NUTRITIONAL, MANAGERIAL, PHYSIOLOGICAL, AND ENVIRONMENTAL FACTORS AFFECTING MILK UREA NITROGEN IN QUÉBEC HOLSTEIN COWS: A FIELD TRIAL

by

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SUGGESTED SHORT TITLE:

MILK UREA NITROGEN

AND

THE FACTORS AFFECTING ITS VARIATION

In loving memory of my father

Michel Depatie

...

ABSTRACT

Master of Science — Animal Science

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Nutritional, Managerial, Physiological, and Environmental Factors Affecting Milk Urea Nitrogen in Québec Holstein Cows: A Field Trial

This trial was carried out in order to elucidate factors affecting milk urea nitrogen (MUN). Twenty-five herds were selected for MUN testing. Three sampling periods were chosen. The first occcured during the months of March and April, the second during July and August, and the third during November and December 1997. A total of 2,686 samples were collected and analyzed. Two different methods were employed for MUN analysis and were referred to as the Macdonald Campus method (MUN-MAC) and the Programme d'Analyse des Troupeaux Laitiers du Québec method (MUN-P.A.T.L.Q.). The MUN-MAC consists of an enzymatic method while the P.A.T.L.Q. method is an infra-red method. Prior to initiation of the trial, the MUN-MAC method was validated and found suitable for use in this experiment. Thirty-five milk samples were spiked either with 5, 10 or 15 mg/dl of urea nitrogen. The recovery was 99.10% and the coefficient of variation 2.25%. Analysis of milk samples from the 25 herds used in this study revealed intra-assay variations of 2.01%, 1.90%, and 2.48% for the Accutrol™ Normal, the standard of 30 mg/dl, and the 2% milk fat UHT milk. Inter-assay coefficients of variation were 10.79%, 5.99%, and 9.46% for the Accutrol™ Normal, the standard of 30 mg/dl, and the 2% milk fat UHT milk. The MUN coefficient of variation of the 2,686 milk samples analyzed was 1.85%. Differences in low MUN values were found between

the two methodologies and the cause of this variation could not be elucidated. Two similar models were ran, one for the MUN-MAC method and the other for the P.A.T.L.Q. method. Milk samples analyzed by the MAC method yielded a MUN average of 12.52 ± 3.99 mg/dl while the P.A.T.L.Q. method was 13.22 ± 3.39 mg/dl. The results demonstrated that the factors which significantly contributed to the models were the ration's net energy of lactation, season, region, somatic cell count, total dry matter, neutral detergent fiber, non-structural carbohydrates, total fat, crude protein, protein to energy ratio, starch to protein ratio, parity and days in milk. The overall findings of this study have undoubtedly contributed to a better understanding of nutritional, managerial, physiological, and environmental factors influencing MUN by providing further research findings on their relationships with MUN, especially in Québec Holstein cows.

RÉSUMÉ

Maîtrise en Sciences Sciences Animales

Catherine Depatie

Les facteurs nutrionnels, de gestion, physiologiques et saisonniers qui influencent le taux d'urée du lait chez les vaches Holsteins au Québec: une étude de terrain

Cette étude a été entreprise dans le but d'élucider les différents facteurs influençant l'urée du lait. Vingt-cinq troupeaux ont été sélectionés pour l'analyse de la concenration d'urée du lait. Trois périodes d'échantillonnage ont été choisies. La première s'est déroulée au cours des mois de mars et avril, la seconde en juillet et août et la troisième entre novembre et décembre 1997. Un total de 2,686 échantillons ont été recueillis puis analysés. Deux méthodes différentes indentifiées par celle provenant du Macdonald Campus (MUN-MAC) et celle provenant du Programme d'Analyse des Troupeaux Laitiers du Québec (MUN-P.A.T.L.Q.) ont été employées pour l'analyse de l'urée du lait. La methode MUN-MAC faisait appel à un principe enzymatique tandis que la méthode MUN-P.A.T.L.Q. à l'infrarouge. Avant le commencement de l'étude de terrain, la méthode MUN-MAC a d'abord été validée et jugée adéquate pour son utilisation lors de cette étude. Des concentrations de 5, 10 ou 15 mg/dl d'azote uréique ont été ajouté à trente-cinq échantillons de lait. Un pourcentage de 99.10 a été récuperé et le coefficient de variation était de 2.25%. Les échantillons de lait provenant des 25 troupeaux ont révélé des variations intra-essai de 2.01%, 1.90% et 2.48% pour l'Accutrol™ Normal, le standard de 30 mg/dl et le lait 2% de matière grasse UHT. Les coefficients de variations inter-essai étaient 10.79%, 5.99% et 9.46% pour l'Accutrol™ Normal, le standard of

