Holstein cows. J Dairy Sci 1993; 76(2):525-534.

34 Wattiaux MA, Combs DK, Shaver RD. Lactational responses to ruminally undegradable protein by dairy cows fed diets based on alfalfa silage. J Dairy Sci 1994; 77(6):1604-1617.

35 Wittwer FG, Gallardo P, Reyes J, Opitz H. Bulk milk urea concentrations and their relationship with cow fertility in grazing dairy herds in Southern Chile. Prev Vet Med 1999; 38:159-166.

36 Eicher R, Bouchard E, Tremblay A. Cow level sampling factors affecting analysis and interpretation of milk urea concentrations in 2 dairy herds. Can Vet J 1999; 40(7):487-492.

37 Godden SM, Lissemore KD, Kelton DF, Leslie KE, Lumsden T, Walton JS. A study to evaluate the test characteristics of Milk Urea Nitrogen assay and its relationship to reproductive performance in dairy cows. Proc Am Assoc Bov Prac 1996; 29.

38 Moore DA, Varga GA. BUN and MUN: Urea nitrogen testing in dairy cattle. Comp Cont Ed Pract Vet 1996; 18(6):712-720.

39 Miettinen PVA, Juvonen RO. Diurnal variation of serum and milk urea nitrogen levels in dairy cows. Acta Agric Scand Sect A Animal Sci 1990; 40:289-296.

40 Godden SM, Lissemore KD, Leslie KE, Lumsden T, Walton JS, Kelton DF. Preliminary result from a study evaluating the test characteristics of a milk urea nitrogen assay and the influence of factors related to sample collection and handling. Proc Am Assoc Bov Prac 1996; September:169-170.

41 Oltner R, Sjaunja L. Evaluation of a rapid method for the determination of urea in cow's milk. Acta vet scand 1982; 28:39-45.

42 Coggins CRE, Field AC. Diurnal variation in the chemical composition of plasma from lactating beef cows on three dietary energy intakes. J Agric Sci Camb 1976; 86:595-602.

43 Ciszuk P, Gebregziabher T. Milk urea as an estimate of urine nitrogen of dairy cows and goats. Acta Agric Scand Sect A Animal Sci 1994; 44:87-95.

44 Ferguson JD. Milk urea nitrogen. Ferguson JD, editor. http://cahpwww.nbc.upenn.edu/mun/mun_info.html , 1-6. 6-29-1999.

45 Manston R, Rowlands GJ, Little W, Collis KA. Variability of the blood composition of dairy cows in relation to time of day. J Agric Sci Camb 1981; 96:593-598.

46 Thomas PC, Kelly JM. The effect of frequency of feeding on milk secretion in the Ayrshire cow. J Dairy Res 1976; 43:1-7.

47 Hof G, Vervoorn MD, Lenaers PJ, Tamminga S. Milk urea nitrogen as a tool to monitor the protein nutrition of dairy cows. J Dairy Sci 1997; 80(12):3333-3340.

48 Godden SM. Evaluation of a milk urea assay, and the relationship between milk urea concentrations and nutritional management and performance in Ontario dairy herds. [PhD dissertation], Guelph, Ontario, University of Guelph, 1998.

49 Gustafsson GA. Current status concerning the use of milk urea concentration: analytical methods, use in different countries and how to utilize the results. Proceeding of the 30th Biennial Session of ICAR, Velhoven, The Netherlands, 1996:223-228

50 de Jong EAM, Klomp H, Hemert HV. Evaluation of a segmented flow method for the routine determination of urea in milk. Neth Milk Dairy J 1992; 46:115-122.

51 Rodriguez LA, Stallings CC, Herbein JH, McGilliard ML. Effect of degradability of dietary protein and fat on ruminal, blood and milk components of Jersey and Holstein cows. J Dairy Sci 1997; 80(2):353-363.

52 Hammond AC. Effect of dietary protein level, ruminal protein solubility and time after feeding on plasma urea nitrogen and the relationship of plasma urea nitrogen to other ruminal and plasma parameters. J Anim Sci 1983; 57[supple 1], 435.

53 Cerbulis J, Farrell HM. Composition of milks of dairy cattle. 1.Protein, lactose, and fat contents and distribution of protein fraction. J Dairy Sci 1975; 58(6):817-827.

54 Grexton B. MUN data on Ontario herds offers up some interesting information. http://www.ontdhi.com/mundata.htm . 1999.

55 Ferguson JD, Thomsen N, Slesser D, Burris D. Pennsylvania DHIA milk urea testing. J Dairy Sci 1997; 80[suppl.1], 161.

56 Carlsson J, Bergstrom J, Pehrson B. Variation with breed, age, season, yield, stage of lactation herd in the concentration of urea in bulk milk and individual cow's milk. Acta vet scand 1995; 36:245-254.

57 Diab IAK, Hillers JK. Effect of selection for milk yield and dietary energy on yield traits, bovine somatotropin, and plasma urea nitrogen in dairy cows. J Dairy Sci 1996; 79(4):682-688.

58 Jonker JS, Kohn RA, Erdman RA. Using milk urea nitrogen to predict nitrogen excretion and utilization efficiency in lactating dairy cows. J Dairy Sci 1998; 81:2681-2692.

59 Eicher R, Bouchard E, Bigras-Poulin M. Factors affecting milk urea nitrogen and protein concentrations in Quebec dairy cows. Prev Vet Med 1999; 39:53-63.

60 Kaim M, Folman Y, Neumark H, Kaufmann W. The effect of protein intake and lactation number on post-partum body weight loss and reproductive performance of dairy cows. Anim Prod 1983; 37:229-235.

61 Ng-Kwai-Hang KF, Hayes JF, Moxley JE, Monardes HG. Percentages of protein and nonprotein nitrogen with varying fat and somatic cells in bovine milk. J Dairy Sci 1985; 68(5):1257-1262.

62 Emanuelson M, Wiktorsson H. Long-term feeding of rapeseed meal and full-fat rapeseed of double low cultivars to dairy cows. Livest Prod Sci 1993; 33:199-214.

63 Liebich HG, Dirksen G, Arbel A, Dori S, Mayer E. Feeding dependent changes of the ruminal mucosa of high producing dairy cows during the dry period and first eight weeks post partum. J Vet Med A 1987; 34:661-672.

64 Ubertalle A, Profiti M, Battaglini LM, Mimosi A, Fortina R. Milk urea nitrogen of Italian Friesian and Valdostana Red Pied dairy cattle. Scienza-e-Tecnica-Lattiero-Casearia. 1998; 49[5], 249-265.

65 Schepers AJ, Meijer RGM. Evaluation of the utilization of dietary nitrogen by dairy cows based on urea concentration in milk. J Dairy Sci 1998; 81:579-584.

66 Hewett C. On the causes and effects of variations in the blood profile of Swedish dairy cattle. Acta vet scand 1974; suppl. 50. 1-151

67 Kaufmann W. Variation in composition of raw milk with special regard to the urea content. Milchwissenschaft 1982; 37[1], 6-9.

68 Carlsson J, Pehrson B. The relationships between seasonal variations in the

concentration of urea in bulk milk and the production and fertility of dairy herds. J Vet Med A 1993; 40:205-212.

69 Licata E. Subclinical mastitis and urea determination in cows' milk . Obiettivi e Doc -Vet 1985; 6(12):65-67.

70 Ide Y, Shimbayashi K, Yomenura T. Effect of dietary conditions upon serum and milk urea nitrogen in cows. 1. serum and milk urea nitrogen as affected by protein intake. Jap J Vet Sci 1966; 28:321-327.

71 Carroll DJ, Barton BA, Smith RD. Influence of protein intake and feeding strategy on reproductive performance of dairy cows. J Dairy Sci 1988; 71(12):3470-3481.

72 Howard HJ, Aalseth EP, Adams GD, Bush LJ. Influence of dietary protein on reproductive performance of dairy cows. J Dairy Sci 1987; 70(8):1563-1571.

73 Jordan ER, Chapman TE, Holtan DW, Swanson LV. Relationship of dietary crude protein to composition of uterine secretions and blood in high-production postpartum dairy cows. J Dairy Sci 1983; 66(9):1854-1862.

74 Oldham JD, Napper DJ, Smith T, Fulford RJ. Performance of dairy cows offered isonitrogenous diets containing urea or fishmeal in early and in mid-lactation. Br J Nutr 1985; 53:337-345.

75 Garcia AD, Linn JG, Stewart SC, Olson JD, Olson WG. Evaluation of milk urea nitrogen (MUN) as a dietary monitor for diary cows. J Dairy Sci 1997; 80[suppl.1], 162.

76 Grings EE, Roffler RE, Deitelhoff DP. Response of dairy cows in early lactation to additions of cottonseed meal in alfalfa-based diets. J Dairy Sci 1991; 74(8):2580-2587.

77 Kirchgessner M, Kreuzer M, Roth-Maier DA. Milk urea and protein content to diagnose energy and protein malnutrition of dairy cows. Arch Anim Nutr (Berlin) 1986; 36(2-3):192-197.

78 Refsdal AO, Bxvre L, Bruflot R. Urea concentration in bulk milk as an indicator of the protein supply at the herd level. Acta vet scand 1985; 26:153-163.

79 Hespell RB, Bryant MP. Efficiency of rumen microbial growth: influence of some theoretical and experimental factors or ^{Y}ATP. J Anim Sci 1979; 49(6):1640-1659.

80 Russell JB, Strobel HJ. Concentration of ammonia across cell membranes of mixed rumen bacteria. J Dairy Sci 1987; 70(5):970-976.

81 Nocek JE, Russel JB. Protein and energy as an integrated system.Relationship of ruminal protein and carbohydrate availability to microbial synthesis and milk production. J Dairy Sci 1988; 71(8):2070-2107.

82 Hovingh EP. The summer-fall slump:seasonal variation in average dairy milk production in PEI, Canada. [PhD dissertation], Charlottetown, Prince Edward Island, University of Prince Edward Island, 1998.

83 Peterson RG, Waldern DE. Repeatabilities of serum constituents in Holstein Friesian affected by feeding, age, lactation, and pregnancy. J Dairy Sci 1981; 64(5):822-831.

84 Rowlands GJ, Little W, Stark AJ, Manston R. The blood composition of cows in commercial dairy herds and its relationship with season and lactation. Br Vet J 1979; 135:64-74.

85 Verdi RJ, Barbano DM, Dellavalle ME, Senyk GF. Variability in true protein, casein, nonprotein nitrogen, and proteolysis in high and low somatic cell milks. J Dairy Sci 1987; 70(2):230-242.

86 Franke AA, Bruhn JC, Lawrence CM. Distribution of protein in California milk in 1983. J Dairy Sci 1988; 71(9):2373-2383.

87 Blanchard T, Ferguson JD, Love L, Takeda T, Henderson B, Hasler J. Effect of dietary crude-protein type on fertilization and embryo quality in dairy cattle. Am J Vet Res 1990; 51(6):905-908.

88 Sonderegger H, Schurch A. A study of the influence of the energy and protein supply on the fertility of dairy cows. Livest Prod Sci 1977; 4:327-333.

89 Folman Y, Neumark H, Kaim M, Kaufmann W. Performance, rumen and blood metabolites in high-yielding cows fed varying protein percents and protected soybean. J Dairy Sci 1981; 64(5):759-768.

90 Canfield RW, Sniffen CJ, Butler WR. Effects of excess degradable protein on postpartum reproduction and energy balance in dairy cattle. J Dairy Sci 1990; 73(9):2342-2349.

91 Ferguson JD, Galligan DT, Blanchard T, Reeves M. Serum urea nitrogen and conception rate : The usefulness of test information. J Dairy Sci 1993; 76:3742-3746.

92 Barton BA, Rosario HA, Andersson GW, Grindle BP, Carroll DJ. Effects of dietary crude protein, breed, parity, and health status on the fertility of dairy cows. J Dairy Sci 1996; 79(121):2225-2236.

93 Kim HS, Park SB, Kim CK, Chung YC, Lee JW, Kim CH. Effect of dietary crude protein level on blood urea nitrogen, milk yield and conception rate of dairy cows. Korean Journal of Dairy Science 1998; 20(3):163-168.

94 Duby RT, Tritschler JP, Prange RW, Abdul-Wahid FT. Effect of dietary crude protein on urea in fluids of the reproductive tract of ewes and dairy cows. J Anim Sci 1984;

59[Suppl. 1], 339.

95 Dasgupta PR, Kar AB, Dhar ML. Spermicidal activity of urea. Indian J Exp Biol 1971; 9:414-415.

96 Ferguson JD. Impact of protein nutrition on reproduction in dairy cows. J Dairy Sci 1989; 72(3):746-766.

97 Bishonga C, Robinson JJ, McEvoy TG, Aitken RP, Findlay PA, Robertson I. The effects of excess rumen degradable protein in ewes on ovulation rate, fertilization and embryo survival *in vivo* and during *in vitro* culture. Anim Prod 1994; 58: 447.

98 Breau WC, Boice ML, Tritschler JP, Prange RW, Duby RT. Migration ability through synthetic cervical mucus and acromal integrity of bull sperm after incubation with urea in vitro. Biol Reprod 1985; 32 (Suppl 1): 219.

99 Larson SF, Butler WR, Currie WB. Reduced fertility associated with low progesterone postbreeding and increased milk urea nitrogen in lactating cows. J Dairy Sci 1997; 80(7):1288-1295.

100 Elrod CC, Butler WR. Reduction of fertility and alteration of uterine pH in heifers fed excess ruminally degradable protein. J Anim Sci 1993; 71:694-701.

101 Elrod CC, Van Amburgh M, Butler WR. Alterations of pH in response to increased dietary protein in cattle are unique to the uterus. J Anim Sci 1993; 71:702-706.

102 Magdus M, Fekete S, Frenyo LV, Miskucza O, Kotz L. Milk production and certain parameters of energy metabolism in dairy cows fed rations of varying energy and crude protein contents and fat. Acta Vet Hung 1988; 36(1-2):43-59.

103 Clark JH, Davis CL. Some aspects of feeding high producing dairy cows. J Dairy Sci 1980; 63(6):873-885.

104 Sklan D, Tinsky M. Production and reproduction responses by dairy cows fed varying undegradable protein coated with rumen bypass fat. J Dairy Sci 1993; 76(1):216-223.

105 Bazeley K, Pinsent PJN. Preliminary observations on a series of outbreaks of acute laminitis in dairy cattle. Vet Rec 1984; 115:619-622.

106 Manson FJ, Leaver JD. The influence of dietary protein intake and of hoof trimming on lameness in dairy cattle. Anim Prod 1988; 47:191-199.

107 Seglar WJ. Maximum forage quality. Comp Cont Ed Pract Vet 1997; 19(10):s254-s261.

108 Ropstad E, Refsdal AO. Herd reproduction performance related to urea concentration in bulk milk. Acta vet scand 1987; 28:55-63.

109 Ontario DHI. Bulk MUN milk - Why not? http://www.ontdhi.com/bulk%20milk.html. 1999.

110 Ferguson JD, Terry Blanchard, Galligan DT, Hoshall DC. Infertility in dairy cattle fed a high percentage of protein degradable in the rumen. JAVMA 1988; 192:659-662.

111 Carlsson J, Pehrson B. The influence of the dietary balance between energy and protein on milk urea concentration: Experimental trials assessed by two different protein evaluation systems. Acta vet scand 1994; 35(2):193-205.

112 Nelson AJ. Practical application of MUN analyses. Proc Am Assoc Bov Prac 1996; 29(September):85-95.

113 Godden SM, Lissemore KD, Kelton DF, Leslie KE, Lumsden JH, Walton JS. Results of an observational study describing the relationship between milk urea concentrations and feeding management and performance in Ontario dairy herds. Proc Am Assoc Bov Prac 1999; 32:196-197.

114 Hutjens MC. Tools for the veterinarian to increase herd performance and profitability. Bovine Pract 1998; 32(1):68-72.

115 Lefebvre D. Urea in milk. Another tool at your disposal. Producteur-de-Lait-Quebecois 1996; 16[5], 13-15.

116 Little W, Sansom BF, Manston R, Allen WM. The effects of reducing the water intake of lactating dairy cows by 40% for 3 weeks. Anim Prod 1978; 27:79-87.

117 Bartley EE, Avery TB, Nagaraja TG, Watt BR, Davidovich A, Galitzer S Ammonia toxicity in cattle. V. Ammonia concentration of lymph and portal, carotid and jugular blood after the ingestion of urea. J Anim Sci 1981; 53(2):494-498.

118 Whitaker DA, Smith EJ, Kelly JM. Milk production, weight changes and blood biochemical measurements in dairy cattle receiving recombinant bovine somatotrophin. Vet Rec 1989; 124:83-86.

119 Gustafsson AH, Carlsson J. Effects of silage quality, protein evaluation systems and milk urea content on milk yield and reproduction in dairy cows. Livest Prod Sci 1993; 37:91-105.

120 Roseler DK, Ferguson JD, Sniffen CJ. The effects of dietary protein degradability/undegradability on milk urea, milk NPN and blood urea in lactating dairy cows. J Dairy Sci 1990; 73[suppl.1.], 168.

121 Jordan ER, Swanson LV. Serum progesterone and luteinizing hormone in dairy cattle fed varying levels of crude protein. J Anim Sci 1979; 48(5):1154-1158.

122 Voss VL, Stehr D, Satter LD, Broderick GA. Feeding lactating dairy cows proteins

resistant to ruminal degradtion. J Dairy Sci 1988; 71(9):2428-2439.

123 Butler WR, Everett RW, Coppock CE. The relationships between energy balance, milk production and ovulation in postpartum Holstein cows. J Anim Sci 1981; 53(3):742-748.

124 Sato H, Nishiguchi Y, Kato T. Relation between milk urea levels and conception in lactating dairy cows. Animal Science and Technology 1996; 67(1):58-63.

125 Melendez P, Donovan A, Hernandez J. Milk urea nitrogen and infertility in Florida dairy cows. J Dairy Sci 2000; 83(3):459-463.

126 Tamminga S. Nutrition management of dairy cows as a contribution to pollution control. J Dairy Sci 1992; 75(1):345-357.

127 Edgson A. Metabolic profile studies in the bovine animal. Bovine Pract 1973; 8:25-29.

Chapter 3

Evaluation of the Fossomatic 4000 MilkoScan analyzer for MUN Testing

3.1 Introduction

Direct and indirect spectrophotometric analyses based on wet chemistry, the most common tests to measure serum and milk urea concentrations, have been used for many decades (1). The direct method determines urea nitrogen levels by using a chemical agent, diacethylmonoxine, to react with urea molecules to form a color, which is measured spectrophotometrically (2). The indirect method measures ammonia after an enzymatic digestion of the urea to ammonia with urease (3). This ammonia level is selected by spectrophotometry after a reagent is added which binds to ammonia to give a color change.

A more precise, less complicated wet chemistry method for detecting milk and serum urea concentration is the Eurochem (CL10). In this test, the amount of urea originally present in the sample is estimated by measuring the change in the pH of the sample associated with the addition of urease that converts urea to ammonia (Foss North America, Brampton, Ontario). This method has generally been accepted as the gold standard for urea determination in North America (5).

The cost of all wet chemistry methods in a commercial large-scale setting has been relatively high due to the time-consuming handling of the samples (4). As a result, it has been impractical to measure milk urea concentration in dairy herds on a wide-scale basis

using wet chemistry.

Recently, rapid methods of MUN determination, based on an infrared technology, have become available (6). Use of this technology within dairy herd improvement laboratories (DHI) offers the best potential for a cost-effective MUN determination (4). The same instrument can be used for determination of milk fat, milk protein, lactose, somatic cell count, and MUN content, and, therefore, no separate handling of samples is needed.