30 mg/dl et le lait 2% matière grasse UHT. Le coefficient de variation des 2,686 échantillons de lait analysés était 1.85%. Les deux méthodologies ont démontrés des différences entre les valeurs faibles d'urée du lait et la cause de cette variation n'a pu être déterminée. Deux modèles similaires ont donc été employés, l'un pour la méthode MUN-MAC et l'autre pour MUN-P.A.T.L.Q.. La moyenne des échantillons analysés par la méthode MUN-MAC était de 12.52 ± 3.99 mg/dl alors quelle celle de la méthode MUN-P.A.T.L.Q. était de 13.22 ± 3.39 mg/dl. Les facteurs qui ont contribués de façon significative aux modèles sont l'énergie, la saison, la région, les cellules somatiques, la matière sèche, les fibres par détergent neutre, les hydrates de carbone non structuraux, le gras de la ration, la protéine brute, le ratio protéine/énergie, le ratio amidon/protéine, la parité et le stade de lactation. Les résultats de cette étude ont sans aucun doute contribué à une meilleure compréhension des facteurs nutrionnels, de gestion, physiologiques et saisonniers qui influencent le taux d'urée du lait en fournissant des données aditionnelles sur la relation entre ces derniers et l'urée du lait plus, particulièrement dans les vaches Holsteins au Québec.

LIST OF ABBREVIATIONS

BUN.....blood urea nitrogen
CP.....crude protein
CV.....coefficient of variation
DCP.....digestible crude protein
DHIA.....dairy herd improvement analysis
DIM.....days in milk
DMI.....dry matter intake
IR.....infrared
MAC.....Macdonald Campus, McGill University
MUN.....milk urea nitrogen
N.....nitrogen
NPN.....non protein nitrogen
NSC.....non structural carbohydrates
P.A.T.L.Q.....Programme d'Analyse des Troupeaux Laitiers du Québec
RDIP.....rumen degradable intake protein
RUIP.....rumen undegradable intake protein
SCC.....somatic cell count
U.H.T.....ultra high temperature

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CHAPTER 1. GENERAL INTRODUCTION

Over the past decade, non-protein nitrogen (NPN) in milk and especially milk urea nitrogen (MUN) have received increased attention in the dairy industry and in research. It is now recognised that low levels of NPN in milk are often related to better cheese yields (Moore and Varga, 1996). If it were the same for MUN, its monitoring would offer new possibilities to the industry. Moreover, growing concerns of maintaining an equilibrium between agriculture practices and the environment have resulted in increasing demands towards reduction of nitrogen output into the environment. When an animal demonstrates a high level of blood NPN, thus milk urea, a large amount of urea ends up in the urine. Milk urea nitrogen could prove to be a useful tool in controlling environmental pollution by nitrogenous compounds. Furthermore, causes underlying a reduction in fertility remain difficult to identify. However with regards to urea, there seems to be a range of values in which reproductive problems are minimised (Gustafsson and Carlsson, 1993). In such cases, using milk urea as an indicator would be beneficial for optimisation of reproductive performance and health status.

Although there exist a variety of fields in which knowledge of MUN could be applied, the most interesting aspect of milk urea analysis lies in its potential as a nutritional indicator. Efficient utilisation of dietary protein is, without a doubt, one of the greatest challenges in dairy nutrition. The lack of information on the protein status of certain feedstuff due to variations in climatic conditions, stage of maturity at harvest, and utilisation of fertiliser adds to this challenge. Monitoring MUN could therefore be useful if it can provide additional information for nutritional optimisation. This would contribute to improving the general metabolic

efficiency of the animal and would also help in reducing economic costs related to overfeeding protein.

Many methods of milk urea analysis have been developed and are presently available on the market. In order to enable the interpretation of the values obtained, these methods must first be well established and reliable. Then, norms must be defined in order to establish recommendations. However, in order to do so, a thorough understanding of the factors responsible for changes in MUN is primordial.

In spite of the above mentioned, causes of variation in MUN are not fully understood. Concerns about interpreting MUN values have risen and caution must be used because many aspects remain difficult to grasp. This project will try to elucidate nutritional, managerial, physiological, and environmental factors affecting MUN in Québec Holstein dairy cows.

CHAPTER 2. LITERATURE REVIEW

2.1. PRINCIPLES OF NITROGEN METABOLISM RELATED TO MILK UREA NITROGEN

Once ingested, nitrogenous compounds from the diet may take two different routes depending on their degradabilities. On one hand, rumen undegradable intake protein (RUIP) ends up in the small intestine where part of it can be digested. Nitrogenous compounds resulting from this digestion are then absorbed through the intestinal wall and finally reach the blood circulation through the hepatic vein. These nutrients can then reach various tissues including those from the mammary gland and be available for metabolic use and protein synthesis. As for the non digestible fraction, it is eliminated in the feces. On the other hand, rumen degradable intake protein (RDIP) is broken down to ammonia in the rumen. Utilisation of the ammonia for microbial protein synthesis depends on the availability of energy, generally non fibrous carbohydrates and digestible fiber (Lefebvre, 1996). It is also proportional to the rate of growth of microbial flora in the rumen. An excess of ammonia, surpassing the capacity of the bacteria to use it, will result in an increased diffusion across the rumen wall into the hepatic vein and the liver. In addition, higher pH will further increase ammonia absorption because tissue membranes are permeable to the lipid-soluble form NH_3 and impermeable to the ammonium ion NH_4^+ (Bartley et al., 1976). Therefore, it is important to note that ruminal ammonia may be high but not necessarily absorbed if ruminal pH is maintained low.