The Fossomatic 4000 MilkoScan analyzer (FOSS4000 - Foss North America, Brampton, Ontario) is one such machine that uses infrared technology to produce an estimate of the urea content of milk samples. Infrared light is passed through a filter to produce a beam of a specific wavelength for the milk component being measured. The beam is subsequently passed through a milk sample and the amount of light absorbed by the sample is recorded. A computer algorithm then adjusts this urea measurement for the concentrations of other milk components, known as interfering substances, which are known to also absorb some light at the urea wavelength. This calculation produces an estimate of the concentration of urea in a milk sample.

When a new method is developed, it is necessary to evaluate whether the new method can consistently produce the same results derived from the historically accepted method (7). It is important to compare the agreement between test results from identical samples tested by the new test and by the gold standard test. It is also important to determine the repeatability of the method, that is, the ability of the method to consistently produce the same

results of the same samples upon repeated testing. This chapter evaluates these two measures of test performance.

The first objective of this study was to determine, using routine samples, the agreement of MUN measurement using the infrared method at the PEI Milk Quality Laboratory (PEIMQL) compared with an enzymatic reference method conducted at the laboratory of the Ontario Dairy Herd Improvement Corporation (ODHIC). The second objective was to determine the repeatability of the same infrared method by using routine samples measured twice at the PEIMQL.

3.2 Materials and Methods

3.2.1 Comparison between infrared method and enzymatic method

The milk samples were selected by PEIMQL staff to represent a large number of herds, and a broad range of MUN concentrations. They were selected during 5 different time periods of the year, 29-36 samples per time period. Each sample was preserved with a Bronopol tablet (6 mg:2-Bromo-2-Nitro-Propane-1,3 Diol/tablet- one tablet per sample) to inhibit the growth of bacteria and yeast. Each milk sample was divided into paired duplicate samples. One of each of the duplicate samples was analyzed for MUN (mg/dl) using the infrared FOSS4000 at the PEI Milk Quality Laboratory. The second duplicate sample was analyzed for MUN (mg/dl) using the enzymatic Eurochem CL 10 test (CL10 - Foss North America, Brampton, Ontario) at the laboratory of the Ontario Dairy Herd Improvement

Corporation. Staff at this lab were blinded to the results from the PEIMQL. There was only 1 day between testing by the two methods. The one hundred and sixty one samples utilized for comparison of the infrared method with the enzymatic method were part of the quality control program of the PEIMQL.

Descriptive statistics for the MUN results of the 161 samples analyzed by each method were calculated. Significant differences between MUN mean values and standard deviations were determined using the paired t test. A scatter plot was created by plotting the MUN results from the infrared assessment against those from the enzymatic method, and a regression line was fit to the data (Figure 3.1). A line of perfect agreement (hatched line at 45 ° and intercept zero) was imposed on this figure. Agreement between the two tests was assessed using two methods: calculation of the concordance correlation coefficient (7) and a graphical procedure proposed by Bland and Altman (8) (Figure 3.2), as described below. All analyses were carried out using Stata release 6 (STATA Corporation, College Station, Texas)(9).

The concordance correlation coefficient (Pc) was calculated as a means of measuring overall agreement (accuracy & precision) between the enzymatic method and the infrared method by measuring the variation of the data from the line of perfect agreement (accuracy - based on the bias correction factor - Cb) and the reduced major axis of the data (precision - based on the Pearson correlation coefficient - P) (7;10). The formula for determining Pc is $Pc = Cb * P$.

The Bland and Altman method of assessing agreement involved plotting the mean of the paired measurements (x axis) against their difference (y axis). The 95% limits of agreement were computed as the mean difference plus or minus 1.96 times the standard deviation of the difference (8;10).

3.2.2 Repeatability within the PEI Milk Quality Lab

Two hundred composite milk samples from individual cows were collected by random sampling from routine work at PEIMQL. Each milk sample was split to create 2 identical replicate samples. Each replicate sample was preserved with a Bronopol tablet (6 mg:2-Bromo-2-Nitro-Propane-1,3 Diol /tablet- one tablet per sample) to inhibit the growth of bacteria and yeast. All samples were analyzed using the FOSS4000. Both samples were run in the same batch on the same day, after standardization of the equipment. The PEIMQL staff were blinded to the identity of the samples. The statistical approach used in the previous section was also used here, along with calculating an estimate of the coefficient of variation (CV), a measure of test precision for the infrared machine.

3.3 Results

3.3.1 Comparison between the infrared and enzymatic methods

As shown in Table 3.1, the MUN concentrations from the infrared method significantly lower standard deviations but not significantly different means than by the enzymatic method ($P < 0.05$). The enzymatic assessment had a somewhat higher minimum and maximum MUN value when compared with the infrared test. Comparisons, by test day, of mean MUN values from the infrared and enzymatic methods are shown in Table 3.2

When MUN values from the infrared method were plotted against those of the enzymatic test (Figure 3.1), points tended to cluster at or near the line of perfect agreement. The concordance correlation coefficient was 0.972 (95% confidence interval = 0.964, 0.980). The bias correlation factor (C_b) that measured how far the best-fit deviated from the 45° line (measure of accuracy), was 0.998 which showed that the best-fit line was very close to the perfect agreement line. The Pearson correlation coefficient (P) was 0.973, showing good precision.

Using the Bland and Altman method of assessing agreement, the mean difference between the two tests was 0.051 mg/dl (Std.Dev. = 1.18). The 95% limits of agreement were -2.29 and 2.19 mg/dl, respectively, indicating that 95% of pairs of results differed by less than approximately 2.2 mg/dl (Figure 3.2).

Table 3.1 Descriptive statistics for milk urea nitrogen results from 161 milk samples analyzed using the infrared method and the enzymatic method

Method of Analysis	Mean MUN (mg/dl)	Standard Deviation	Minimum (mg/dl)	Maximum (mg/dl)
Infrared method	13.78 [a]	4.63 [a]	4.1	26.3
Enzymatic method	13.73 [a]	4.84 [b]	4.5	29.1

[a,b] values with the same superscript were not statistically significantly different at p=0.05

Table 3.2 Comparisons, by test day, of mean milk urea nitrogen values (n=161) determined by the enzymatic method and infrared method

Date	# samples	Enzymatic method		Infrared method	
		Mean	SD.	Mean	SD.
June 07/99	29	13.45 [a]	5.63 [a]	13.50 [a]	5.16 [a]
Aug 11/99	32	15.39 [a]	5.83 [a]	15.89 [b]	5.47 [a]
Oct 28/99	36	14.14 [a]	3.85 [a]	13.71 [a]	3.84 [a]
Feb 16/00	33	12.57 [a]	4.37 [a]	12.67 [a]	4.13 [a]
May 11/00	31	13.67 [a]	4.23 [a]	13.34 [a]	4.06 [a]

[a,b] pairs of values within the same row with the same superscript were not statistically significantly different at p=0.05

Figure 3.1 Scatter plot of milk urea nitrogen results from the enzymatic method (CL10) and infrared method (FOSS4000).

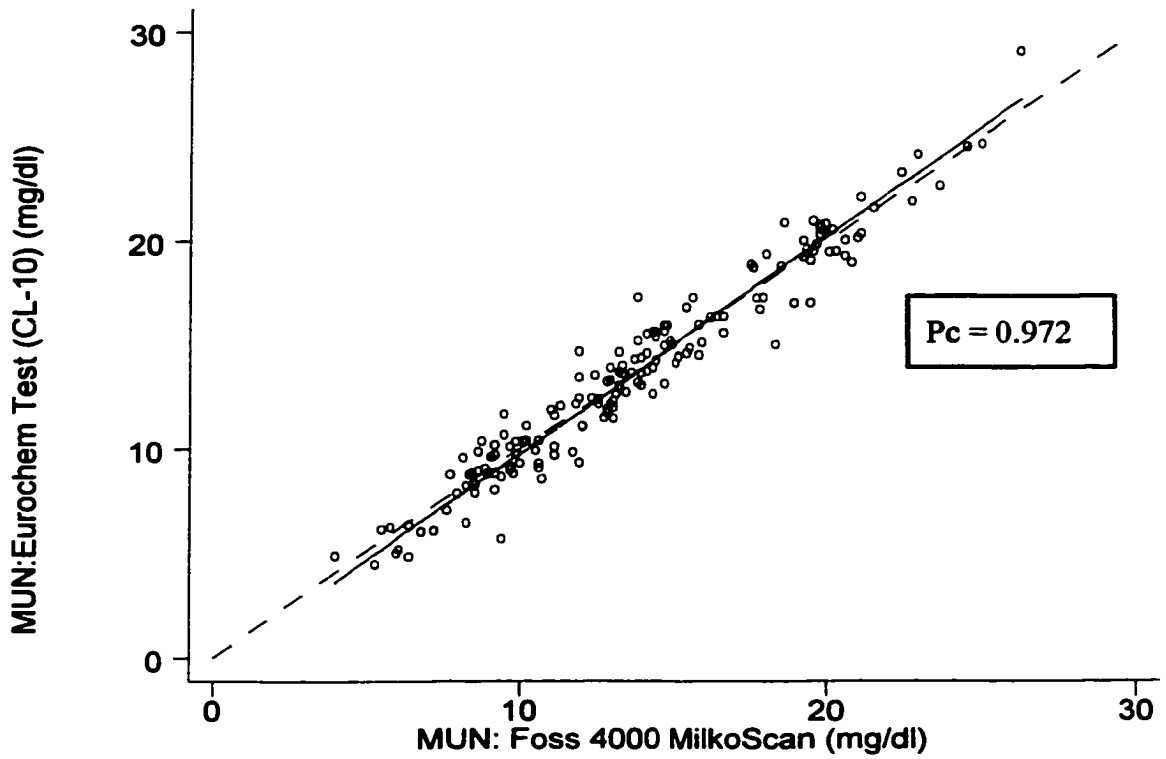
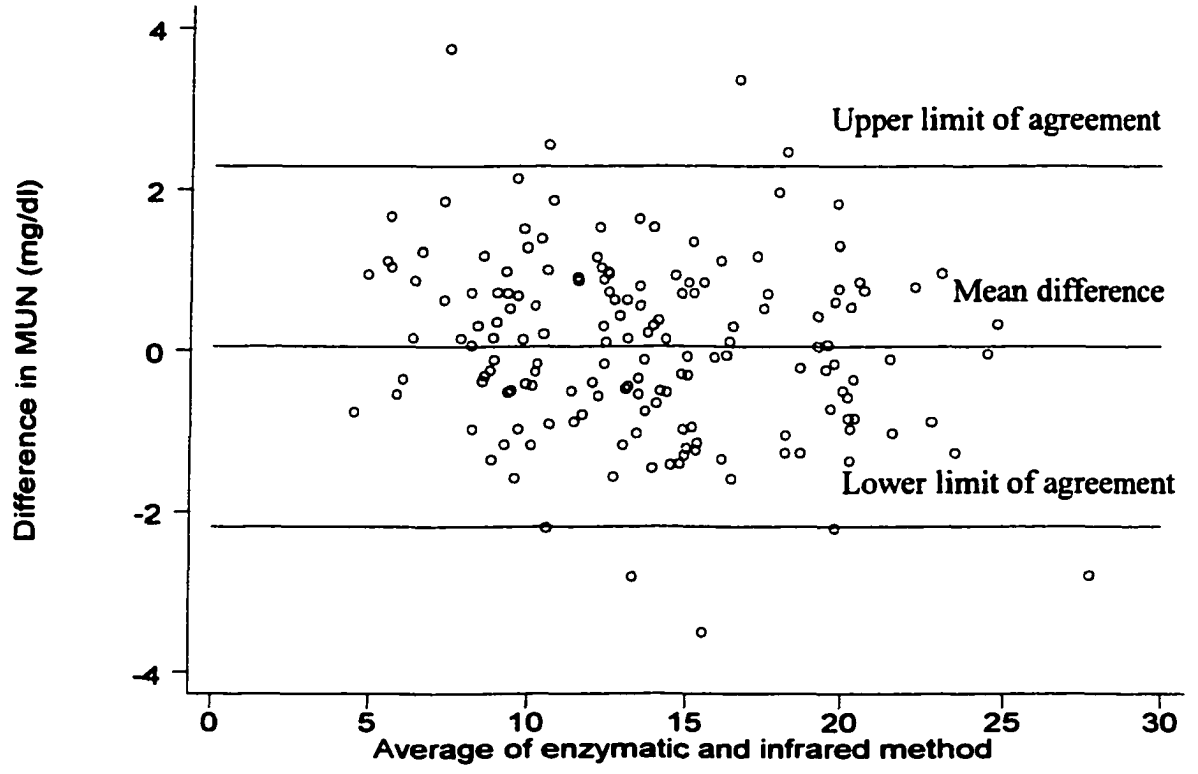


Figure 3.2 Difference between the milk urea nitrogen values from the enzymatic method and infrared method plotted against the mean value from the two methods, with horizontal lines showing the 95% limits of agreement.



3.3.2 Repeatability within the PEI Milk Quality Lab

The sets of MUN concentrations from the repeatability trial had significantly different means but not significantly different standard deviations (Table 3.3). When MUN values from the infrared test one were plotted against those of infrared test two (Figure 3.3), points tended to cluster at or near the line of perfect agreement. The concordance correlation coefficient was 0.983 (95% confidence interval = 0.978 - 0.988). The Pearson correlation was 0.988 showing good precision and C_b was 0.995 which showed that the best-fit line was close to the perfect line. Using the Bland and Altman method of assessing agreement, the mean difference between the two tests was 0.29 mg/dl (Std. Dev. = 0.49). The 95% limits of agreement were -0.68 and 1.27 mg/dl, respectively, indicating that 95% of pairs of results differed by less than approximately 1 mg/dl (Figure 3.4). The coefficient of variation for the Fossomatic 4000 Milkoscan Analyzer was 2.2%.

Table 3.3 Descriptive statistics for milk urea nitrogen results from 200 replicate milk samples analyzed using the infrared method.

Foss 4000 Milkoscan	Mean MUN (mg/dl)	Standard Deviation	Minimum (mg/dl)	Maximum (mg/dl)
Test 1	15.48 [a]	3.08 [a]	6.7	25.3
Test 2	15.18 [b]	3.09 [a]	5.7	24.5

[a,b] values with the same superscript were not statistically significantly different at p=0.05

Figure 3.3 Comparison of milk urea nitrogen results from 200 replicated samples using the infrared method.

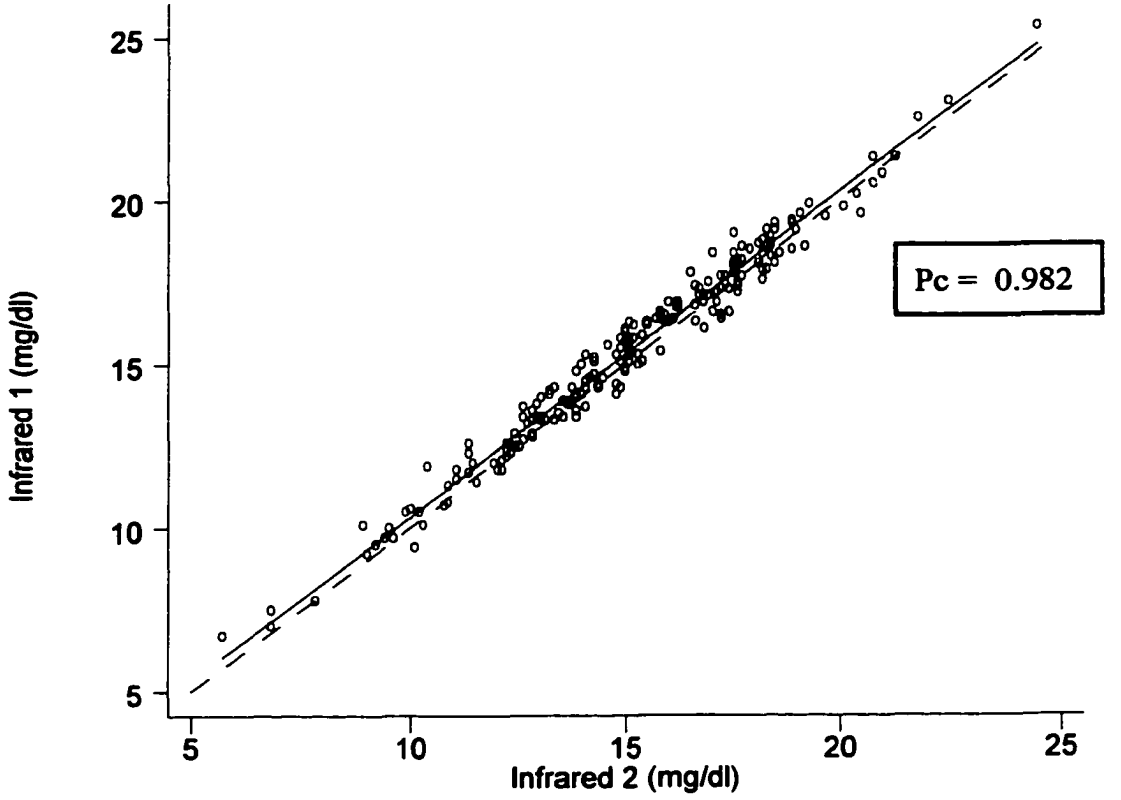
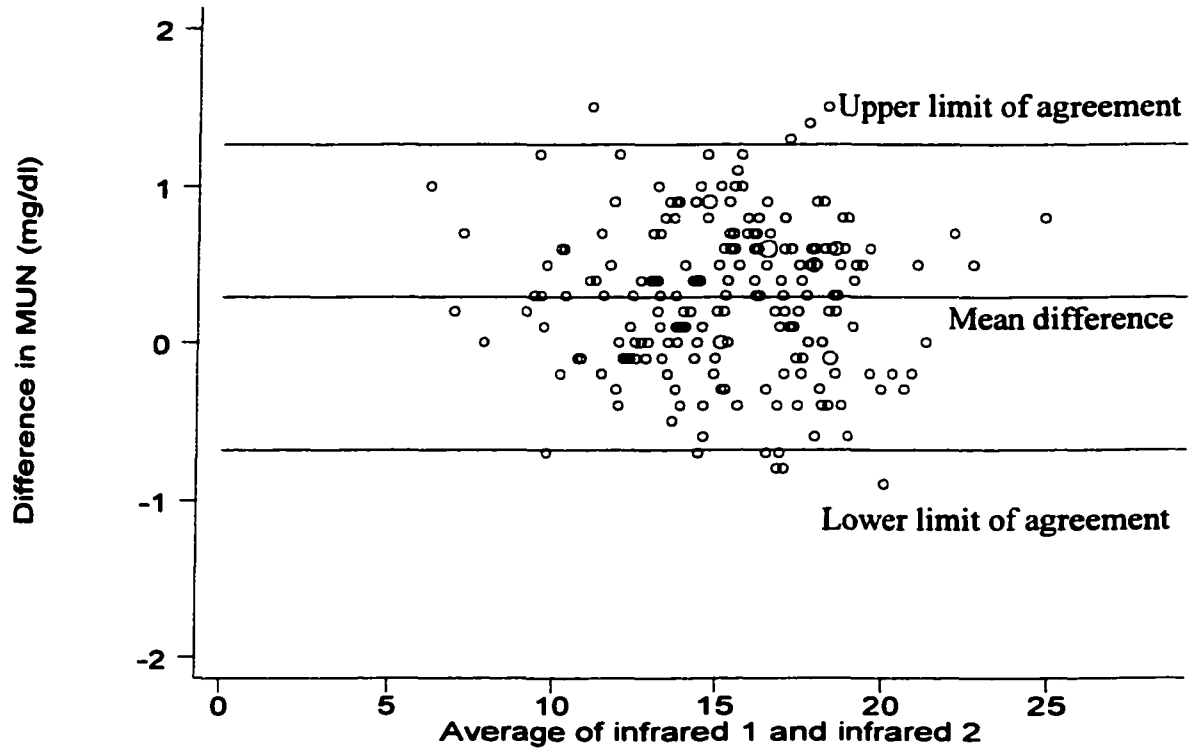


Figure 3.4 Difference between the milk urea nitrogen values from test one and test two replicate samples using the infrared method, plotted against the mean value, with horizontal lines showing the 95% limits of agreement.