2.1.1. The Urea Cycle

In mammals, as in dairy cows, it is the liver which synthesizes almost all the of urea. This organ has two ways of disposing excess nitrogen, by forming glutamine from glutamate or by forming urea (Reeds and Beckett, 1996). Glutamine synthesis is known to occur in the perivenous hepatocytes whereas ureagenesis happens in the periportal hepatocytes (Häussinger et al., 1992). In the latter case, using the energy from a first molecule of ATP, a molecule of ammonia combines with one bicarbonate and then fixes to a molecule of ornithine to form citrulline in the liver mitochondria (Devlin, 1997). Citrulline diffuses to the cytoplasm of the cell where energy is once more used to add a molecule of ammonia as an amine group from aspartic acid. This leads to the eventual formation of arginine. Arginine is degraded to liberate a molecule of urea and ornithine which diffuses to the mitochondria, and the cycle starts again. The liver may also use amino acids which are in excess in the blood, deaminate them and incorporate the amino group in a urea molecule. Carbamoyl phosphate synthase, the major rate controlling enzyme of the cycle, has a high K_m for ammonia making the urea cycle a low affinity system in contrast to the high affinity glutamine synthesis system (Häussinger et al., 1992).

Urea can be excreted by various ways. The majority ends up in urine but a small quantity also shows up in the uterine fluid and milk (Hutjens and Barmore, 1995). Nearly everywhere that water can go, urea can also. When milk accumulates in the mammary gland, urea diffuses into and out of the stored milk in order to equilibrate with blood plasma urea nitrogen (Kohn et al. 1999). Some believe urea output in milk results from passage through leaky junctions in the mammary gland (Metcalf et al., 1994). Urea may also be recycled via the

saliva. It returns to the gastrointestinal tract, especially the rumen, to be used by the microorganisms. The urea which is recycled by the saliva will only be useful if ammonia in the rumen is limiting microbial growth. This recycling of urea represents 12-33% of the nitrogen digested (Lapierre and Bernier, 1996).

Interestingly it has been demonstrated, with the discovery of arginase, that other organs also synthesize urea but in smaller quantities than the liver. There is urea formation in the intestines, pancreas, kidneys, lungs, brain (Cynober et al., 1995), and even the mammary gland. However, only part of the urea cycle is present. Indeed, in 1937, a study demonstrated by arteriovenous difference that the mammary gland of lactating goats could produce urea (Graham et al., 1937). Another group also working with these ruminants observed an excessive absorption of arginine by the mammary gland (Mepham and Linzell, 1966; Mepham and Linzell, 1967). With the use of radioactive markers, they demonstrated that arginine was catabolised to urea and ornithine. The latter, plus the one directly absorbed from the blood will be used to synthesize proline. When the absorption of non essential amino acids is insufficient to satisfy the requirements of the mammary gland for production of proteins, this deficit can be eliminated by the synthesis of other precursors, like proline (Mepham, 1982). Moreover, it was demonstrated in the rat that the absorption of proline alone was insufficient for production of caseins (Yip and Knox, 1972). Another study confirmed production of urea by the mammary gland but this time in the bovine specie (Basch et al., 1997). The enzymes present in the mammary gland contribute to the conversion of arginine to ornithine. Here again, ornithine is transformed into precursors of proline, which in turn will serve for making caseins. Thus the bovine mammary gland has the capability of producing urea. However, the quantities produced

remain minimal compared to the ones absorbed from the blood. Production of urea by the mammary gland is therefore not a major source of variation of milk urea (DePeters and Ferguson, 1992).

2.2. FEEDING AND FEED MANAGEMENT

Several factors in the diet can influence the quantity of urea in milk. However, many studies omit to acknowledge all these factors, which makes the interpretation of the results very difficult.

2.2.1. Dry Matter Intake and Crude Protein

Total dry matter intake only affects MUN slightly (Cannas et al., 1998; Ide et al., 1966; Oltner and Wiktorsson, 1983; Oltner et al., 1985). Therefore, the amount of feed ingested has very little influence on MUN. Several authors believe that it is rather the level of crude protein (CP) in the ration that affects MUN (Cannas et al., 1998; Ide et al., 1966; Refsdal et al., 1985). However, Lewis (1957) reported that CP was not the major factor influencing changes in MUN. Many also support the idea that digestible crude protein influences MUN (Erbersdobler et al., 1980; Gustafsson and Carlsson, 1993; Refsdal et al., 1985; Ropstad et al., 1989). For many years, countries such as the Netherlands, Norway, and Sweden used a Digestible Crude Protein (DCP) system to express protein requirements for dairy cattle. Prior to 1991, the Netherlands' DCP system was based on the difference between ingested CP(N x 6.25) and CP(N x 6.25) found in feces (CVB, 1990) and therefore studies conducted during that time refer to MUN findings in relation to DIP. That system had some drawbacks as it did not describe the amount of CP degraded in the rumen nor did it account for microbial protein synthesis in the rumen.

The fact that no ruminal N-transactions were included in such a system rendered it a poor predictor of the amount of true protein absorbed in the small intestine. This led to the development of the new Dutch protein evaluation system: the DVE/OEB-system (Tamminga et al., 1994). During that same period, a new protein evaluation system also became official in Sweden: the AAT/ PVB-system (Gustafsson and Carlsson, 1993). These new systems express the protein value of a feedstuff as the true protein digested in the intestine and thus have recognised the importance of estimating the sum of digestible microbial true protein derived from rumen degradable protein and rumen undegradable protein (Hof et al., 1994).

2.2.2. Rumen Degradable Intake Protein and Rumen Undegradable Intake Protein

MUN may therefore also be influenced by the levels of rumen degradable intake protein (RDIP) and rumen undegradable intake protein (RUIP). Ruminal degradation of the intake CP can vary from approximately 20% for blood meal to 100% for urea (Harris, 1995). Therefore, two rations having the same level of CP may vary with regards to their ruminal degradation and thus influence MUN differently. Forages, wheat, oat, and non heated soybean meal are examples of RDIP currently used in ration formulation. As for RUIP, blood meal, feather meal, fish meal, and heated soybean are commonly used.

A lot of research has been carried out with various protein sources, differing in the degradation of their protein fraction, in order to study the effects on MUN. An increase in milk NPN and MUN was achieved with diets containing either an excess of CP or an excess of RDIP along with a deficiency in RUIP (Baker et al., 1995). Moreover, an excess of RDIP, RUIP, or even both may also result in an increase in MUN (Roseler et al., 1993). However, CP was not

held constant during this experiment which makes the interpretation of the results difficult as well as the differentiation of the effects from RDIP, RUIP, and total protein. In a recent study, no effect of RDIP and RUIP on MUN was observed (Rodriguez et al., 1997). This time CP was held constant at 16.2% across treatments and the diets were isocaloric. As it can be seen, studies conducted on the effects of RDIP and RUIP show different results. This difference may reside in the fact that not all sources of MUN variations are held constant besides RDIP and RUIP. It is hence very difficult to define a trend as to the effect of RDIP and RUIP on MUN.

2.2.3. Energy

Energy is negatively correlated to MUN. The level of energy will influence the quantity of protein and NPN utilised by micoorganisms (Moore and Varga, 1996). Therefore an increase in energy supplied by the ration results in a decrease in MUN (DePeters and Ferguson, 1992). Most grains (corn, oats, wheat, barley) are good sources of energy in the form of non fibrous carbohydrates because of their high level of starch. Moreover, good quality forages and beet pulp are excellent sources of digestible fibre (cellulose and hemicellulose). Inclusion of these in the diet enable the rumen microflora to capture the surplus of ammonia and produce microbial proteins. This then leads to a reduction in MUN. However, in a recent study with lactating ewes, unexpected results demonstrated no effect of dietary energy levels on MUN (Cannas et al., 1998). This may partly be explained by the fact that intake was high in this trial and the passage rate of feeds was also probably high. A large amount of protein may have escaped from the rumen and reached the intestine. It is recognized that in well-balanced diets, estimated ammonia losses from true protein digested in the small intestine are quantitatively the most important ones, and the amount becomes higher when intake exceeds requirements (Hof et al.,

1994). However, recent studies have demonstrated that MUN concentration is representative of the surplus of N for microbial synthesis in the rumen (Hof et al., 1997; Schepers and Meijer, 1998). Therefore, MUN cannot be used as an indicator for the utilisation of absorbed true protein (Hof et al., 1997). Thus this might partially explain the results obtained by Cannas et al. (1998).

2.2.4. Protein to Energy Ratio

The protein to energy ratio seems to be the nutritional factor that has the most influence on MUN (Carlsson and Pehrson, 1994; Gustafsson and Carlsson, 1993; Oltner and Wiktorsson, 1983; Refsdal et al., 1985). Lewis (1957) first demonstrated the importance of this ratio by changing the protein source (casein vs zein) and consequently ruminal ammonia levels. By adding an energy source in the form of starch, a rapid decrease of ruminal ammonia and blood urea followed. Later, another group demonstrated that urea concentrations varied only slightly when the quantity of CP was increased or decreased, as long as the protein to energy ratio was held constant (Oltner and Wiktorsson, 1983). However, as soon as the ratio was changed, the concentration of milk urea varied. Results from a recent study, using isonitrogenous and isoenergetic diets, showed that elevating the non-structural carbohydrates (NSC):protein ratio by increasing total carbohydrate intake was more effective in improving nitrogen utlization in the rumen than was elevating the NSC:structural carbohydrates ratio without increasing carbohydrate intake (Carruthers et al., 1997). It is very clear that the relation between protein and energy in the ration has a greater influence on MUN than total dry matter, CP, RDIP, RUIP, or even energy.