3.4 Discussion

3.4.1 Comparison between infrared method and enzymatic method

There is no official reference method available to measure milk urea nitrogen concentration (4). This study selected the Eurochem test (CL-10), as the reference method for this evaluation, because it has been well accepted and used for many years and is the official calibration tool for infrared techniques (4).

The means of the MUN test results from both methods, by month, were only slightly different, but the range of MUN levels for the enzymatic test was wider than for the infrared assessment. The standard deviations within each month were not statistically significantly different but in the whole data set (Table 3.1), the standard deviations were significantly different due to the larger sample size. While this difference is statistically significant it was small in magnitude and not biologically important. The maximum possible concordance correlation coefficient is 1 (range -1 to 1). In this study, the concordance correlation coefficient was 0.972 which suggested that there was very good overall agreement between the two methods. The major axis of the data effectively fell on the line of perfect concordance, reflecting excellent accuracy in the infrared results when compared with the enzymatic method. This concordance correlation coefficient was higher than that reported by Godden *et al* (5), who found a concordance correlation coefficient, based on 89 samples, of 0.86.

Using the Bland and Altman method of agreement, the mean difference between the two tests was 0.051 mg/dl (Std.Dev. = 1.18), while 95% of pairs of results differed by less than approximately 2.2 mg/dl. This is indicative of good agreement between the MUN measure from the infrared assessment and the enzymatic method.

Appendix B contains data from Lefebvre (11), who examined the mean differences and standard deviations of differences between infrared methods and enzymatic methods conducted at three different laboratories. By comparison, the PEIMQL had a smaller mean difference (0.05 mg/dl) and standard deviation (1.18 mg/dl) than any of the other laboratories. The values of the minimum and maximum MUN concentrations among the regions were quite different, with the ODHIC laboratory having the highest MUN values. These differences among regions may be due to both nutritional and non-nutritional factors.

Sauve (12) evaluated the reliability of 15 infrared MUN analyzers around North America compared to an enzymatic method. Twelve samples were divided into duplicate samples and 1 of each duplicate sample was analyzed by each method. In his study, only two of 15 infrared analyzers had standard deviations of the differences that were less than 1.5 mg/dl, indicating large regional differences in quality control of MUN testing. Nine of 15 infrared laboratories had mean differences greater than zero, showing a positive bias in results of the infrared method. However, there was virtually no bias in results from the PEIMQL infrared method compared to the enzymatic method.

The reasons for this superior quality control at the PEIMQL are likely numerous,

including such issues as initial calibration and maintenance. All of the main components of milk interfere spectrophotometrically with each other such that variation in concentration of any one component will affect all of the others in the infrared method. A calibration equation is used to correct for this interference. The slope, bias and coefficient values produced by the interference filter and utilized in this equation are different from instrument to instrument (13). The quality of the original calibration of the equipment, and its continued maintenance, will determine how well the infrared method can account for and remove the effects of these interfering factors, when producing a urea estimate (6). As a result, it is important to compare infrared assessments to reference methods, not to another laboratory which uses the infrared method.

3.4.2 Repeatability within the PEI Milk Quality Lab

The results from replicated samples showed a high level of repeatability for the infrared machine. A scatter plot (with equal scales) between the first test and the second test of the infrared assessment showed that all points were close to a 45^{O} line through the origin (Fig. 3.3). The concordance correlation coefficient of 0.983 (possible range -1 to 1.0) also suggested that there was a very good overall agreement between the replicate samples. The coefficient of variation result showed that the infrared method had a very high level of precision.

3.5 Conclusions

Results from the comparison of the infrared assessment and enzymatic test for measurement of milk urea nitrogen indicated good agreement. The repeatability of test results from the infrared technique were very high, with a high level of precision. Therefore, the infrared technique provides strong potential for a cost-effective method for determining MUN values, because the same instrument can be used for all compositional analyses of milk. The quality control program of the PEI Milk Quality Laboratory is producing MUN results that are reliable and repeatable. However, the accuracy and precision information presented from this study should not be extrapolated to MUN data produced by other DHI laboratories due to the potential differences in calibration procedures.

3.6 References

1 Payne JM, Dew SM, Manston R. The use of a metabolic profile test in dairy herds. Vet Rec 1970; 87:150-158.

2 de Jong EAM, Klomp H, Hemert HV. Evaluation of a segmented flow method for the routine determination of urea in milk. Neth Milk Dairy J 1992; 46:115-122.

3 Ferguson JD. Milk urea nitrogen. Ferguson JD, editor. http://cahpwww.nbc.upenn.edu/mun/mun_info.html , 1-6. 6-29-1999.

4 Gustafsson, AH. Current status concerning the use of milk urea concentration: analytical methods, use in different countries and how to utilize the results. Proceeding of the 30th Biennial Session of ICRA, Velhoven, The Netherlands, 1996:223-228

5 Godden SM, Lissemore KD, Kelton DF, Lumsden JH, Leslie KE, Walton JS. Analytic validation of an infrared milk urea assay and effects of sample acquisition factors on milk urea results. J Dairy Sci 2000; 83(3):435-442.

6 Grappin R, Packard VS, Ginn RE. Repeatability and accuracy of dye-binding and infrared methods for analyzing protein and other milk components. J Food Prot 1980; 43(5):374-375.

7 Lin LI-K. A concordance correlation coefficient to evaluate reproducibility. Biometrics 1989; 45:255-268.

8 Bland JM, Altman DG. Comparing methods of measurement: why plotting difference against standard method is misleading. The Lancet 1995; 346(October 21):1085-1087.

9 Stata Corp. Stata statistical software. [Release 6]. 1999. College Station (TX), Stata Corporation.

10 Steichen TJ, Cox NJ. Concordance correlation coefficient to evaluate reproducibility. Stata Technical Bulletin. 1998: 137-143.

11 Lefebvre D. Development Agent - Nutrition, PATLQ. 2000. Personal Communication

12 Sauve JP. General Manager of the Canadian Laboratory Accreditation Program. 2000. 6-27-2000. Personal Communication

13 McKenna D. Foss Electric. North America. 2000. Personal Communication

Chapter 4

The effect of cow and test factors on Milk Urea Nitrogen

4.1 Introduction

Concentrations of urea in milk are variable from herd to herd and within cows in the same herd. While differences in feed composition and dairy cattle nutritional management practices are likely to be the major influence on milk urea concentrations, other factors have been associated with milk urea concentrations (1). To interpret the milk urea concentration correctly, it is important to appropriately account for other factors besides the cow's diet. Therefore, characteristics of the animal or herd, cow factors, method of sample collection, sample storage, and sample processing must be considered (2-4).

There are limited and often conflicting reports dealing with the effect of cow factors on milk urea concentrations. Some researchers have found that breed significantly influences milk fat, milk protein (5), milk non-protein nitrogen (MNPN) (6), and plasma urea nitrogen (PUN) (7), and may influence MUN (1;8). A significant effect of parity (first vs. second lactation or later) on plasma urea nitrogen was found in several studies (7;9;10). Studies have also reported that MUN values were lower in heifers than in older cows (1;11-14). However, other studies have reported no association to exist between parity and MUN concentrations (8;15-19). Many studies have shown that MUN concentrations were much lower during the first month of lactation than later (1;2;8;20-23). Conversely, Schepers (24), Hoffmann (19) and Faust (25) found no differences between milk urea concentration at

different stages of lactation. Milk yield has also been shown to affect MUN. Urea levels have tended to be higher in high producing herds when compared to low producing herds (1;14;18), but this effect may be due to confounding from herd level factors such as feeding management. In contrast, other studies (4;12;15;26;27) found no significant correlation between milk urea nitrogen levels and milk yield. There are few reports that deal with the relationship between high somatic cell counts and NPN content of milk (13) and also there is little information about the influence of udder infection on MUN.

The conflicting results reported in the studies referred to above, may be due to limitations in the study design, such as failure to adjust for small sample sizes or herd level clustering effects of different feeding or management strategies. Therefore, comprehensive studies on the effect of cow level factors on MUN are still lacking.

The goal of this study was to determine how cow level factors' such as breed, parity, days in milk, milk production, milk quality and milk components affect MUN.

4.2 Materials and methods

4.2.1 Sampling frame and laboratory methods

A total of 199 dairy farms in PEI, containing 11,837 lactating dairy cows participated in the project. The herds were all subscribers to the milk recording service provided by the Atlantic Dairy Livestock Improvement Corporation (ADLIC). Individual cow milk samples

(n = 77,395) were collected monthly from July, 1999 to June 2000 from each farm. The herds in this study utilized one of four different individual cow milk sampling protocols. Milk samples were either collected by a technician or the farm owner, and consisted of either a mixture of equal amounts of the morning and afternoon milkings, or alternatively, milk sample from the morning milking one month and evening milking the next month. All the samples were preserved with bronopol and kept at 4 °C while being transported to the laboratory. Milk urea nitrogen levels were measured using a Fossomatic 4000 Milkoscan Analyzer (Foss North America, Brampton, Ontario) at the PEI Milk Quality Laboratory. Milk fat, milk protein and somatic cell counts (SCC) were also analyzed by the same machine during the same period for these routine measurements. Milk production, days in milk, and parity data from each cow for each test date were also obtained from ADLIC, along with the cow breed.

4.2.2 Variables

Seven variables were investigated to determine their effect on MUN values: breed, parity, days in milk (DIM), milk production, milk fat percent, milk protein percent and SCC. Herds were classified as having one of 6 breeds: Holstein, Ayrshire, Guernsey, Jersey, Milking Shorthorn and Mixed (herds that had multiple breeds in the herd). Parities were separated into 4 categories: first, second, third, and fourth plus. Centering the DIM variable (DIM2 = DIM - mean (DIM)), and then squaring it (DIM2_sq = DIM2* DIM2) normalized the days in milk data. Milk fat was also normalized through centering (fat2 = fat - mean(fat)), and then squaring it (fat2_sq = fat2 * fat2). Somatic cell count (SCC) data were transformed

to a linear score (ie. logarithm of SCC to the base 2, starting at 12,500, such that a SCC of 25,000 produced a linear score of 1, a SCC of 50,000 produced a linear score of 2, etc. - producing a score of 0-9) because the original distribution of data was right skewed. Milk production and milk protein on the test date were kept on their original scales. Finally, the test date was converted into the number of months past the start of the project, and this variable was included in the model to account for the expected seasonal variation in MUN.

4.2.3 Statistical methods

Certain observations were excluded from statistical analyses because of being biological anomalies. An observation was excluded if: days in milk was over 600 days; milk protein was below 1.5% or over 5%; milk fat was below 1.5% or above 6% ; milk production values were less than or equal to 0.1 kg (missing code); or MUN tests were less than or equal to 0.1 mg/dl. This produced a reduced dataset of 75, 565 observations from 11,837 lactating cows in 199 PEI dairy herds. Description statistics (eg. means of the other variables) of the excluded observations, by exclusion category, were calculated for comparison purposes.

Descriptive statistics for MUN, parity, days in milk, milk yield, fat and protein were calculated. A frequency distribution of MUN values, by MUN concentration categories, was created to determine whether the values followed a normal distribution.

Regression analyses were conducted to determine relationships between MUN levels and measured cow and test factors. For all regression analyses, in addition to the exclusions

already mentioned, records from cows that were not Holstein were also excluded (except for unconditional analyses between MUN and breed) due to the relatively small number of herds and cows of other breeds, producing unreliable statistics, as described below. The final dataset used for all regression analyses had 68,158 observations from 10,688 lactating Holsteins in 177 herds.

To identify if independent variables for regression analyses were highly correlated, simple correlation coefficients among cow and test factors were determined. Generalized estimating equations (GEE) models with a completely unstructured correlation matrix were used to evaluate the nature of the within cow correlation of test day MUN values. The resulting estimated correlation matrix suggested that any time dependent correlation was due to seasonal effects, not the sequence of tests within the cow. Consequently, random effects regression models with an exchangeable (compound symmetry) correlation structure were used in all simple (unconditional) and multivariable regression modeling.

Random effects linear regression models were used to investigate the relationship between MUN and the measured cow factors, while adjusting for clustering within cows and within herds. The variables "herd" and "cow" were included as random effects to control for the effect of clustering of MUN test dates within cow, and clustering of cows within herd, respectively. Initially, variables were assessed individually and then included in a multivariable model. Variables were retained in the model if they were significant at $p < 0.05$. The following fixed effect variables were examined in the modeling process: parity (first lactation versus older cows), centered days in milk, centered days in milk squared,

milk yield, centered milk fat percent, centered milk fat percent squared, milk protein percent, and month. The proportion of the variation occurring at the herd, cow and test levels was calculated for the null model, final model with all significant fixed effects except season, and final model with significant fixed effects including season. Model diagnostics were performed, examining a normal score plot of the residuals of the models.

A list of the dependent and independent variables, and their definitions, for the random effects linear regression modeling is shown in Table 4.1. All descriptive statistics, data manipulation and GEE modeling were performed using STATA version 6 (28) while MLwiN (29) was used to fit the random effects regression models.

Table 4.1 Variables included in the random effects linear regression model for analysis between test day milk urea nitrogen concentration and test day cow factors, controlling for season (month).

Variable	Type	Definition
mun	continuous	MUN concentration (dependent variable)
ls	continuous	Linear score - logarithmic transformation of SCC
dim2	continuous	Days in milk centered
dim2_sq	continuous	Days in milk centered and then squared
hr_24yl	continuous	Milk yield (kg) on test date
fat2	continuous	% Milk fat centered
fat2_sq	continuous	% Milk fat centered and then squared
protein	continuous	% Milk protein
parity	class	1 = parity 1 = baseline
		2 = parity 2 +
month	class	12 months, numbered 1-12, the first month as baseline

4.3 Results

4.3.1 Descriptive Statistics

Table 4.2 presents the number of observations and mean values of the other variables for the records that were excluded on the basis of breed and outlier values for MUN, days in milk, milk yield, fat and protein. Large differences in other parameters can be seen between the averages of the excluded observations versus the retained observations, suggesting that the excluded observations were abnormal in many respects, justifying their exclusion.

Table 4.3 shows the descriptive statistics for MUN, milk yield, milk fat, milk protein, linear score, parity and days in milk for the 75,565 individual milk samples in the reduced dataset (includes all breeds), collected over a one year period , from 11,837 lactating cows distributed in 199 dairy herds across PEI. The frequency distribution of MUN values by MUN concentration categories (Figure 4.1) shows a normal distribution.

For assessing the correlation among variables for the regression analyses, the Pearson correlation coefficients among all independent variables still remaining in the final dataset of 68,185 observations were calculated (see Table 4.4). Although all correlations were statistically significant ($p < 0.01$), most were small in value, and therefore multicollinearity was not a major concern for the regression analyses. Milk yield had moderate negative correlation with days in milk ($r=-0.61$) and milk protein ($r=-0.54$). Milk protein was moderately positively correlated with days in milk ($r = 0.56$) and milk fat ($r= 0.48$).

Table 4.2 Mean values of other variables for the records excluded from, and retained for, regression analysis, by exclusion/inclusion category.

Reasons for Exclusion	#of Records	Protein %	Fat %	SCC cell $*10^3$/ml	Milk yield kg	MUN mg/dl
Protein <1.5%	5	X	0.36	20.66	22.34	32.4
Protein >5.0 %	70	X	6.2	1592.9	10.31	8.78
Fat < 1.5%	165	2.94	X	223.06	30.80	12.47
Fat > 6.0%	1,060	3.80	X	504.47	21.24	11.15
Milk yield ≤ 0.1 kg	161	4.06	5.02	671.86	X	9.64
MUN ≤ 0.1 mg/dl	115	4.18	4.49	2598.97	15.91	X
DIM > 600	254	3.70	4.24	342.28	16.5	10.04
Ayrshire	4,080	3.41	4.16	246.95	22.82	11.17
Guernsey	193	3.54	4.44	538.99	20.99	11.48
Jersey	274	3.73	4.99	238.16	20.06	12.81
Milking Short Horn	1,024	3.35	3.72	186.66	23.62	13.81
Mixed [a]	1,836	3.32	4.06	264.69	21.58	10.85
Records retained (Valid & Holstein)	68,158	3.28	3.80	258.23	28.01	11.79

[a] herds which have different purebred cows in the herd.

Table 4.3 Descriptive statistics for milk urea nitrogen and other variables for 75,565 milk samples from 11,837 lactating cows (all breeds) in 199 PEI dairy herds.

Variable	Mean	S.D.	Min.	Max.
MUN (mg/dl)	11.76	3.75	0.2	39.4
Milk yield (kg)	27.5	9.58	1.2	78.1
Milk fat (%)	3.83	0.77	1.5	6.0
Milk protein (%)	3.29	0.38	1.82	5.0
Linear score	3.06	1.78	0	9
Parity	2.8	1.9	1	13
Days in milk	178	112	2	600

Figure 4.1 Distribution of milk urea nitrogen concentrations for 75,565 milk samples from 11,837 lactating cows in 199 PEI dairy herds.

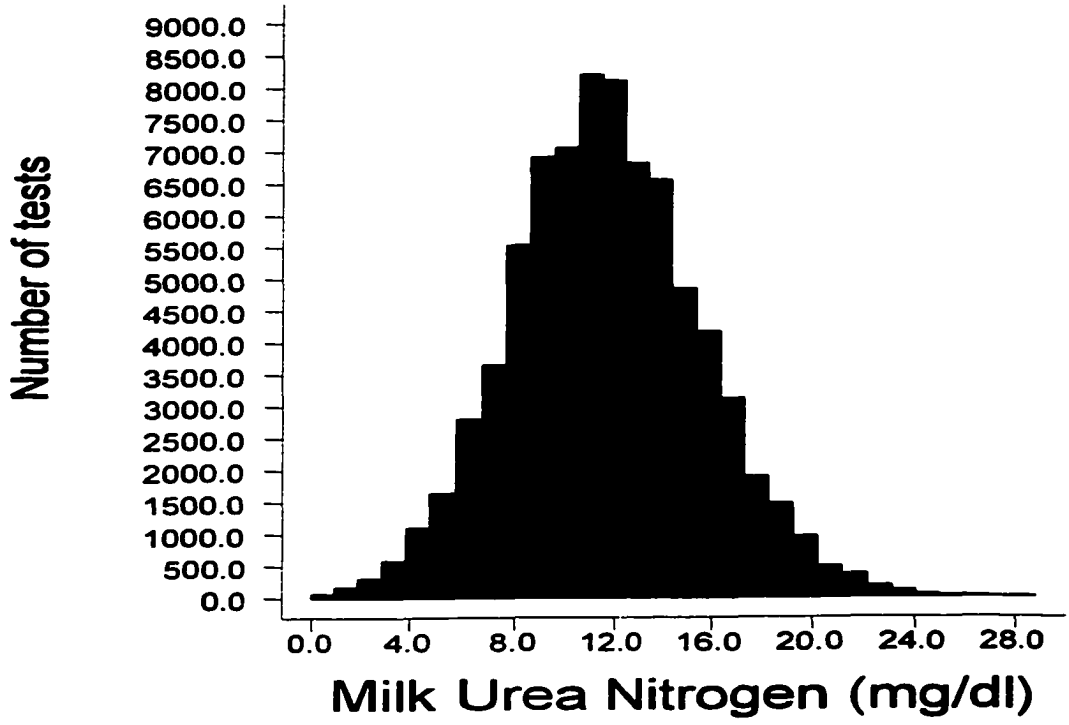


Table 4.4 The Pearson correlation coefficients among milk urea nitrogen and other variables for 68,158 milk samples from 10,688 lactating Holstein cows in 177 PEI herds.