2.2.5. Frequency of Feeding

Very few studies exist on the effect of feeding frequency on blood urea nitrogen and, to the best of our knowledge, practically none exists on its effects on MUN. In a study conducted with four dairy cows, the animals were fed twice daily or continuously (4 meals of hay and hourly meals of concentrate for 24 hours) (Thomas and Kelly, 1976). The diets were balanced to meet 80% and 100% of NRC requirements for energy. The results demonstrated that the frequency of feeding influenced blood urea. Clear peaks of plasma urea were observed 2 to 4 hours after feeding when animals were fed twice daily. No effects were observed when cows were continuously fed. Others also observed peaks in blood urea when animals were fed twice daily (Coggins and Field, 1976; Manston et al., 1981). Since sampling for milk urea testing is normally executed at milking, it is important to take into account the time laps between the last meal and milking when interpreting milk urea values.

2.3. PHYSIOLOGY

2.3.1. Breed

Research on the effect of breeds on milk urea is rare. No effect of breed was reported in three studies (Carlsson et al., 1995; Erbersdobler et al., 1979; Mariani, 1974). However, a difference between two German breeds was observed (Wolfshoon-Pombo et al., 1981). Others have equally seen differences between breeds. Pennsylvania DHIA in collaboration with the University of Pennsylvania School of Veterinary Medicine has analyzed over 2,822,495 milk samples for MUN from September 25, 1995 to July 31, 2000. The results indicated that Brown Swiss and Jersey breeds had the highest average MUN values of 15.01 and 14.69 mg/dl,

respectively, while the Ayrshire breed had the lowest average of 12.57 mg/dl (Center for Animal Health and Productivity, 2000). Due to scarce and contradicting results, the effect of breeds on milk urea warrants further investigations.

2.3.2. Live Weight and Mammary Health

A study demonstrated that urea was negatively correlated to live body weight (Oltner et al., 1985). These authors believed that it might partially be a simple dilution effect. If the same quantity of urea is produced regardless of the cow's size, the urea concentration in blood and milk will be higher in cows of a smaller body weight. Others found no effect of body weight on milk urea (Ropstad et al., 1989).

Some researchers found that somatic cell count (SCC) did not significantly influence MUN (Eicher et al., 1997b). Furthermore, in this same study, no significant differences were reported between the quarter samples. However, a significant but small correlation between SCC and NPN as well as a lower milk urea value has been detected in cows positive to the California mastitis test compared to those negative to the test (Licata, 1985). MUN values were the lowest for samples with the largest SCC (Faust et al., 1997b). It may be suggested that cows having a high SCC should not be incorporated to group or herd averages in MUN (Hutjens, 1996).

2.3.3. Stage of Lactation

Total nitrogen in milk decreases during the first two months following parturition. Then

it increases until the end of lactation to finally come back to the initial level (Ng-Kwai-Hang et al., 1985). Similarly, urea concentration is lower during the first month of lactation and increases throughout lactation to finally decrease at the end of lactation (Carlsson et al., 1995; Whitaker et al., 1995). Some even recommend that cows should not be included for MUN testing in the first month after calving (Agsource, 2000). The lower values of milk urea for the beginning of lactation may be the result of an overall decrease in feed intake or of a consumption of diets rich in grains (Adam and Cloutier, 1999). It is important to notice that changes in milk urea follow changes in energy balance but in opposite directions. This is logical since it has previously been stated that urea is negatively influenced by energy. Stage of lactation has an effect on milk urea especially when the animals are kept indoors and that the diet is well balanced and the intake is controlled.

2.3.4. Age and Parity

The majority of studies do not distinguish between age and parity. This could eventually lead to wrongful interpretation of results. Primiparous cows generally have a lower milk urea concentration due to their lower feed intake (Adam and Cloutier, 1999; Whitaker, 1995). Similarly, others have found that multiparous cows had higher urea values than primiparous cows (Oltner et al., 1985). The authors explained these results by the fact that primiparous cows have a drive to grow and thus probably use amino acids more efficiently or differently thus leading to less deamination by the liver. However, it is important to note that the animals used for this experiment were in early lactation. It has been previously stated that milk urea is low at that time and thus could explain the lower MUN values for the primiparous cows. Some have observed no effect of parity on blood urea and milk urea (Ropstad et al.,

1989). One study found no effect of lactation number but noticed a difference between primiparous and multiparous cows (Eicher et al., 1997a). They attributed these findings to different management practices.

2.4. DIURNAL AND SEASONAL VARIATIONS

Studies conducted on diurnal variations and milk urea have shown quite variable results. Some researchers have observed an increase in ruminal ammonia, serum urea, and milk urea when animals were fed once daily (Gustafsson and Palmquist, 1993). Another group found an increase in milk urea after the first meal but not the second (Carlsson and Bergström, 1994). However, in another study, an increasing concentration of milk urea was seen after the first meal followed by a decrease after the second (Miettinen and Juvonen, 1990). Others have demonstrated an increase in MUN 2 hours after the first meal while a decrease came only 6 hours after the second feeding (Rodriguez et al., 1997). All of these results remain difficult to interpret since they are confounded with the effect of feeding.

Season may also influence MUN. It has been clearly demonstrated that the average MUN level was higher when cows were grazing (Carlsson and Pehrson, 1993). Authors of another study observed three peaks of MUN when cows were put on pasture (Refsdal et al., 1985). The first peak, in June, was the result of changing from a conventional winter feeding to grazing. The second, in the middle of July, might be explained by the use of grass well dressed after the first cut of grass for silage. The final peak, at the end of September, probably reflected the use of diets with a large amount of green fodder within that area. Others have observed a single increase in MUN during the first week out on pasture and a return to normal three weeks

later (Vignon et al., 1978). The high level of MUN during the first week may be the result of the high level of soluble N in young grass. The decrease in the following weeks may be due to a rapid decrease of soluble nitrogen as the plant matures and to the adaptation of the rumen microflora to fermentable nitrogenous compounds. A group of researchers has noticed that the general aspect of the milk urea curve was more uniform when cows were housed inside (Carlsson et al., 1995). Moreover, concentration of milk urea was higher and the variation greater when the animals were kept outdoors. Others believed that work itself, performed by the cow, either by grazing or by maintaining body temperature during a heat stress resulted in the breakdown of body reserves thus leading to an increase in MUN (Garcia and Linn, 1997). Thus a seasonal variation on MUN seems to exist. Concentrations of MUN tend to be more elevated in summer and lower in winter. Nevertheless as seen previously, it is very difficult to separate effects of season (light, temperature, humidity, etc.) from those of feeding.

2.5. REPRODUCTION

Studies undertaken to explore the relationship between concentrations of milk urea and reproduction are relatively numerous. However, the results are often varied and may sometimes be contradictory. A team of researchers concluded that a concentration too high or too low of bulk milk urea was associated with a lower fertility in dairy cows (Gustafsson and Carlsson, 1993). A study conducted in Norway associated high urea values with an increased incidence of ovarian cysts in a cow population (Ropstad and Refsdal, 1987). A decrease in pregnancy rate was also associated with MUN values above 19 mg/dl (Butler et al., 1996). However, herds with a low urea concentrations had a longer interval between calving and first insemination (Carlsson and Pehrson, 1993). During this experiment, no difference in terms of fertility or

ovarian cysts was detected in groups having an intermediate or high level of milk urea. These findings may be due to the fact that bulk milk was used. Another study showed no relationship between high urea values and changes in reproduction and concluded that the only disadvantage to high urea values may just be economics (Carlsson, 1989). Researchers have shown that an excess in RDIP, by an unknown mechanism, leads to a decrease in uterine pH during the luteal phase which leads to a reduction in fertility (Elrod and Buttler, 1993). The same authors reported that heifers with plasma urea nitrogen (PUN) greater or equal to 16mg/dl had conception rates 30% lower than those with PUN levels less than 16 mg/dl. However, a study has demonstrated a positive relationship between the rate of conception and milk urea especially in primiparous cows (Butler et al., 1995). These researchers demonstrated that heifers that had MUN values above the group mean of 17.2 mg/dl had the highest conception rates. Results from that trial also indicated that only 5 of the 19 herds tested had a mean MUN value equal or above 19 mg/dl which might be considered detrimental according to previous research results. Thus the authors did not observe a strong negative relationship between MUN and conception rates and concluded that cows could still be fed and managed for high milk production while conserving MUN concentrations that indicated good balance and use of dietary protein. There exists a relationship between reproduction and milk urea. Values too high or too low seem to be related to various reproductive problems.

2.6. SIGNIFICANCE OF THIS RESEARCH PROJECT

Blood urea nitrogen (BUN) has been used for many years to diagnose nutritional and reproductive problems in dairy cattle. Recently, an interest in MUN has emerged. Many authors have reported correlations between milk urea and urea present in different blood components.

For example, correlations ranging from 0.79 to 0.98 were observed for milk urea and blood urea (DePeters and Ferguson, 1992; Erbersdobler et al., 1980; Harris, 1995; Hutjens and Barmore, 1995; Lefebvre, 1996; Oltner and Wiktorsson, 1983; Oltner et al., 1985; Roseler et al., 1993). Others have found correlations varying from 0.86 to 0.96 for serum urea and milk urea (Miettinen and Juvonen, 1990) and of 0.98 between plasma urea and milk urea (Oltner and Wiktorsson, 1983). Weaker correlations such as 0.46 and 0.75 have been cited for ruminal ammonia and milk urea (Carlsson and Pehrson, 1994; Ropstad et al., 1989). A delay of 1.5 to 2 hours exists between concentrations of urea present in blood and in milk (Lefebvre, 1996; Lefebvre et al., 1995; Moore and Varga, 1996). From these findings, it is clear that a very strong relationship exists between BUN and MUN. Furthermore, MUN analysis has many advantages over that of BUN. It is simpler, faster and cheaper because it may be sampled during monthly routine milk testing and thus requires no extra labour. Milk sampling is also less stressful for the animal in comparison to blood sampling for BUN.