Parameter	MUN	Parity	Days in milk	Milk Fat	Milk Protein	Linear Score	Milk Yield
MUN	1.000						
Parity	0.028	1.000					
Days in milk	-0.047	-0.004	1.000				
Milk fat	-0.117	-0.013	0.228	1.000			
Milk protein	-0.212	-0.069	**0.563**	**0.476**	1.000		
Linear score	-0.166	0.245	0.183	0.101	0.193	1.000	
Milk Yield	0.173	0.168	**-0.606**	-0.305	**-0.536**	-0.242	1.000

4.3.2 Unconditional associations

Mean and standard deviation for milk urea nitrogen levels for the various breeds are shown in Table 4.5. Numerically, Milking Shorthorns appeared to have the highest average MUN values, Jerseys had the second highest, and mixed breed herds had the lowest MUN concentrations. However, a random effects regression model showed no significant differences among the breeds ($P = 0.165$). This is potentially a function of power due to the small number of non-Holstein herds and cows in the data set.

The first parity animals had a lower MUN value than the older animals (Table 4.6), with third parity cows having the highest average MUN. However, the age effect was very small, and the unconditional random effects regression model showed no significant differences among the parities ($P = 0.377$).

There was considerable variation in the MUN concentration with days in milk. MUN was lowest in the first month of lactation and increased rapidly during the next two month period (Figure 4.2). The peak MUN value was about four months after calving and then it gradually decreased with the largest declines occurring in prolonged lactation (>305 days). A random effects regression model showed a significant association between MUN and days in milk ($P < 0.001$).

Table 4.5 Descriptive statistics of milk urea nitrogen by breed, based on 75,565 observations from 11,837 lactating cows in 199 PEI dairy herds.

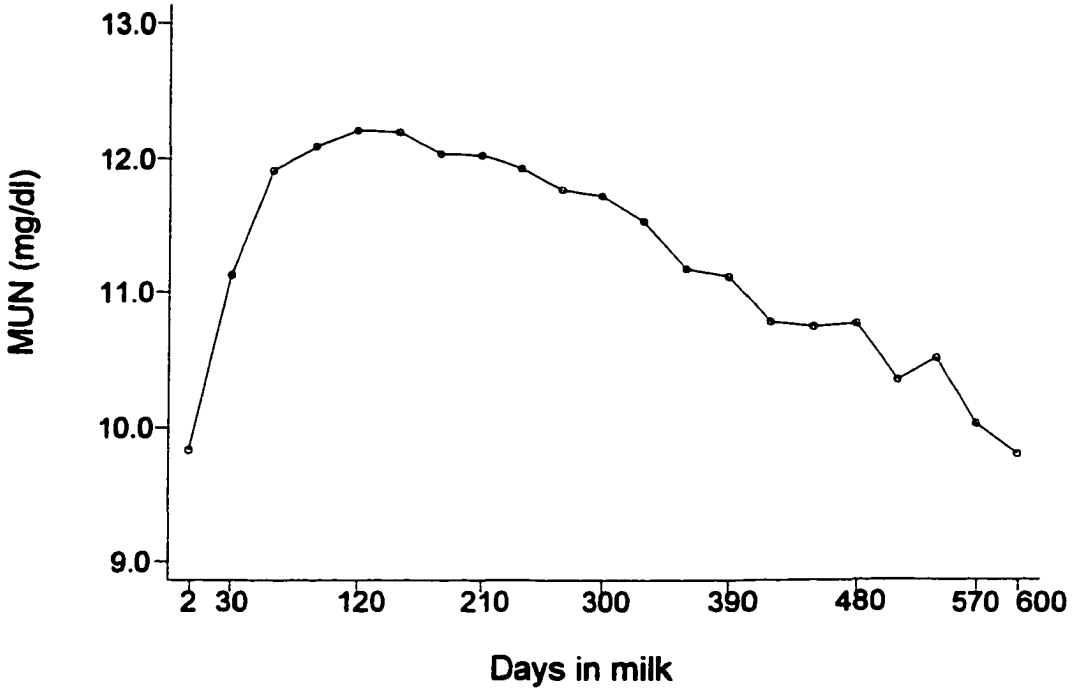
Breed	Observations	Mean (mg/dl)	SD. (mg/dl)	Min. (mg/dl)	Max. (mg/dl)
Holstein	68,158	11.79	3.76	0.2	39.4
Ayrshire	4,080	11.17	3.40	0.2	30.6
Guernsey	193	11.48	3.67	0.7	21.4
Jersey	274	12.81	3.78	5.9	23.8
Milking Shorthorn	1,024	13.82	3.97	0.5	24.7
Mixed [a]	1,836	10.85	3.45	0.9	24.6

[a] herds that had multiple breeds in the herd

Table 4.6 Descriptive statistics of milk urea nitrogen by parity based on 68,158 observations from 10,688 lactating Holstein cows in 177 PEI dairy herds.

Parity number	Observations	Mean (mg/dl)	SD. (mg/dl)	Min. (mg/dl)	Max. (mg/dl)
Parity one	20,337	11.59	3.63	0.2	28.6
Parity two	15,434	11.86	3.79	0.2	30.6
Parity three	11,954	11.93	3.82	0.3	35.0
Parity four or more	20,433	11.86	3.82	0.2	39.4

Figure 4.2 The relationship between days in milk and milk urea nitrogen, based on 68,158 observations from 10,688 lactating cows in 177 PEI dairy herds.



Note: Rather than plot every point on the graph, average MUN values were calculated for day 2-7, 8-30, and then every 30 day period after that.

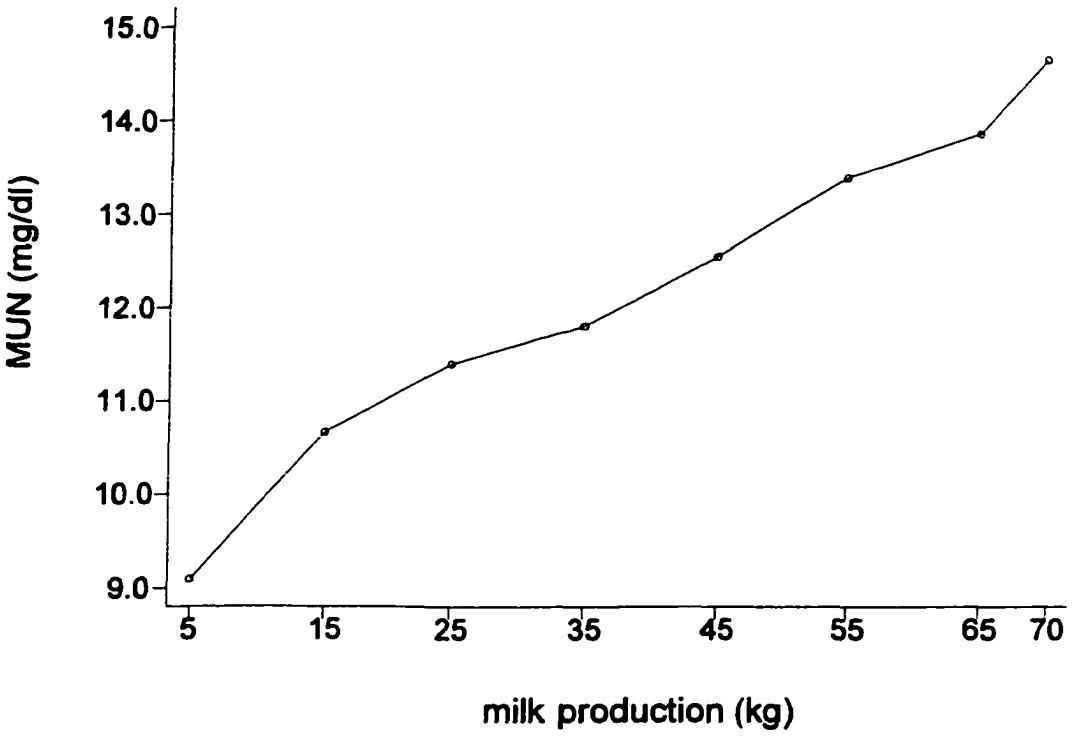
MUN in high producing cows was higher than in low producing cows (Fig. 4.3) with the relationship between MUN and production being linear in nature. A random effects regression model showed a significant association between MUN and milk production (P <0.001).

The relationship between milk protein % and MUN was approximately linear in nature (Figure 4.4), except for deviations at very high and very low levels of protein. However, there were relatively few observations at these extreme values. The relationship was highly significant and negative in the unconditional random effects model ($P < 0.001$).

The relationship between milk fat % and MUN was not linear (Figure 4.5), so the model was fit using centered milk fat and centered milk fat squared. However, the effect of milk fat % on MUN was generally small.

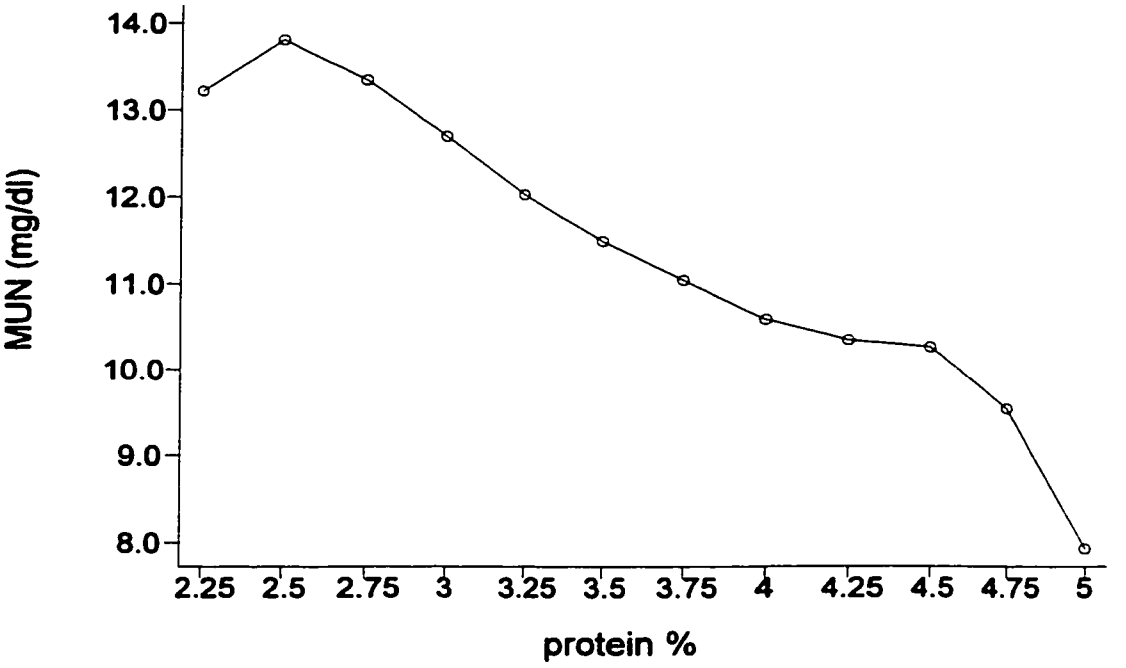
Linear score appeared to have a strong and linear relationship with MUN (Figure 4.6). A random effects regression model showed a significant negative association between MUN and linear score (P <0.001).

Figure 4.3 The relationship between milk production and milk urea nitrogen based on 68,158 observations from 10,688 lactating Holstein cows in 177 PEI dairy herds.



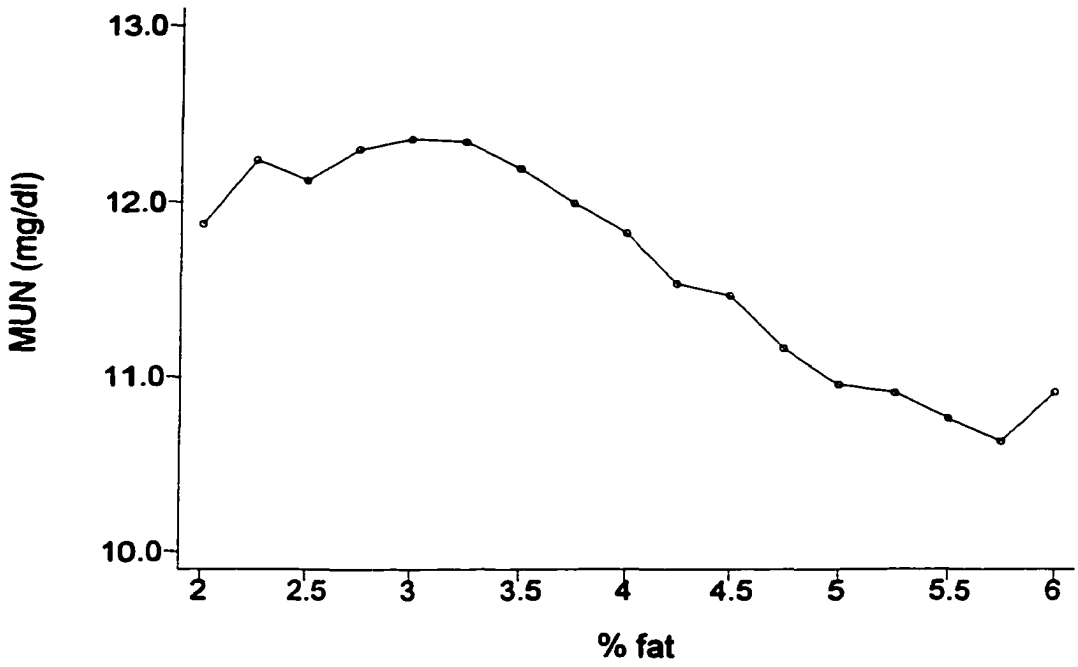
Note: Rather than plot every point on the graph, average MUN values were calculated for milk production between 1.2 - 5 kg, 5.01 - 15 kg, 15.01 - 25 kg, 25.01-35 kg, 35.01- 45 kg, 45.01-55 kg, 55.01- 65 kg, 65.01-78.1 kg.

Figure 4.4 The relationship between milk protein and milk urea nitrogen based on 68,158 observations from 10,688 lactating Holstein cows in 177 PEI dairy herds.



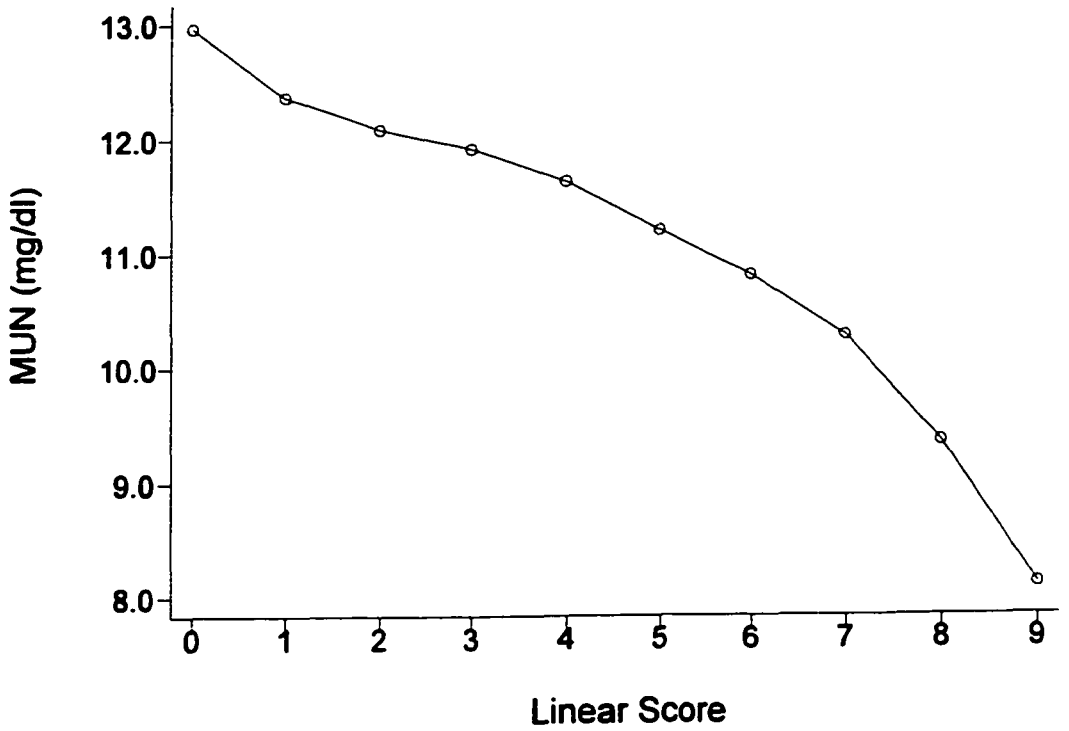
Note: Rather than plot every point on the graph, average MUN values were calculated for milk protein between 1.5-2.25 %, 2.26-2.5%, 2.51-2.75%, 2.76-3.0%, 3.01-3.25%, 3.26 - 3.5%, 3.51-3.75%, 3.76-4.0%, 4.01-4.25%, 4.26-4.5% 4.51-4.75%, 4.76-5.0%.

Figure 4.5 The relationship between milk fat and milk urea nitrogen based on 68,158 observations from 10,688 lactating Holstein cows in 177 PEI dairy herds.



Note: Rather than plot every point on the graph, average MUN values were calculated for milk fat between 1.5-2.0 %, 2.01-2.5%, 2.51-3.0%, 3.01-3.5%, 3.51-4.0%, 4.01-4.5%, 4.51-5.0%, 5.01-5.5%, 5.51-6.0%

Figure 4.6 The relationship between linear score and milk urea nitrogen based on 68,158 observations from 10,688 lactating Holstein cows in 177 PEI dairy herds.



4.3.3 Multiple variable regression analysis

The final random effects linear regression model was shown is Table 4.7. Linear score, days in milk, milk production, milk fat, milk protein, parity, and month were associated with test date MUN levels ($P<0.05$). The linear score and milk protein percent had negative relationships with MUN concentration. Days in milk and milk fat had a quadratic association with MUN. A positive relationship existed between MUN and milk production. MUN values were elevated in late winter/early spring (March, April) and through the summer/fall months, with the highest values occurring in July and August.

The variation at the herd and cow levels in the model were 19.7% and 19.0%, respectively, while the variation in test date was 61.3%. When looking at the 3 different models, the herd and cow level variations were relatively consistent (Table 4.8). The normal score plot of residuals (Figure 4.7) of the final model shows that the model fit the data well, producing a straight line through the axis.

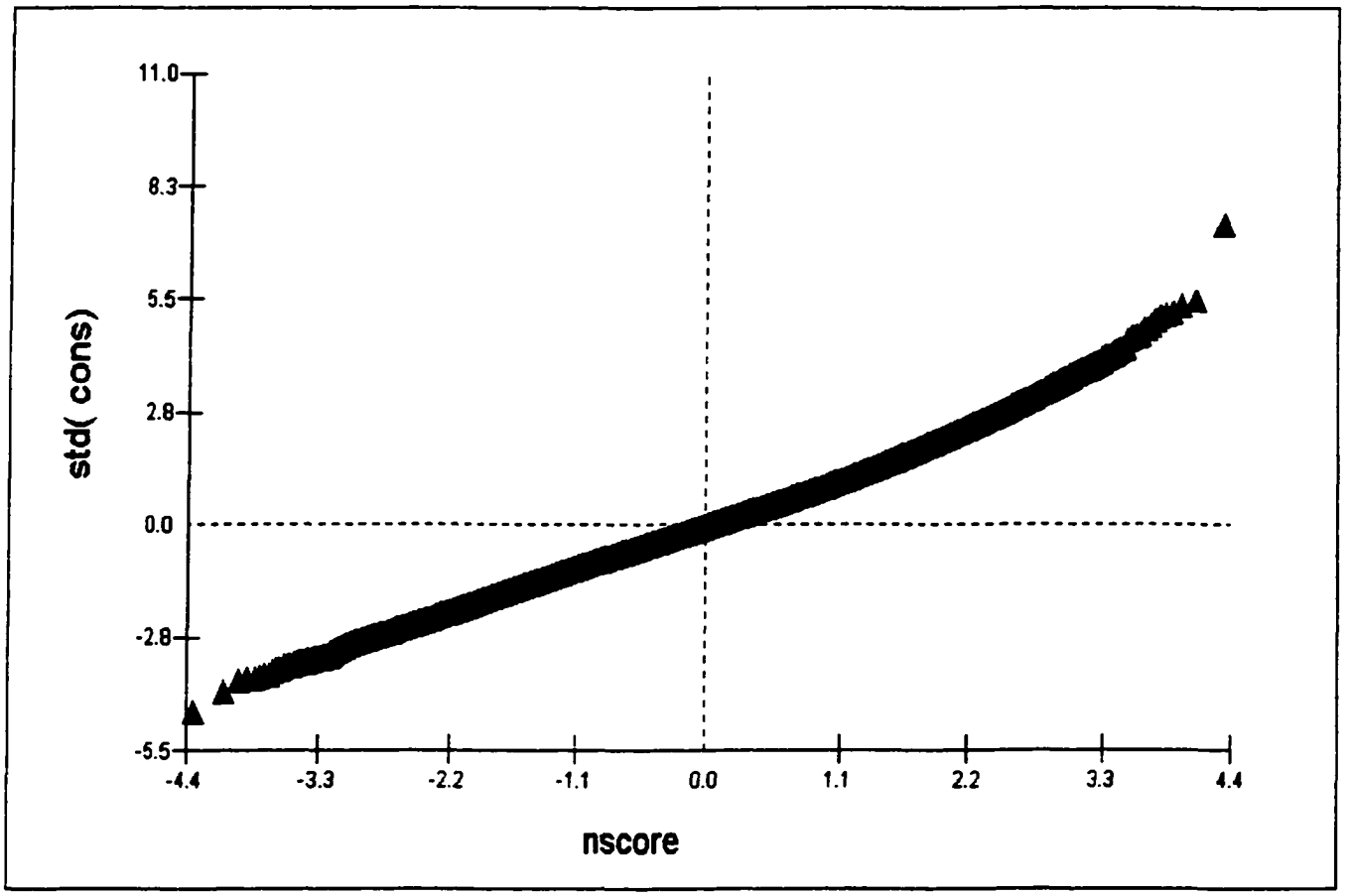
Table 4.7 Random effects linear regression model describing the relationships between test day milk urea nitrogen concentration and cow factors and month.

Variable	level	Estimated coefficient	Standard Error	P-value
Fixed effects				
Constant	-	17.123	0.229	0.000
ls	-	-0.271	0.008	0.000
dim	dim2	0.0055	0.00017	0.000
	dim2_sq	-0.00002	0.00000	0.000
hr_24yl	-	0.039	0.002	0.000
fat	fat2	0.404	0.071	0.001
	fat2_sq	-0.060	0.026	< 0.05
protein	-	-1.747	0.051	0.000
parity	parity 1	baseline		
	parity 2	0.114	0.039	0.000
month	Jan	baseline		
	Feb	-0.638	0.053	0.000
	Mar	0.705	0.051	0.000
	Apr	1.157	0.054	0.000
	May	-0.615	0.052	0.000
	Jun	-0.226	0.052	0.000
	Jul	1.578	0.054	0.000
	Aug	1.547	0.057	0.000
	Sep	0.296	0.052	0.000
	Oct	0.212	0.055	0.000
	Nov	-0.609	0.052	0.000
	Dec	-0.582	0.051	0.000
Random Effects				
herd	-	2.443	0.268	0.000
cow	-	2.378	0.052	0.000
test	-	7.658	0.045	0.000

Table 4.8 Variance components at each level of the multi-level random effects linear regression modeling, based on three different models.

Model Level	Null model	Fixed effects without season	Fixed effects with season
	Variance Estimate (%)	Variance Estimate (%)	Variance Estimate (%)
Herd	2.757 (19.1)	2.507 (19.1)	2.443 (19.6)
Cow	2.625 (18.2)	2.362 (18.0)	2.378 (19.0)
Test	9.034 (62.7)	8.270 (62.9)	7.658 (61.4)
Total variance	14.419	13.139	12.479

Figure 4.7 A normal plot of model residuals



Note: the observations to the far right of the graph represent cows with pathologically high MUN levels, and therefore do not represent a population of healthy cows.

4.4 Discussion

The descriptive analysis suggested that Milking Shorthorns had the highest average MUN values and Jerseys had the second highest levels. However, the differences among breeds were not significant. Ferguson *et al* (30) reported on the MUN results in nine dairy breeds from their Foss 4000 Milkoscan infrared analyzer at the Pennsylvania Dairy Herd Improvement Association (PHDHIA), and also found relatively little difference among breeds. These breed results were as follows (number of samples/ average MUN): Ayrshire (1,324/14.19 mg/dl); Guernsey (4,740/14.49 mg/dl); Holstein (645,131/14.04 mg/dl); Jersey (20,865/15.81 mg/dl); Brown Swiss (3,118/16.51 mg/dl); Milking Short Horn (84/15.80 mg/dl); Mixed breed (5,684/14.51 mg/dl); and Red and White (2,346/14.30 mg/dl). Carlsson (1) reported no differences in MUN concentrations between two Swedish breeds, Swedish Holstein and Swedish Red and White. Grexton (12) reported that Ontario Holsteins had the lowest average MUN values (14.0 mg/dl) while Brown Swiss had the highest at 16.5 mg/dl. The other breeds were between 14.7 mg/dl and 15.4 mg/dl. No significant differences were reported. It is interesting to note that all of the breed values reported from Ontario were higher than those found in PEI. In our study, no observed differences in breed may be due to the small number of herds and cows of other breeds, producing a lack of statistical power to detect a difference. The reported variability among breeds may be due to physiological or genetic differences or feeding management programs. It also might be that there is a breed difference in protein/ energy metabolism. However, overall, breed does not appear to have a large effect on MUN levels.

The relationship between parity and MUN values was not significant in the

unconditional and multivariable (without season as a fixed effect) random effects regression analysis, but significant in the final multivariable random effects regression model that included season. This conditional association may exist because of the reduced feed intake ability of first calf heifers compared to older cows, which may be exacerbated during the pasture season. Regardless of the significance, the difference was quite small (0.25-0.32 mg/dl). These finding are consistent with other studies (1;11-14;31). These other studies have suggested that first lactation animals are still growing and may, therefore, utilize amino acids more effectively. The consequence of this would be reduced deamination and urea formation in the liver (14).

MUN was lowest in the first month of lactation and increased rapidly over the first two months followed by a slower increase over the next 2 months. This result does not agree with Ng-Kwai-Hang *et al.* (13) who reported that MNPN fell rapidly after calving, and then gradually rose through to the end of lactation. The peak MUN value found at four months in lactation was in agreement with Carlsson *et al.* (1) who reported that MUN reached a maximum between three and six months of lactation and then slowly decreased in the later lactation. The factor that may reduce MUN in the first month of lactation may be the inability of cows to ingest sufficient feed early in lactation (1).

The Pearson correlation coefficient between MUN and milk production (0.173) was similar to that reported by Ferguson (30) (0.178). As well, other studies have reported a positive association between MUN and milk yield (1;14;18;31;32). It has been suggested that the reason for this relationship is due to changes in feeding management with increasing

production. However, Hewett (33) showed that urea values may vary directly with lactation milk yield as well. Jonker *et al.* (34) reported that a 2000 kg increase in milk production per lactation was associated with a 2.6 mg/dl increase in mean MUN in their model. In our study, when looking at the unconditional association between milk production and MUN concentration, the coefficient of milk production was 0.05. Therefore, an increase of 1 kg of milk production per day (equal to 305 kg of milk over a 305 day lactation) increased the MUN concentration by 0.05 mg/dl. Therefore, with this calculation, an increase in milk production by 2000 kg over a 305 day lactation would increase MUN concentration by 0.33 mg/dl, which is a much smaller effect than that reported by Jonker *et al.* (34).

The Pearson correlation coefficients for MUN with protein and fat percentage were -0.212 and -0.117. These are stronger correlations than those reported by Ferguson *et al.* (30), who reported correlation coefficients of -0.138 for protein percent and 0.0135 for fat percent. A graph of milk fat against MUN seemed to be the combination of two lines, suggesting a negative non-linear (quadratic) relationship between MUN and milk fat. This form of relationship was also reported by Godden (35). This result was not consistent with that of Faust *et al.* (25) and Carlsson (27), who found no relationship between milk urea nitrogen and percentage of protein and fat, but they did not transform fat to investigate quadratic associations.

In this study, SCC had a strong negative relationship with MUN concentration. This finding agrees with a previous study, which found the lowest MUN concentrations in cows

with the highest SCC (25). Our study results also agree with that of Ferguson *et al.* (30) who reported a Pearson correlation coefficient for MUN with linear score = -0.240 (in our study it was -0.166). Licata (36) reported a decrease of 2.7 mg/dl in quarters that were CMT positive, compared to CMT negative quarters. However, there are other studies that failed to find an association between SCC and MUN (13;16;37). Godden *et al.* (35) observed in her study that it is unknown how much of the negative association observed between MUN concentrations and somatic cells may be attributed to the failure of the computer algorithm to completely control for the negative interfering effect of somatic cells when producing a urea estimate in samples with very high somatic cell counts, and how much may be due to a true biological association. However, with adequate calibration, the IR method should be able to control for the effects of somatic cell count, and other interfering substances. In our study, a strong significant negative relationship was found between MUN and linear score in the random effect linear regression model. Furthermore, results of Chapter Three indicated that there was excellent agreement between the MUN results from the infrared method used in the study, better than in other tested laboratories.

When GEE was used to look at the correlation between test day MUN within a cow, the correlation between test day MUN did not follow a time sequence. However, season had an effect on the correlation. Therefore, cow was added to the regression models as a random effect with an exchangeable correlation to account for correlation of tests within each cow, and the month variable was added to the model to control for season. To account for clustering of cows within herds, herd was also added to the model as a random effect. MLwiN software was used to handle the random effects of herd and cow.

From Table 4.8, on average, 19% of the variation in MUN values was occurring at each of the cow and herd levels. This is different from a study by Gustafsson *et al.* (8) who found that the variation between herds was responsible for 33% of the total variation in milk urea concentration, whereas variation between individual cows was responsible for only 11%. It is unclear why the two studies differ in this respect. When comparing the variance from the null model versus the full model without season, the percentage of variation in the MUN value accounted for by the fixed effects in the model was small at only 8.8% ((14.416-13.139)/14.416). When including the month variable in the model, the percentage of variation in MUN accounted for by the fixed effects and month was only 13.3% ((14.416-12.497)/14.416). It is clear that there are other sources of variation that account for MUN values which were not included in this study.

4.5 Conclusions

The milk urea concentration was lower during the first month of lactation and rose to peak at 4 months of lactation, and decreased later in lactation. A positive relationship existed between MUN concentration and milk yield. A negative relationship existed between MUN and milk protein % and linear score. A quadratic relationship was found between milk fat % and MUN concentration, with lower MUN values occurring at low and high fat percentages. MUN values were elevated in late winter/early spring (March, April) and through the summer/fall months, with the highest values occurring in July and August. Only 13% of the variation in MUN values can be explained by the combination of studied factors.

4.6 References

1 Carlsson J, Bergstrom J, Pehrson B. Variation with breed, age, season, yield, stage of lactation herd in the concentration of urea in bulk milk and individual cow's milk. Acta vet scand 1995; 36:245-254.

2 Moore DA, Varga GA. BUN and MUN: Urea nitrogen testing in dairy cattle. Comp Cont Ed Pract Vet 1996; 18(6):712-720.

3 Godden SM, Lissemore KD, Leslie KE, Lumsden T, Walton JS, Kelton DF. Preliminary result from a study evaluating the test characteristics of a milk urea nitrogen assay and the influence of factors related to sample collection and handling. Proc Am Assoc Bov Prac 1996; September:169-170.

4 Eicher R, Bouchard E, Tremblay A. Cow level sampling factors affecting analysis and interpretation of milk urea concentrations in 2 dairy herds. Can Vet J 1999; 40(7):487-492.

5 Bruhn JC, Franke AA. Monthly variations in gross composition of California Herd Milks. J Dairy Sci 1977; 60(5):696-700.

6 Cerbulis J, Farrell HM. Composition of milks of dairy cattle. 1.Protein, lactose, and fat contents and distribution of protein fraction. J Dairy Sci 1975; 58(6):817-827.

7 Barton BA, Rosario HA, Andersson GW, Grindle BP, Carroll DJ. Effects of dietary crude protein, breed, parity, and health status on the fertility of dairy cows. J Dairy Sci 1996; 79(121):2225-2236.

8 Gustafsson AH., Emanuelson M, Oltner R, Wiktorsson H. Milk urea level, its variations and how it is affected by herd, milk yield, stage of lactation, season and feeding - A field study. Dept of Animal Nutrition and Management, Swedish University of Agricultural Sciences. Report 165 1987; 1-31

9 Carroll DJ, Barton BA, Smith RD. Influence of protein intake and feeding strategy on reproductive performance of dairy cows. J Dairy Sci 1988; 71(12):3470-3481.

10 Peterson RG, Waldern DE. Repeatabilities of serum constituents in Holstein - Friesians affected by feeding, age, lactation, and pregnancy. J Dairy Sci 1981; 64(5):822-831.

11 Canfield RW, Sniffen CJ, Butler WR. Effects of excess degradable protein on postpartum reproduction and energy balance in dairy cattle. J Dairy Sci 1990; 73(9):2342-2349.

12 Grexton B. MUN data on Ontario herds offers up some interesting information. http://www.ontdhi.com/mundata.htm . 1999.

13 Ng-Kwai-Hang KF, Hayes JF, Moxley JE, Monardes HG. Percentages of protein and nonprotein nitrogen with varying fat and somatic cells in bovine milk. J Dairy Sci 1985; 68(5):1257-1262.

14 Oltner R, Wiktorsson H, Emanuelson M. Urea concentrations in milk in relation to milk yield, live weight, lactation number and amount and composition of feed given to dairy cows. Livest Prod Sci 1985; 12:47-57.

15 Ropstad E, Vik-Mo L, Refsdal AO. Levels of milk urea, plasma constituents and rumen liquid ammonia in relation to the feeding of dairy cows during early lactation. Acta vet scand 1989; 30:199-208.

16 Eicher R, Bouchard E, Bigras-Poulin M. Factors affecting milk urea nitrogen and protein concentrations in Quebec dairy cows. Prev Vet Med 1999; 39:53-63.

17 Kaim M, Folman Y, Neumark H, Kaufmann W. The effect of protein intake and lactation number on post-partum body weight loss and reproductive performance of dairy cows. Anim Prod 1983; 37:229-235.

18 Kaufmann W. Variation in composition of raw milk with special regard to the urea content. Milchwissenschaft 1982; 37[1], 6-9

19 Hoffmann VM, Steinhofel O. Possibilities and limitations for appraisal of energy and protein supply through monitoring of milk urea level. Mh Vet Med 1990; 45:223-227.

20 Emanuelson M, Wiktorsson H. Long-term feeding of rapeseed meal and full-fat rapeseed of double low cultivars to dairy cows. Livest Prod Sci 1993; 33:199-214.

21 DePeters EJ, Cant JP. Nutritional factors influencing the nitrogen composition of bovine milk: A review. J Dairy Sci 1992; 75(8):2043-2070.

22 Bruckental I, Oldham JD, Sutton JD. Glucose and urea kinetics in cows in early lactation. Br J Nutr 1980; 44:33-45.

23 Grimard B, Humblot P, Ponter AA, Mialot JP, Sauvant D, Thibier M. Influence of postpartum energy restriction on energy status, plasma LH and oestradiol secretion and follicular development in suckled beef cows. J Reprod Fert 1995; 104:173-179.

24 Schepers AJ, Meijer RGM. Evaluation of the utilization of dietary nitrogen by dairy cows based on urea concentration in milk. J Dairy Sci 1998; 81:579-584.

25 Faust MA, Kilmer LH, Funk R. Effects of laboratories for milk urea nitrogen and other milk components. J Dairy Sci 1997; 80[suppl. 1], 206

26 Gustafsson AH, Palmquist DL. Diurnal variation of rumen ammonia, serum urea and milk urea in dairy cows at high and low yields. J Dairy Sci 1993; 76(2):475-484.

27 Carlsson J Milk urea as a marker of nutritional imbalance in dairy cows with special reference to fertility. Proc. 7th Int. Conf. Prod. Diseases, Cornell University, Ithaca, USA: 1989;397-400

28 StataCorp. Stata statistical software. [Release 6]. 1999. College Station (TX), Stata Corporation.

29 Goldstein H, Rasbash J, Plewis I, Draper D, Browne W, Yang M. A User's Guide to MLwin. -140 pp. 1998. Institute of Education, London.

30 Ferguson JD, Thomsen N, Slesser D, Burris D. Pennsylvania DHIA milk urea testing. J Dairy Sci 1997; 80[suppl.1], 161

31 Ferguson JD. Milk urea nitrogen. Ferguson JD, editor. http://cahpwww.nbc.upenn.edu/mun/mun_info.html , 1-6. 6-29-1999.

32 Macleod GK, Grieve DG, McMillan I, Smith GC. Effect of varying protein and energy densities in complete rations fed to cows in first lactation. J Dairy Sci 1984; 67(7):1421-1429.

33 Hewett C. On the causes and effects of variations in the blood profile of Swedish dairy cattle. Acta Vet Scand 1974; suppl. 50.

34 Jonker JS, Kohn RA, Erdman RA. Milk urea nitrogen target concentrations for lactating dairy cows fed according to National Research Council Recommendations. J Dairy Sci 1999; 82(6):1261-1273.

35 Godden SM. Evaluation of a milk urea assay, and the relationship between milk urea concentrations and nutritional management and performance in Ontario dairy herds. [PhD dissertation], University of Guelph, Ontario, 1998.

36 Licata E. Subclinical mastitis and urea determination in cows' milk . Obiettivi e Doc -Vet 1985; 6(12):65-67.

37 Verdi RJ, Barbano DM, Dellavalle ME, Senyk GF. Variability in true protein, casein, nonprotein nitrogen, and proteolysis in high and low somatic cell milks. J Dairy Sci 1987; 70(2):230-242.

Chapter 5

Bulk Tank MUN versus Herd Average MUN

5.1 Introduction

Urea concentration in milk could potentially be used as a parameter to monitor energy and protein levels and their balance in dairy cow rations. However, there are many factors affecting MUN values from individual cows, making MUN interpretation for individual cows difficult. Researchers have suggested that the mean urea concentration from a group of cows should fall within a specific range (1-4). An alternative to determining the mean MUN level for a group of cows is to test the bulk tank MUN (BTMUN). The cost and labor advantages of testing one BTMUN versus a group of cows seem attractive. BTMUN might be a reliable guide to the average urea concentration of a herd.

There are some data on the mean, standard deviation and range of BTMUN in different regions: Sweden (5), Norway (6), and Chile (7) were 11.2 ± 2.1 (4.76 - 17.08) mg/dl, 13.72 ± 3.36 (4.2 - 32.48) mg/dl, and 13.16 ± 3.64 (3.92 - 28.0) mg/dl, respectively. No data are available from Canadian dairy herds. Two studies have looked at the correlation between BTMUN and herd average blood urea nitrogen, and have reported very different results with values of $r = 0.77$ (8), and $r = 0.95$ (7).

Schepers *et al.* (9) and Hof *et al.* (10) showed that BTMUN is a valuable tool to monitor the rumen-degraded protein balance in the ration. However, Nelson (11) stated that a high normal value of BTMUN was difficult to interpret. Bulk tank samples have been observed to differ from the mean of all cows by as much as 3.0-4.5 mg/dl. Garcia (12) showed results that dietary crude protein or soluble protein were not reliable predictors of BTMUN, and correlation between BTMUN and herd average MUN was low (0.36).

A report from Ontario DHI (13) discussed the reasons why BTMUN may not be a useful substitute for individual cow MUN's. The two most important reasons listed were as follows.