As technology unfolds at an astonishing rate and MUN results are reported in an increasing fashion, confusion exists as to the interpretation of these values. It is of crucial importance to fully understand and grasp this concept as it grows in popularity. As seen previously, many factors may influence the outcome of MUN. This project will focus more specifically on total DMI, CP, RDIP, RUIP, E, P/E ratio, frequency of feeding, stage of lactation, parity and seasonal variations. Relevant information will be obtained by using the P.A.T.L.Q. database. Every month, a multitude of parameters from dairy cows all over the province of Québec are carefully recorded and stored. However, a thorough understanding of how MUN values are obtained must first be achieved.

The discovery of MUN as well as its potential application in various fields has created a strong incentive for development of reliable methods for testing this parameter. Different methods are rapidly being developed for MUN analysis and the use of the appropriate technique is left to the client's discretion since no national method is recognised at the time being. P.A.T.L.Q. would like to offer this very promising service with the use of infra-red technology. This method needs regular calibration and, unfortunately, no national controls exist presently on the market although there is an informal interlab program to ensure the quality of MUN data. Thus, the first part of this project was to develop and validate an enzymatic method for MUN testing at Macdonald Campus (MAC), McGill University. This method could then first be used to validate P.A.T.L.Q.'s infra-red methodology. Secondly, samples from this chemistry based method could then possibly be used as controls to calibrate P.A.T.L.Q.'s system. Controls would have the advantage of being provided from Québec and thus ranging in values that uniquely represent MUN from this province's dairy cows. Furthermore, they could be provided rapidly, regularly or on demand and, at lesser cost.

The second goal of this study was to determine nutritional, managerial, physiological, and environmental factors that influence MUN variation. This was achieved by validating an enzymatic method and an infrared method of measuring MUN and by determining which factors influence MUN.

CHAPTER 3. MATERIALS AND METHODS

3.1. HERD SELECTION CRITERIA

All herds selected were enrolled on the official P.A.T.L.Q. testing program and had the feeding option. This allowed a supervision during sampling as well as control and access to feeding information. Only herds with Holstein cows were selected. Herds had a rolling herd average above 8,000 kg/year which was considered adequate to rule out major management problems. Eleven regions were selected and described in Table1. Herds were chosen per region from the lower third and upper third milk protein production and defined as low or high milk protein production herds. A total of 25 herds were selected according to the aforementioned criteria.

3.2. SAMPLING

Milk samples were collected during three periods. The first being in March and April 1997, the second in July and August, and the third in November and December. A total of 2,686 cows were sampled. All samples were collected in duplicate during an official test by P.A.T.L.Q. supervisors. A preservative, bronopol (2-bromo-2-nitropropane-1,3-diol), was added to the milk as usual procedures require. Samples were sent simultaneously to P.A.T.L.Q. and to MAC by an express courier (Purolator) to ensure they would reach destination in the shortest time possible usually taking one day. Exceptionally, the longest delay was 3 days either because the sample was taken late on a Friday, was coming from a far and remote area or due to a courier strike.

Table 1. Regions selected for this trial.

Region Nomenclature (1)	**Region Description**	
Region 1	- Bonaventure - Kamouraska - Matane - Matapédia	- Rimouski - Rivière-du-loup - Témiscouata
Region 2	- Bellechase - Charlevoix-Est - Lévis - L'islet - Lobinière	- Montmagny - Montmorency - Portneuf - Québec
Region 3	- Beauce - Dorchester	- Frontenac - Mégantic-Sud
Region 4	- Arthabaska - Drummond - Nicolet-Est	- Nicolet-Ouest - Yamaska - Mégantic-Nord
Region 5	- Brôme - Compton - Richmond - Shefford	- Sherbrooke - Stanstead - Wolfe
Region 6	- Bagot - Missisquoi - Richelieu	- Rouville - St-Hyacinthe - Verchères
Region 7	- Beauharnois - Châteauguay - Huntingdon - Iberville - Laprairie	- Napierville - Soulanges - St-Jean - Vaudreuil
Region 9	- Abitibi-Est - Abitibi-Ouest	- Rouyn-Noranda - Témiscamingue
Region 10	- Berthier - Deux-Montagnes - Jacques-Cartier - Joliette	- L'Assomption - Montcalm - Terrebonne
Region 11	- Champlain - Maskinongé	- St-Maurice
Region 12	- Chicoutimi - Jonquière - Lac St-Jean	- Roberval - Saguenay

(1) according to P.A.T.L.Q.'s Rapport de production 1993.

3.3. STORAGE AND PRESERVATION OF SAMPLES

Milk samples arriving at P.A.T.L.Q. were analyzed for MUN, by infrared spectroscopy with a Foss 4000 unit from Foss Food Technology, concurrently with the routine testing for protein, fat and SCC. On the other hand, due to the enormous number of samples received and to technical feasibility, both milk and feed samples were frozen at -18°C upon arrival at MAC until analysis. At the end of the sampling period, all feed samples received were then sent to an independant firm, Agri-Food Laboratories (Guelph, Ontario, Canada) for wet chemistry analyses.

3.4. ANALYSIS OF MILK UREA NITROGEN

The instrument used for MUN analysis at MAC was an Abbott-VP Discrete Autoanalyser (Mississauga, Ontario, Canada). It was programmed with the Abbott Laboratories UN test (North Chicago, Illinois, USA) with the exception of the initial absorption being entered as 0.9 instead of 1.3 in order to decrease the initial absorption level.