1.Single sample MUN test results are quite variable, and thus a single MUN test result may not represent what is going on in the dairy herd. For this reason, they recommend that an average MUN value from all cows be used for evaluating nutritional programs. A single bulk milk sample would also be highly variable, and without the ability to average multiple bulk milk values together, the information could not be confidently used to assess nutritional management.

2. Each cow contributes a different volume of milk to the bulk tank. Depending on the age, stage of lactation and health of a cow, the amount of milk (and MUN) that she contributes to the bulk tank will vary. This means that the bulk milk sample will not fairly represent the entire herd.

Therefore, it is necessary to investigate more thoroughly whether a BTMUN can be a reliable measure of the average milk urea concentration of a herd, and whether this

relationship is affected by other factors such as herd size, type of DHI recording that is used on the farm, and season of the year.

The seasonal variation in BTMUN also needs clarification. Earlier studies, which have looked at variation of milk non-protein nitrogen (MNPN - of which urea is a part) levels in bulk tanks in different areas (New York (14), Quebec (15), Netherlands (16), and Ontario (17)) found that MNPN levels were high in August, September, and October, decreased gradually after that, reached minimum values in April, and then slowly increased again.

The objectives of this study were: 1) to determine if BTMUN can represent the status of the whole herd average of individual cow MUN levels and whether this relationship is affected by other factors such as herd size, type of DHI recording that is used on the farm, and season of the year; and 2) to determine the seasonal variation in BTMUN concentrations in PEI dairy herds.

5.2 Materials and Methods

5.2.1 Data collection

The study for comparison of BTMUN with herd average MUN took place from July 1999 to June 2000 and used all 199 PEI dairy herds on monthly, individual cow milk testing for MUN and milk weights (among other parameters) through the Atlantic Dairy Livestock Improvement Corporation (ADLIC). The average herd size was 45 cows, ranging from 10 to 180.

All of the herds had bulk milk samples obtained every milk pick up by milk truck drivers as part of the regular quality control program of the dairy to which each farm shipped their milk. In general, the samples of bulk milk were composed of two morning and two afternoon milkings. MUN analyses on bulk tank milk (as with individual cow milk samples) were normally done within 1-2 days following sample collection. The MUN analyses were performed using a Fossomatic 4000 Milkoscan analyzer at the PEI Milk Quality Laboratory (PEIMQL). Normally, a bulk milk sample was tested for MUN every 14 days for each farm.

Data on individual milk production and MUN levels were obtained from ADLIC records. These data were stratified by pasture season (yes/no), and five herd size groups based on the number of milking cows in the herd [group one (<40), group two (41-60), group three (61-80), group four (81-100) and group five (>100)]. The herds in this study were also stratified according to one of four different individual cow milk sampling protocols employed on the farm: 1. official 2X test - milk sampled by a technician and collected 2 times, 12 hours apart (OFF-2X), 2. official 1X test - milk sampled by a technician and

collected in the morning one month and in the evening the next month (OFF-1X), 3. non-official 2X test - milk sampled by the farmer and collected 2 times, 12 hours apart (UNOFF-2X), 4. non-official 1X test - milk sampled by the farmer and collected in the morning one month and in the evening the next month (UNOFF-1X)

The data for assessing seasonal variation in BTMUN were collected from July 1999 to February 2001 from 199 herds, totalling 11,223 bulk tank samples. The period of data collection was extended to February, 2001 to examine seasonal effects between years. These data were divided into 8 categories; 1. mid-pasture period (July 1 - August 31, 1999), 2. late pasture period (September 1 - October 31, 1999), 3. early stable period (November 1, 1999 - February 29, 2000), 4. late stable period (March 1 - May 15, 2000), 5. early pasture period (May 16 - June 30, 2000), 6. second mid-pasture period (July 1 - August 31, 2000), 7. second late pasture period (September 1 - October 31, 2000), and 8. second early stable period (November 1, 2000 - February 28, 2001).

5.2.1 Statistical Analysis

Along with unweighted herd average MUN values (UNWHMUN), weighted herd average MUN levels (WHMUN) were calculated for each herd each month (weighted by cow milk production). The following cow observations were excluded from these calculation: milk fat tests < 1.5% or > 6%; milk protein tests < 1.5% or > 5%; milk production values of 0.1 kg (missing code); and MUN tests ≤ 0.1 mg/dl. Descriptive statistics for the BTMUN, WHMUN and UNWHMUN for the July 1999 to June 2000 period were calculated.

UNWHMUNs and WHMUNs were compared to the BTMUN closest to it in time (maximum of 7 days before or after the WHMUN). Significant differences ($P < .05$) between BTMUN and UNWHMUN and WHMUN mean values and standard deviations were determined using a t test.

A scatter plot was created by plotting the BTMUN results against WHMUN. A line of perfect agreement (45 ° and intercept zero) was imposed on this figure. Significant agreement ($p \leq 0.05$) between BTMUN and WHMUN was assessed using two methods: calculation of the concordance correlation coefficient (18) and its 95% confidence interval, and a graphical procedure proposed by Bland and Altman (19) as described in Chapter 3. Significant agreement between BTMUN and WHMUN was also assessed within each of the strata for the following variables: milk sampling protocols (1 of 4 options), herd size groups (<40, 41-60, 61-80, 81-100, and >100 cows), and pasture season (pasture and non-pasture groups).

Testing for significant differences ($p \leq 0.05$) between BTMUN (and WHMUN) values among strata requires adjustment due to the clustering of repeated BTMUN (and WHMUN) tests within herds. Generalized estimating equations (GEE) were used to determine if there were significant differences between BTMUN (and WHMUN) values among strata, adjusting for this correlation, using an exchangeable (compound symmetry) correlation structure.

To determine seasonal patterns in BTMUN, significant differences in BTMUN

among periods were also assessed using generalized estimating equations (GEE), again to using an exchangeable correlation structure to adjust for repeated data within herds. All analyses were carried out using Stata release 6 (College Station, Texas)(20).

5.3 Results

There were 1,772 complete observations in the final dataset for the period of July 1999 to June, 2000, giving almost 10 WHMUN-BTMUN paired observations per herd, on average. As shown in Table 5.1, the BTMUN mean was slightly higher (but not statistically significant at p=0.05) than the WHMUN mean, but significantly higher than the mean UNWHMUN. The BTMUN standard deviation was slightly lower than the WHMUN and UNWHMUN standard deviations but these difference was not statistically significant.

With all farms and herd tests included, the concordance correlation coefficient showed a moderately high correlation (Pc = 0.81). Figure 5.1 demonstrates this moderate correlation, showing the solid regression line being very close to the hatched 45 degree line of perfect agreement, but also showing numerous data points falling off of the line. However, there is an even distribution of points about the line, and leading to a mean difference between BTMUN and WHMUN of only 0.06 mg/dl (sd = 1.76). With the dispersion of points about the line, the 95% of limits of agreement were -3.52 and 3.40 mg/dl, respectively (Fig 5.2), meaning that 95% of the differences between BTMUN and WHMUN fell within this seven unit range, while 5% fell outside this range.

Table 5.1. Descriptive statistics for bulk tank milk urea nitrogen (BTMUN) and weighted herd average milk urea nitrogen (WHMUN) in 199 PEI dairy herds.

Type	No. Samples	Mean (mg/dl)	SD (mg/dl)	Min. (mg/dl)	Max. (mg/dl)
BTMUN	1772	11.70 [a]	2.84 [a]	3.6	21.0
WHMUN	1772	11.64 [a]	2.91 [a]	3.4	21.8
UNWHMUN	1772	11.52 [b]	2.87 [a]	3.3	21.5

[a,b] values with the same superscript were not statistically significantly different at p=0.05

Figure 5.1 Scatter plot comparison of bulk tank milk urea nitrogen (BTMUN) and weighted herd average milk urea nitrogen (WHMUN) in 199 PEI dairy herds.

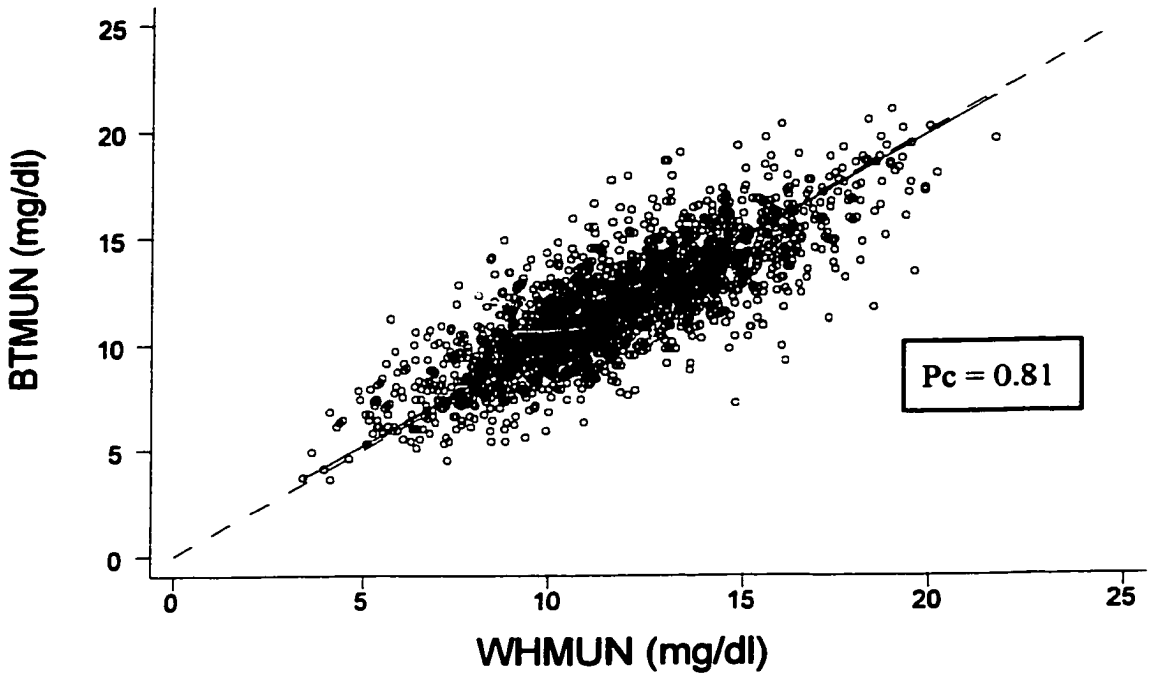
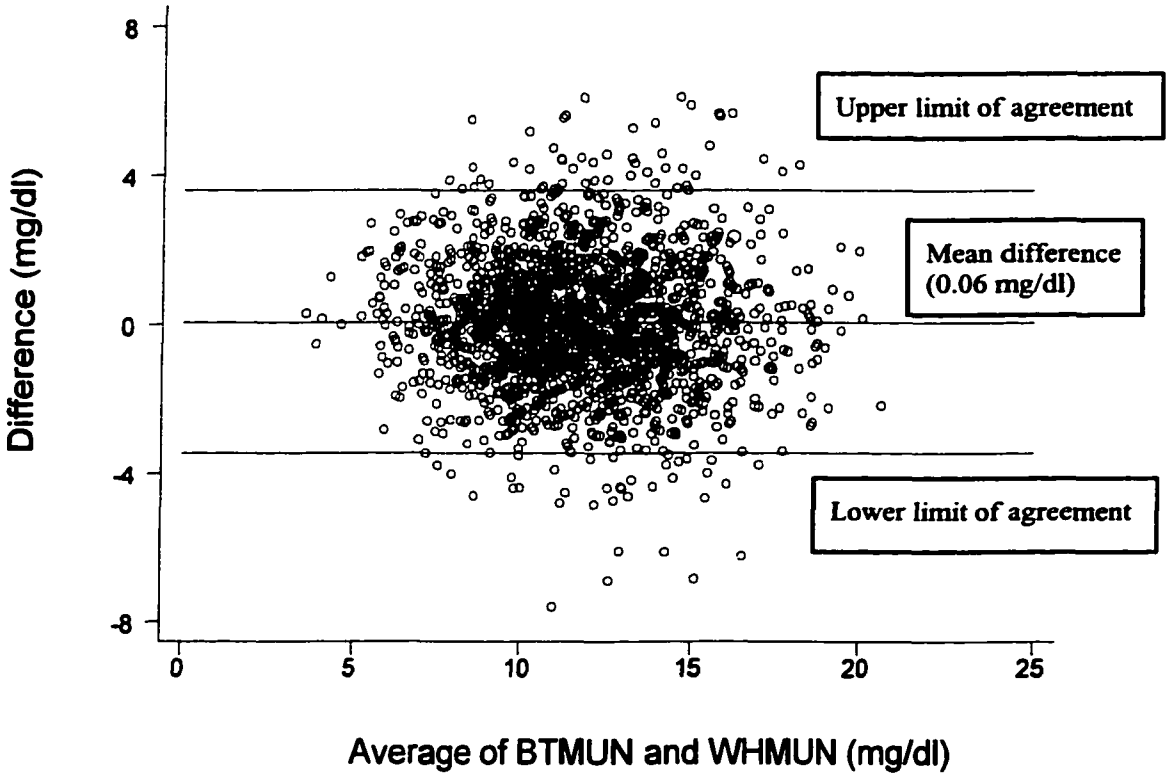


Figure 5.2 Difference between bulk tank milk urea nitrogen (BTMUN) and weighted herd average milk urea nitrogen (WHMUN), plotted against the mean value with horizontal line, showing the 95% limits of agreement, in 199 PEI dairy herds.



When stratifying the data by milk sampling protocols, the concordance correlation coefficient was slightly higher for OFF-2X sampling than for the other three options (Table 5.2 & Appendix C.1), but the differences among the protocols were very small may have been due to chance alone. The 95% limits of agreement between BTMUN and WHMUN for herd tests that were officially sampled by a technician were somewhat smaller (-3.20 to 3.45 and -3.35 to 3.45) than for non-officially sampled herd tests (-4.09 to 3.24 and -3.81 to 3.13) (Appendix C.2). The GEE results showed that there were no significant differences in BTMUN or WHMUN among sampling protocols.

When stratifying the data by pasture season, the concordance correlation coefficient for BTMUN and WHMUN was slightly higher in the non-pasture season (0.82) than pasture group (0.79) (Table 5.3 & Appendix C.3, C.4), but this difference was not statistically significant. The limits of agreement were somewhat wider for the pasture season (-3.80 to 3.80) than for the non-pasture season (-3.30 to 3.09). The GEE result showed that there were strongly significant differences in BTMUN and WHMUN among pasture and non-pasture groups. While herds were on pasture, their BTMUNs and WHMUNs were significantly higher than when not on pasture.

When stratifying the data by herd size groups, the concordance correlation coefficient for BTMUN and WHMUN was numerically higher in small and medium sized herds (Pc = 0.83 and 0.81, respectively) than large herds (Pc = 0.71 - 0.77) (Table 5.4 & Appendix C.5), but this difference was not statistically significant. Herds with less than 40 cows had the lowest BTMUN and WHMUN, on average while herds with 81-100 cows had the

highest. The GEE result showed that there were significant differences in BTMUN and WHMUN among herd sizes.

Table 5.5 shows the descriptive statistics of BTMUN values for July, 1999 to February, 2001 (n = 11,223), stratified by the eight seasonal periods. Again, during the pasture season, herds had a higher BTMUN than during the non-pasture period, particularly during the mid- and late pasture season of 2000. The GEE results showed that there were strongly significant (P <.05) differences among seasonal groups.

Table 5.2 Descriptive statistics for bulk tank milk urea nitrogen (BTMUN) and weighted herd average milk urea nitrogen (WHMUN) in 199 PEI dairy herds, categorized by milk sampling protocols

Sampling Option	No. of Observations	BTMUN Mean (Sd.) (mg/dl)	WHMUN Mean (Sd.) (mg/dl)	Pc [a] (95% CI)
OFF-2X [1]	485	11.84 (2.85)	11.96 (2.90)	0.83 (0.80-0.85)
OFF-1X [2]	744	11.88 (2.75)	11.93 (2.81)	0.81 (0.78-0.83)
UNOFF-2X [3]	280	11.38 (2.93)	10.96 (2.89)	0.79 (0.74-0.83)
UNOFF-1X [4]	263	11.26 (2.89)	10.92 (2.99)	0.81 (0.77-0.85)

[1] OFF-2X = official-sampled by a technician and milk collected 2 times 12 hours a part.

[2] OFF-1X = official-sampled by a technician and milk collected in the morning one month and evening the next month.

[3] UNOFF-2X = nonofficial-sampled by the farmer and milk collected 2 times 12 hours a part

[4] UNOFF-1X. = nonofficial-sampled by the farmer and milk collected in the morning one month and evening the next month.

[a] Pc = Concordance correlation coefficient

Table 5.3 Descriptive statistics for bulk tank milk urea nitrogen (BTMUN) and weighted herd average milk urea nitrogen (WHMUN) in 199 PEI dairy herds, categorized by pasture and non pasture season.

Strata	No. of Observations	BTMUN Mean (Sd.) (mg/dl)	WHMUN Mean (Sd.) (mg/dl)	Pc (95% CI)
Pasture	740	12.22 [a] (2.93) [a]	12.21 [a] (3.07) [a]	0.79 (0.77-0.82)
Non pasture	1032	11.33 [b] (2.71) [b]	11.22 [b] (2.71) [b]	0.82 (0.80-0.84)

[a,b] values with the same superscript were not statistically significantly different at p=0.05

Table 5.4 Descriptive statistics for bulk tank milk urea nitrogen (BTMUN) and weighted herd average milk urea nitrogen (WHMUN) in 199 PEI dairy herds, categorized by herd size.

Strata	No. of Observations	BTMUN Mean (Sd.) (mg/dl)	WHMUN Mean (Sd.) (mg/dl)	Pc (95% CI)
Group one (cow <40)	989	11.40 (2.86) [a]	11.25 (2.92)	0.81 (0.78-0.83)
Group two (cow 41-60)	494	12.14 (2.87) [b]	12.15 (2.96)	0.83 (0.80-0.86)
Group three (cow 61-80)	159	11.81 (2.81) [a]	12.07 (2.72)	0.77 (0.71-0.84)
Group four (cow 81-100)	68	12.73 (2.32) [b]	12.38 (2.46)	0.77 (0.67-0.87)
Group five (cow > 100)	62	11.50 (2.04) [a]	11.86 (2.24)	0.71 (0.60-0.84)

[a,b] values with the same superscript were not statistically significantly different at p=0.05

Table 5.5 Descriptive statistic for bulk tank milk urea nitrogen (BTMUN) values in 199 PEI dairy herd, by season.

Type	No. of Samples	Mean (mg/dl)	SD (mg/dl)	Min. (mg/dl)	Max. (mg/dl)
Mid pasture (July 1 - Aug 31, 1999)	176	13.40	3.16	5.3	21.0
Late pasture (Sep 1 - Oct 31, 1999)	348	12.11	2.81	4.9	20.1
Early stable (Nov 1 - Feb 29, 2000)	2050	10.86	2.52	3.6	19.7
Late stable (Mar 1 - May 15, 2000)	1999	12.10	2.72	4.6	20.9
Early pasture (May 16 - June 30, 2000)	1191	11.17	2.41	3.3	18.8
2 nd Mid pasture (July 1 - Aug 31, 2000)	1572	14.69	3.50	6.2	27.6
2 nd Late pasture (Sep 1 - Oct 31, 2000)	1585	14.94	3.30	6.3	28.3
2 nd Early stable (Nov 1 - Feb 28, 2001)	2302	9.85	2.28	3.2	19.6

5. 4 Discussion

In this study, the mean BTMUN of 11.70 mg/dl recorded was slightly lower than the values recorded in other studies (5-7). The difference may have been due to different feeds, sources of feeding, and feeding procedures utilized in the PEI dairy herds. The wide variation of urea concentrations between herds in bulk milk samples likely reflects variation in the quality of the rations fed to the herds.

This study showed that the WHMUN and BTMUN had a moderately high correlation (Pc = 0.81) and low mean difference of 0.06 mg/dl, indicating that a measurement of the concentration of urea in bulk milk is a satisfactory but not totally reliable measure of the mean concentration of urea in the milk of a whole herd. This agrees with Carlsson *et al.* (21), who suggested that BTMUN might be a useful guide to the average urea concentration of a herd. Reports from Ontario DHI suggest that a single bulk milk sample is highly variable for MUN, and multiple BTMUN values should be averaged to make interpretation reliable (9).

The correlation in this study was much higher than that found by Garcia *et al.* (12), who reported a Pearson correlation coefficient of 0.36 between BTMUN and the herd average of DHIA samples for all individual cows in the herds. However, the authors did not weight the herd average MUN for milk production. Intuitively, it would make sense that milk weighted herd average MUN values should be closer to BTMUN values than unweighted herd average MUN. We found that the WHMUN had a slightly (and

statistically significant) higher mean than UNWHMUN. However, the numerical differences in all of means (BTMUN, WHMUN and UNWHMUN) were very small, with very little biological relevance.

We looked at the difference between official milk collection and unofficial milk collection, and whether the samples had been collected one time or two times per test day to determine if they influenced the agreement between BTMUN and WHMUN. When correlations by milk sampling protocols were determined, official tests from two milk collection times per sample had the highest correlation between BTMUN and WHMUN but the differences were small and not significant. The reason for these differences are not obvious. One possible explanation is that a BTMUN is usually based on data from 4 milkings compared to 1 or 2 milkings in a WHMUN. However, if this explained the difference, one would expect the concordance correlation between BTMUN and WHMUN values based on a single milking (OFF-1X and UNOFF-1X) to have a lower correlation than with those based on a 2 milkings. This was not the case in our study, and the difference in agreement by number of milkings was very small. There were no significant difference among sampling protocols in term of average BTMUN.

When comparing BTMUN and WHMUN by season, the non-pasture season had a slightly but not statistically significantly ($p<0.05$) higher concordance correlation than the pasture season (Table 5.3). WHMUN values during the pasture season were significantly higher and had significantly more variation (sd = 3.07) than during the non-pasture season (sd = 2.71). Amount and quality of pasture consumption can vary from day-to-day,

depending on weather, pasture rotation, etc., explaining this difference.

The small (<40) and medium (41-60) size herds had a better correlation between their BTMUN and WHMUN values than larger sized herds, although the differences were not statistically significant ($p<0.05$). The largest group had the lowest correlation between BTMUN and herd average MUN (Table 5.4), but the number of observations was small in this group of herds.

Significant differences in BTMUN values were found across seasons. The crude protein on pasture may be higher in soluble and degradable fractions in grass relative to energy content compared to stored feeds, resulting in a higher BTMUN concentration during the grazing period (7). The highest BTMUN values were found during the mid- and late pasture seasons of 2001 likely because the precipitation was unusually high during this period, enhancing pasture growth. More detailed analyses of the effect of nutrition on MUN will be evaluated in another portion of the MUN project.

5.5 Conclusions

The results from this study showed that BTMUN had moderate correlation and agreement with milk weighted herd average MUN values. This moderate reliability extends to both the pasture and non-pasture seasons, various milk sampling protocols, and herd sizes seen in Atlantic Canada. The reasons why BTMUN and WHMUN are not more highly correlated remain unknown.

BTMUN values provide a moderately reliable indicator to the urea status of the whole herd. Bulk tank milk samples are taken every time milk is picked up from a dairy farm, and therefore, BTMUN could be tested more frequently than once a month, and at considerably less cost than individual cow testing, providing more timely and inexpensive information on urea status of a herd. However, BTMUN values do not correlate perfectly with WHMUN values, as we discovered, and BTMUN is not very sensitive to changes in group average MUN values within a herd. Because groups of cows within a herd are often managed very differently, group average MUN values within a herd are necessary for a more thorough assessment of urea levels for various groups within a herd. Only individual cow MUN values that are averaged for groups of cows within a herd can provide this necessary information. Therefore, BTMUN test values cannot replace group averages of individual cow MUN values, but they may provide useful information for the periods between test dates of individual cow MUN tests. Therefore, research into associations between variation in MUN values and feeding management factors should be conducted on group averages of individual cow MUN values. Additional research is needed to determine herd level factors

(feeding or otherwise) that would be associated with BTMUN values because of their possible use in between test dates of individual cow MUN values.

5.6 References

1 Broderick GA, Clayton MK. A statistical evaluation of animal and nutritional factors influencing concentrations of milk urea nitrogen. J Dairy Sci 1997; 80(11):2964-2971.

2 Cannas A, Pes A, Mancuso R, Vodret B, Nudda A. Effect of dietary energy and protein concentration on the concentration of milk urea nitrogen in dairy ewes. J Dairy Sci 1998; 81(2):499-508.

3 Ferguson JD. Milk urea nitrogen. Ferguson JD, editor. http://cahpwww.nbc.upenn.edu/mun/mun_info.html , 1-6. 6-29-1999.

4 Kolver ES, MacMillan KL. Short term changes in selected metabolites in pasture fed dairy cows during peak lactation. Proceedings of the New Zealand Society of Animal Production 1993; 53:77-81.

5 Gustafsson AH, Carlsson J. Effects of silage quality, protein evaluation systems and milk urea content on milk yield and reproduction in dairy cows. Livest Prod Sci 1993; 37:91-105.

6 Ropstad E, Refsdal AO. Herd reproduction performance related to urea concentration in bulk milk. Acta vet scand 1987; 28:55-63.

7 Wittwer FG, Gallardo P, Reyes J, Opitz H. Bulk milk urea concentrations and their relationship with cow fertility in grazing dairy herds in Southern Chile. Prev Vet Med 1999; 38:159-166.

8 Refsdal AO. Urea in bulk milk as compared to the herd mean of urea in blood. Acta vet scand 1983; 24:518-520.

9 Schepers AJ, Meijer RGM. Evaluation of the utilization of dietary nitrogen by dairy cows based on urea concentration in milk. J Dairy Sci 1998; 81:579-584.

10 Hof G, Vervoorn MD, Lenaers PJ, Tamminga S. Milk urea nitrogen as a tool to monitor the protein nutrition of dairy cows. J Dairy Sci 1997; 80(12):3333-3340.

11 Nelson AJ. Practical application of MUN analyses. Proc Am Assoc Bov Prac 1996; 29(September):85-95.

12 Garcia AD, Linn JG, Stewart SC, Olson JD, Olson WG. Evaluation of milk urea nitrogen (MUN) as a dietary monitor for diary cows. J Dairy Sci 1997; 80[suppl.1], 162

13 Ontario DHI. Bulk MUN milk - Why not? http://www.ontdhi.com/bulk%20milk.html. 1999

14 Verdi RJ, Barbano DM, Dellavalle ME, Senyk GF. Variability in true protein, casein, nonprotein nitrogen, and proteolysis in high and low somatic cell milks. J Dairy Sci 1987; 70(2):230-242.

15 Ng-Kwai-Hang KF, Hayes JF, Moxley JE, Monardes HG. Percentages of protein and nonprotein nitrogen with varying fat and somatic cells in bovine milk. J Dairy Sci 1985; 68(5):1257-1262.

16 de Koning PJ, Koops J, van Rooijen PJ. Some features of the heat stability of concentrated milk. III. Seasonal effects on the amounts of casein, individual whey protein and NPN and their relation to variations in heat stability. Neth Milk Dairy J 1974; 27:186-202.

17 Szijarto L, Biggs DA, Irvine DM. Variability of casein, serum protein and nonprotein nitrogen in plant milk supplies in Ontario. J Dairy Sci 1973; 56(1):45-51.

18 Lin LI-K. A concordance correlation coefficient to evaluate reproducibility. Biometrics 1989; 45:255-268.

19 Bland JM, Altman DG. Comparing methods of measurement: why plotting difference against standard method is misleading. The Lancet 1995; 346(October 21):1085-1087.

20 StataCorp. Stata statistical software. [Release 6]. 1999. College Station (TX), Stata Corporation.

21 Carlsson J, Bergstrom J, Pehrson B. Variation with breed, age, season, yield, stage of lactation herd in the concentration of urea in bulk milk and individual cow's milk. Acta vet scand 1995; 36:245-254.

Chapter 6

Conclusions

6.1 Introduction

More efficient use of protein feed supplements can potentially reduce the impact of nitrogen(N) in feed, excretion of N in manure, and losses to the environment (1). Unfortunately, only about 25 to 30% of the N consumed by lactating cows is transferred to the milk produced; the remainder is excreted in feces and urine (2). This is partly caused by inefficient use of rumen degradable protein (RDP), leading to the absorption of ammonia N rather than protein N. The ammonia N is detoxified by the liver through the urea, the metabolic end product of protein catabolism in the body (3).

Urea nitrogen concentrations circulating in the bloodstream are measured in either plasma or serum fractions (PUN or SUN, respectively) and are often referred to generically as blood urea nitrogen (BUN). BUN has long been known to reflect inefficient utilization of dietary crude protein by ruminants (4). Milk urea nitrogen (MUN) is strongly correlated with blood urea nitrogen (5-8) because urea easily diffuses across the mammary tissue bed (9). MUN provides a rapid, noninvasive and inexpensive means of assessing the dynamic of BUN (7), and of monitoring overall protein metabolism in lactating cows (10). Also, the measurement of urea concentration in milk may represent a useful tool to monitor the efficiency of protein utilization in a dairy herd.

The main goal of this project was to evaluate the predictive ability of MUN to indicate nutritional imbalances in dairy herds under a range of feeding management systems in Atlantic Canada. It is hoped that the outcome of this project will not only make a useful contribution to evaluation of milk urea nitrogen as an indicator of efficiency of diets for dairy cows, but also develop criteria to accurately interpret MUN measurements under commercial conditions.

The development of new infrared methods provides a rapid and inexpensive method to measure MUN in milk samples that are routinely collected by milk recording agencies (11). However, before any new test is adopted, its performance should be rigorously and impartially evaluated under laboratory and field conditions (12). In evaluating test performance, it is helpful to know how well the method compares with the reference procedure, and how repeatable the method is on duplicated milk samples. There are many factors in addition to the methodology that can potentially affect MUN. In order to use MUN as a measure of efficiency of protein feeding, factors that influence MUN need to be more clearly understood.

This thesis addresses some of the questions regarding the use of MUN. The three specific objectives of this thesis were:

1. to validate an infrared-based cow level test through a comparison with a gold standard test;
2. to determine the effects of cow factors on MUN; and
3. to determine how well bulk tank MUN levels (BTMUN) reflect the weighted herd

average MUN levels (WHMUN) and unweighted herd average MUN level (UNWHMUN).

6.2 Method of determination

The first objective was to evaluate the performance of the infrared method for measuring MUN using the Fossomatic 4000 Milkoscan analyzer at the PEI Milk Quality Laboratory (PEIMQL) and to compare it with an enzymatic reference method conducted at the laboratory of the Ontario Dairy Herd Improvement Corporation (ODHIC). The concordance correlation coefficient (Pc) was used as a means of measuring overall agreement. The results showed that there was a very good agreement (Pc = 0.972) between the two tests.

We also determined the repeatability of the same infrared method by using routine samples measures twice at the PEIMQL. The coefficient of variation, a measure of precision, for MUN values from replicated samples, was very small (2.2%). The concordance correlation coefficient of 0.983 suggested that there was a very good overall agreement between the replicated samples.

6.3 Cow factors

The second objective of this thesis was to describe the relationship between MUN concentration and cow factors such as breed, parity, days in milk, milk quality, milk

components, and milk yield. Holstein cows had slightly lower MUN levels than Jerseys or Milking Shorthorns but the differences were not statistically significant. The power to determine differences was limited by the low number of non-Holstein herds. MUN concentrations were lowest among first parity cows but differences among parities were not significant. MUNs were lowest during the first month of lactation, dramatically increased during the second month of lactation, peaked at 4 months, and then decreased again through to the end of the lactation. A significant positive relationship existed between MUN and milk yield. A significant negative relationship existed between MUN and percent milk fat, percent milk protein and linear score. Although these associations were statistically significant due to the large data set, their biological significance was limited. Only 9% of the variation in test day MUN was explained by the combination of cow factors described above. Herd factors (eg. nutrition) or other unmeasured cow factors (eg. body condition score) account for the remainder of the variation in individual cow MUN levels.

6.4 Bulk tank MUN

The third objective of this thesis was to determine how well BTMUN compares with WHMUN. The mean difference between BTMUN and WHMUN was 0.06 mg/dl but the moderate correlation between BTMUN and WHMUN indicated that measurement of urea in bulk milk is only a satisfactory (not excellent) measure of the mean concentration of urea in the milk of a herd. When stratified by milk sampling protocols, pasture season, and herd size, BTMUN still had only a moderate correlation and agreement with WHMUN. BTMUNs were significantly associated with season, with concentration being highest during

the pasture season.

6.5 SUMMARY

The MUN project was conducted to determine the sources of variation in MUN values measured at the cow and herd level under management systems located in Atlantic Canada. Although feeding management was expected to be a large source of the variation (as reported in a companion thesis soon to be published at this same institution), other sources of variation needed to be investigated in order to be confident in conclusions regarding variation in MUN values associated with feeding management factors. Other sources of variation that were investigated here included laboratory measurement error, production factors at the cow and test day level, and herd factors such as herd size and milk recording protocol.

The results from replicated milk samples showed a high level of repeatability for the infrared machine and there was very good overall agreement between the gold standard method and the infrared method used at the PEIMQL. Therefore, the quality control program of the PEIMQL is producing MUN results that are highly reliable and repeatable for dairy farmers in the Atlantic region. Automatic infrared instrumentation at PEIMQL can be used satisfactorily to measure milk urea nitrogen for dairy herds in Atlantic Canada.

When looking at the relationship between MUN and cow and test day factors', only 9% of the variation in individual cow MUN was explained by the list of factors investigated

in this study. Therefore, it is unlikely that conclusions regarding variation in MUN values associated with feeding management factors would be a result of laboratory error or confounding effects of cow and test day factors.

A number of cow and test day factors were found to be significantly associated with MUN values, however two of these, parity and percent fat in milk, had small biological significance. The milk urea concentration was significantly lower during the first month of lactation and rose to peak at 4 months of lactation, decreasing later in lactation. A strong positive relationship existed between MUN concentration and milk yield. A strong negative relationship existed between MUN and percent protein in milk and linear score. MUN values were elevated in late winter/early spring (March, April) and through the summer/fall months, with the highest values occurring in July and August.

Although group averages of individual cow MUN values are frequently used to monitor urea status of a herd, bulk tank MUN values are sometimes used for several reasons. Bulk tank milk samples are taken every time milk is picked up from a dairy farm, and therefore, BTMUN could be tested more frequently than once a month, and at considerably less cost than individual cow testing, providing more timely and inexpensive information on urea status of a herd. However, BTMUN values do not correlate perfectly with WHMUN values, regardless of herd size, pasture season or milk recording protocol, as we discovered, and BTMUN is not very sensitive to changes in group average MUN values within a herd. Therefore, BTMUN can be used to supplement group average MUN values, but should be interpreted with caution because they may mask variation that is occurring in average MUN

values among groups of cows.

In conclusion, the MUN values determined by the infrared method at the PEI Milk Quality Laboratory were found to be very reliable. Certain cow and test day factors (days in milk, milk yield, milk protein percentage, linear score and season) were significantly and biologically associated with MUN values. Bulk tank MUN values should only be interpreted in conjunction with group averages of individual cow MUN values due to less than ideal correlation between BTMUN and WHMUN.

Further research will be performed to better describe the relationship between MUN and reproductive performance, the determination of specific guidelines for interpretation of MUN values in Atlantic Canadian dairy herds, and an intervention trial to determine the effectiveness of a MUN monitoring and advisory program. In a companion thesis written by another graduate student at this institution, there will be a description of feeding programs in ninety intensively monitored Atlantic Canadian dairy herds, an evaluation of the rising plate meter for monitoring pasture quality and dry matter content in a subset of these herds, and a determination of the relationship between MUN values and feed composition, intake and delivery in the ninety dairy herds.

6.6 References

1 Rotz CA, Satter LD, Mertens DR, Muck RE. Feeding strategy, nitrogen cycling, and profitability of dairy farms. J Dairy Sci 1999; 82(12):2841-2855.

2 Wilkerson VA, Mertens DR, Casper DP. Prediction of excretion of manure and nitrogen by Holstein dairy cattle. J Dairy Sci 1997; 80(12):3193-3204.

3 Huber JT, Limin Kung JR. Protein and non-protein nitrogen utilization in dairy cattle. J Dairy Sci 1981; 64(6):1170-1195.

4 Lewis D. Blood urea concentration in relation to protein utilization in the ruminant. J Agric Sci Camb 1957; 48:438-446.

5 Baker LD, Ferguson JD. Response in urea and true protein of milk to different protein feeding schemes for dairy cows. J Dairy Sci 1995; 78(11):2424-2434.

6 Kolver ES, MacMillan KL. Short term changes in selected metabolites in pasture fed dairy cows during peak lactation. Proceedings of the New Zealand Society of Animal Production 1993; 53:77-81.

7 Oltner R, Wiktorsson H. Urea concentrations in milk and blood as influenced by feeding varying amounts of protein and energy to dairy cows. Livest Prod Sci 1983; 10:457-467.

8 Wittwer FG, Gallardo P, Reyes J, Opitz H. Bulk milk urea concentrations and their relationship with cow fertility in grazing dairy herds in Southern Chile. Prev Vet Med 1999; 38:159-166.

9 Gustafsson AH, Palmquist DL. Diurnal variation of rumen ammonia, serum urea and milk urea in dairy cows at high and low yields. J Dairy Sci 1993; 76(2):475-484.

10 Roseler DK, Ferguson JD, Sniffen CJ, Herrema J. Dietary protein degradability effects on plasma and milk urea nitrogen and milk non-protein nitrogen in Holstein cows. J Dairy Sci 1993; 76(2):525-534.

11 Gustafsson, AH Current status concerning the use of milk urea concentration: analytical methods, use in different countries and how to utilize the results. Proceeding of the 30th Biennial Session of ICAR, Veldhoven, The Netherlands, 1996; 223-228

12 Godden SM, Lissemore KD, Kelton DF, Lumsden JH, Leslie KE, Walton JS. Analytic validation of an infrared milk urea assay and effects of sample acquisition factors on milk urea results. J Dairy Sci 2000; 83(3):435-442.

Appendix A. Conversion factors for units of urea

Milk urea mg/dl	x 0.467	= Milk Urea Nitrogen	mg/dl
Milk urea g/L	x 47	= Milk Urea Nitrogen	mg/dl
Milk urea mmol/L	x 2.8	= Milk Urea Nitrogen	mg/dl
Milk urea nitrogen mmol/L	x 6.0	= Milk Urea Nitrogen	mg/dl

Appendix B. Comparison of the mean differences and standard deviations of the difference for various laboratories, comparing their infrared method with an enzymatic method.

Regions	No. sample	MUN value Min. - Max	Mean difference (mg/dl)	SD of differences (mg/dl)
Eastern Lab Services	129	4.2 - 20.2	-0.54	1.73
Ontario DHI [a]	920	4.0 - 35.0	-0.10	1.51
Quebec DHI [a]	988	2.8 - 27.8	0.07	1.33
PEI MQL [b]	161	4.1 - 26.3	0.05	1.18

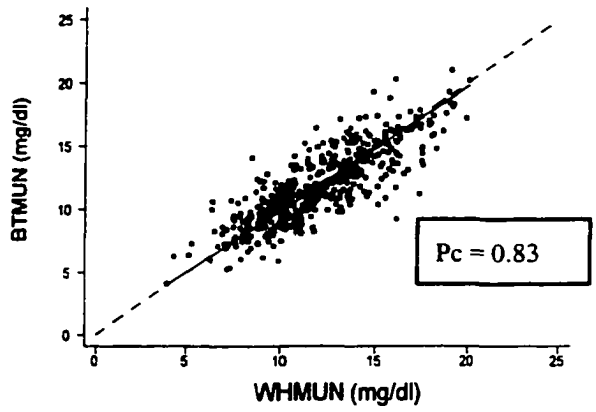
* Lefebvre D. Development Agent - Nutrition, PATLQ. 2000.

[a] = Dairy Herd Improvement

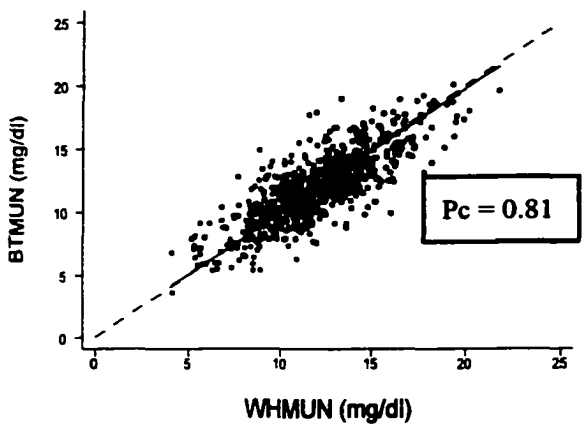
[b] = Prince Edward Island Milk Quality Laboratory

Appendix C.1 Comparison of bulk tank milk urea nitrogen(BTMUN) and weighted herd average milk urea nitrogen (WHMUN), in 199 PEI dairy herds, categorized by milk sampling protocols.

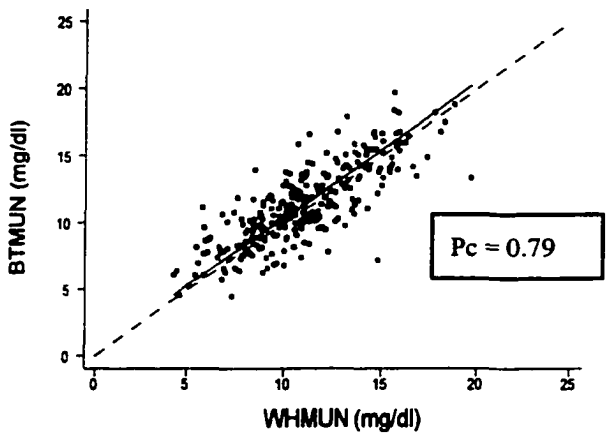
Group 1 official collected 2X



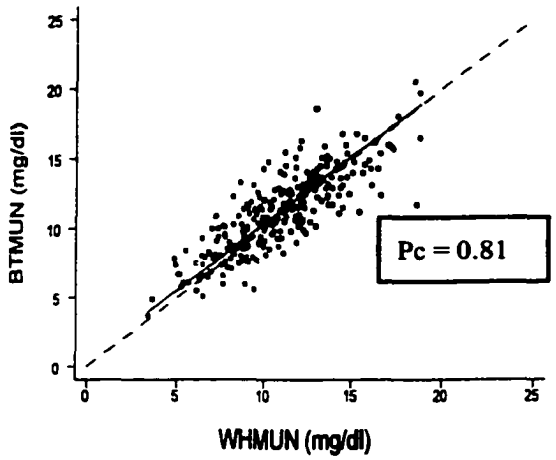
Group 2 official collected AM / PM



Group 3 unofficial collected 2X

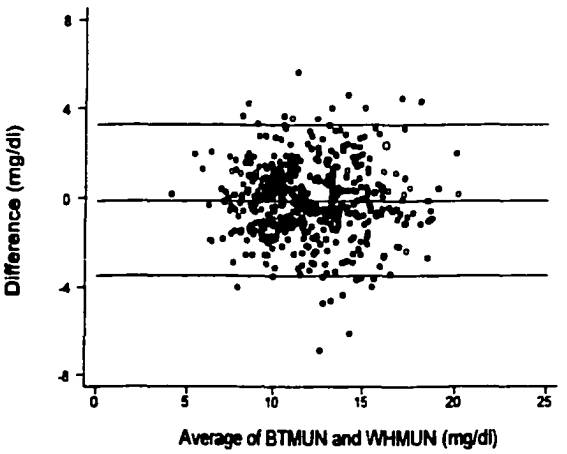


Group 4 unofficial collected AM / PM

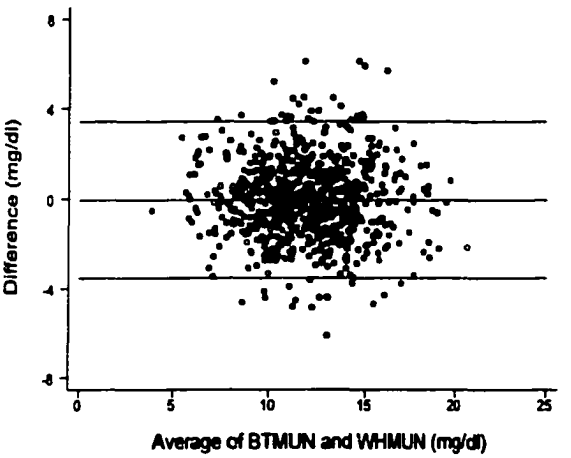


Appendix C.2 Difference between bulk tank milk urea nitrogen (BTMUN) and weighted herd average milk urea nitrogen (WHMUN) plotted against the mean value, with horizontal lines showing the 95% limits of agreement, in 199 PEI dairy herds, categorized by milk sampling protocols.

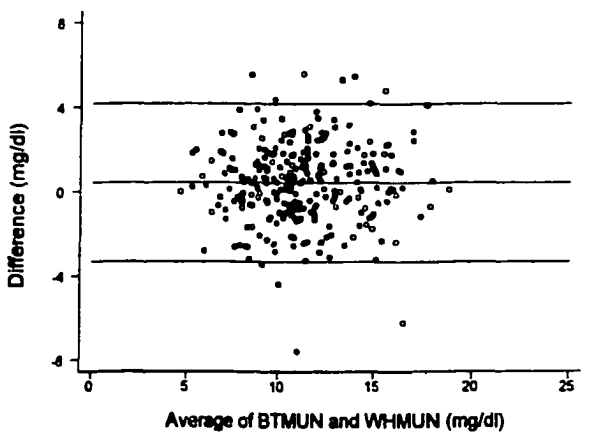
Group 1 Official collected milk 2X



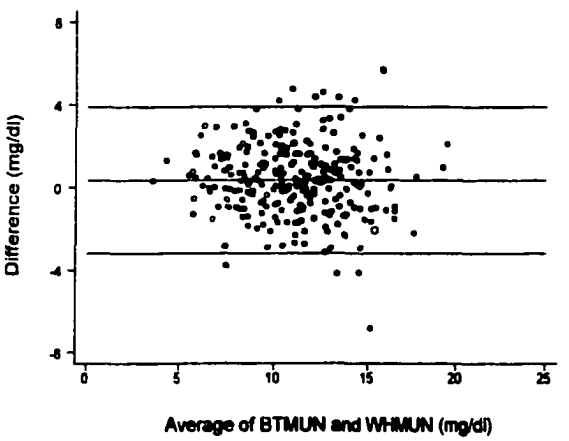
Group 2 Official collected milk AM/PM



Group 3 Unofficial collected milk 2X

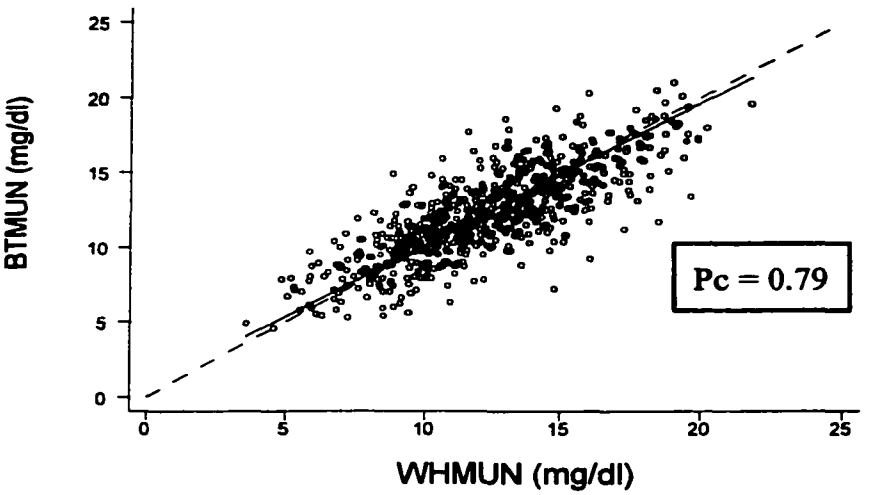


Group 4 Unofficial collected milk AM/PM

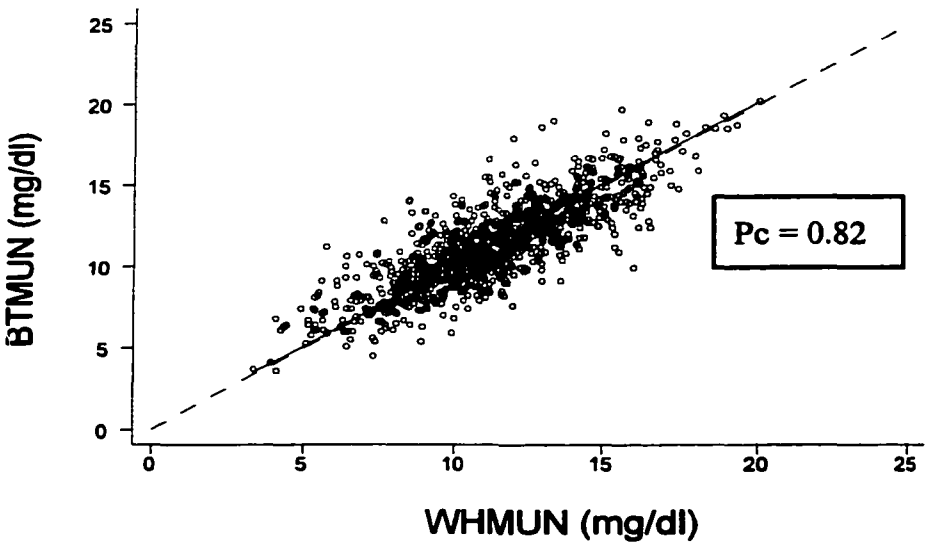


Appendix C.3 Comparison of bulk tank milk urea nitrogen (BTMUN) and weighted herd average milk urea nitrogen (WHMUN) in 199 PEI dairy herds, categorized by pasture season.

Pasture = 1

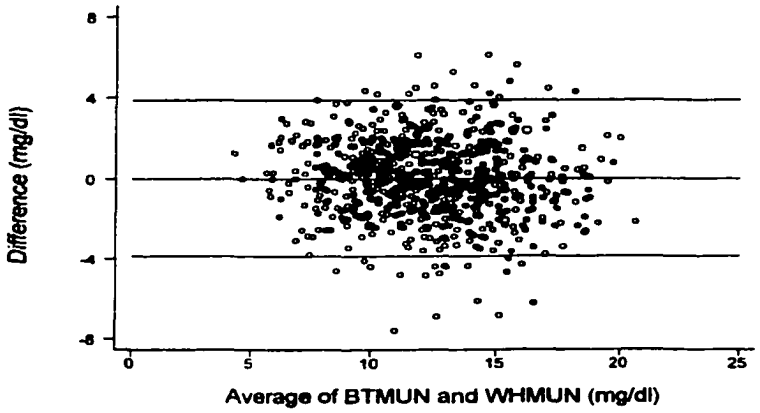


Pasture = 0

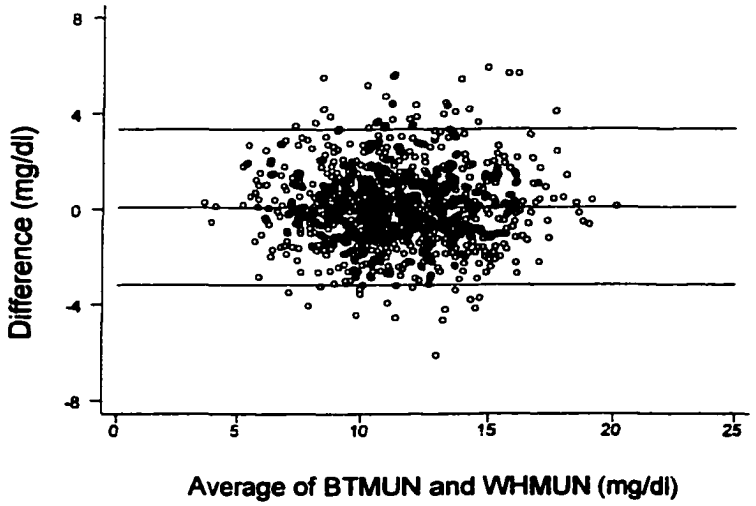


Appendix C.4 Difference between bulk tank milk urea nitrogen (BTMUN) and weighted herd average milk urea nitrogen (WHMUN) plotted against the mean value, with horizontal lines showing the 95% limits of agreement, in 199 PEI dairy herds, categorized by pasture season.

Pasture = 1

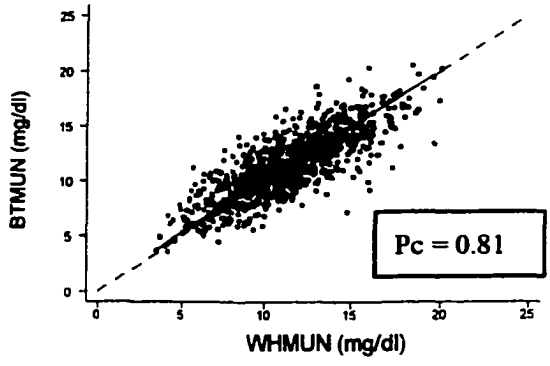


Pasture = 0

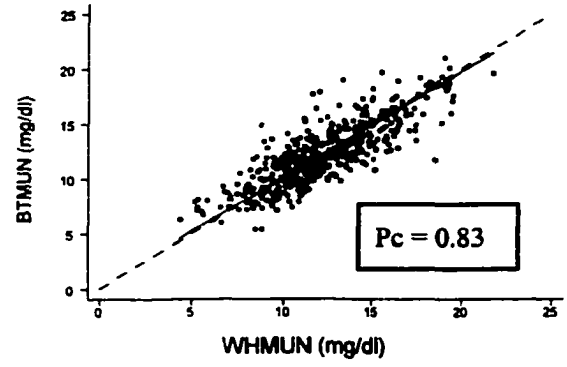


Appendix C.5 Comparison of the bulk tank MUN (BTMUN) and weighted herd average MUN (WHMUN) in 199 PEI dairy herds, categorized by herd size.

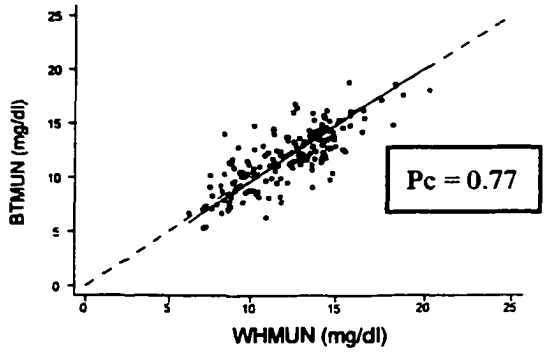
Group 1 = herd size (<40 cows)



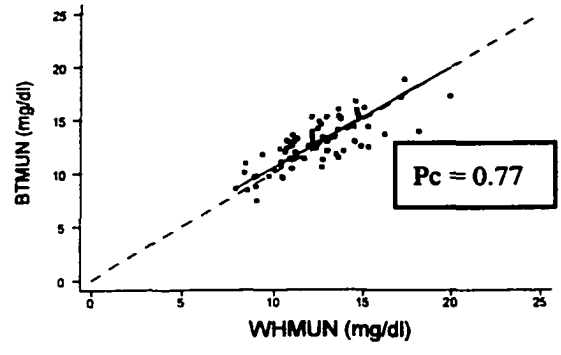
Group 2 = herd size (41-60 cows)



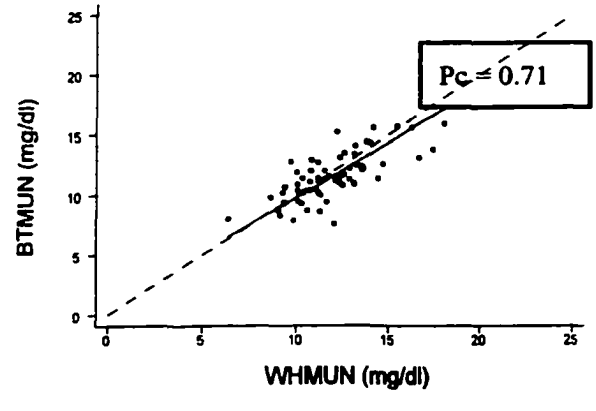
Group 3 = herd size (61-80 cows)



Group 4 = herd size (81-100 cows)

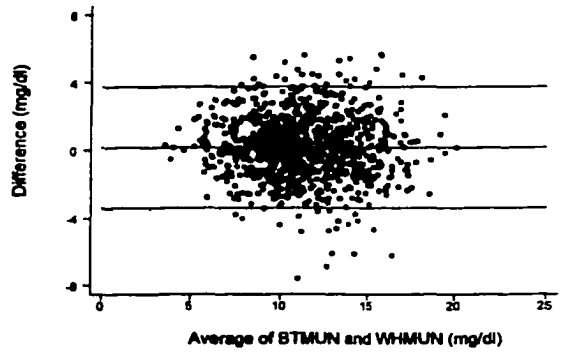


Group 5 = herd size (>100 cows)

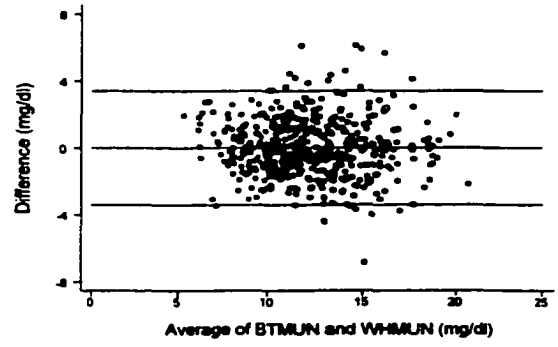


Appendix C.6 Difference between BTMUN and WHMUN plotted against the mean value, with horizontal lines showing the 95% limits of agreement, in 199 PEI dairy herds, categorized by herd size.

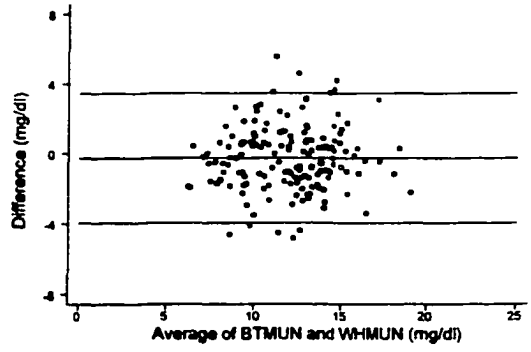
Group 1 = herd size (<40 cows)



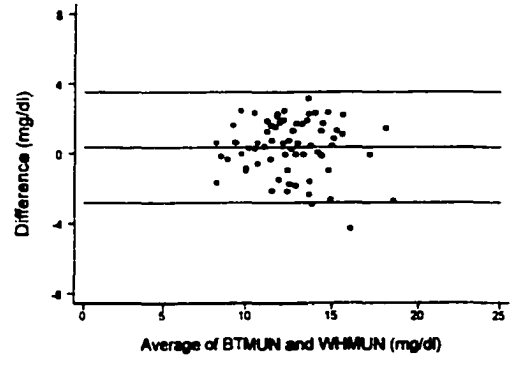
Group 2 = herd size (41-60 cows)



Group 3 = herd size (61-80 cows)



Group 4 = herd size (81-100 cows)



Group 5 = herd size (>100 cows)