A commercially available kit for the determination of urea and ammonia in foodstuffs and other materials from Boehringer Mannheim (Mannheim, Germany) was used. Urea was first hydrolysed to ammonia and carbon dioxide in the presence of the enzyme urease. Then ammonia reacted with 2-oxoglutarate to yield L-glutamate in the presence of glutamate dehydrogenase while nicotinamide-adenine dinucleotide (NADH) was oxidized. The amount of NADH oxidized in the reaction was stoichiometric to the amount of ammonia or with half the amount of urea, respectively. NADH was then determined by its light absorbance at 340 nm.

The chemical reaction was as follows:

1) urea + H_2O + urease → 2 NH_3 + CO_2

2) 2-Oxoglutarate + NADH + NH_4^+ + glutamate dehydrogenase → L-glutamate + NAD^+ + H_2O

Since no national MUN standards existed, standards for glucose/BUN analysis from Abbott Laboratories Diagnostic Division (North Chicago, Illinois, USA) were used. Two standard concentrations were used: 10 mg/dl and 50 mg/dl. Both standards were diluted 1:4 with 5% trichloroacetic acid (TCA) (Fisher Scientific, Québec, Canada) and frozen at -18°C until MUN analysis. As for quality controls, three different types were chosen and used at the beginning, middle, and end of each carousel run in order to monitor the intra- assay as well as the inter-assay variability. The first control used was Accutrol™ Normal from Sigma Diagnostics (St-Louis, MO, USA). It guaranteed readings between 11-17 mg/dl. However, because the range was quite variable, a standard of 30 mg/dl from Abbott Laboratories, Diagnostic Division (North Chicago, Illinois, USA) was employed as a second control. The first two controls were diluted immediately in a 1:4 ratio with TCA. The third control consisted of 2% milk fat UHT milk from Lactel (Sainte-Claire, Québec, Canada). All controls were then frozen at −18°C in quantities sufficient to last for all MUN analysis.

On one occasion, samples from the MAC farm were taken in triplicate and sent to an outside firm, Ontario DHI (Guelph, Ontario, Canada), to P.A.T.L.Q., and to MAC for the MUN analysis using different methods such as wet chemistry, infra-red analysis and enzymatic method, respectively. The wet chemistry method consisted of first deproteinizing the milk. Then distillation was done with and without the addition of urease and urea N was obtained by

difference. Samples from DQCI Services Inc. (St. Paul, Minnesota, USA), were also collected in triplicate, and were sent equally to Ontario DHI, MAC and P.A.T.L.Q. for analyses. Fresh and frozen samples were also compared and analyzed. Recovery of the enzymatic method was assessed with spiked samples.

Finally, milk samples, standards, and controls were thawed at room temperature. All analyses were done in duplicate. Duplicates which had a coefficient of variation of more than 5% were redone. First, in order to precipitate proteins and provide a clear supernatant for the assay, 1 ml of milk was mixed with 4 ml of 5% TCA . Samples were vortexed and let to stand for a minimum of five minutes. These samples were then centrifuged at 3000 RPM at 4°C for 4 minutes. The top fat layer was carefully bypassed with the pipette and 75 µl of milk was inserted into each sample cup. The three controls were inserted at the beginning, middle, and end of each carousel leaving space for 9 duplicate samples per run. Intra-assay and inter-assay coefficients of variation for all controls were calculated.

3.5. CREATING THE DATA BASE

Raw Data

Production and feed management data of 25 herds from three seasons were collected from P.A.T.L.Q.'s data base. Data were selected according to the official P.A.T.L.Q. herd and cow identification. A total of 18 files of raw data comprising 6 files per season were transferred. Production data included test day (TD), milk (kg), fat (%), protein (%), lactation number (LN), days in milk (DIM), somatic cell count (SCC), calving date (CD), and days in gestation (DIG) (Appendix 1). All original feed data were transferred into five files. The first

one was comprised of group number (GN), individual amounts (kg) of energy, protein, mineral and vitamin supplements fed with a maximum of two amounts per supplement category (Appendix 2). However, if more than two supplements per category were fed, the amount fed was reported on a group basis and obtained from the third file. The second file included previous test date (PTD), previous group number (PGN), amount (kg) of forage for a maximum of five different types fed. It also comprised stage of body conditioning score defined as follows: 1= at parturition; 2= at 75 days; 3= at 200 days; 4= at dry off and body condition score (BCS) (Appendix 3). The third file contained herd feed code (FC), quantity required (kg) and a feed catalogue number associated to the quantity recommended (Appendix 4). The fourth file indicated the long and short description of feed along with a column for additional comments. It also described the feed base: 1= as fed; 2= dry matter (Appendix 5). Finally, the last P.A.T.L.Q. file contained the feed analysis data such as feed code (FC), dry matter (DM), acid detergent fiber (ADF), neutral detergent fiber (NDF), net energy of lactation (NEL), non structural carbohydrates (NSC), added fat (%DM), total fat (%DM), crude protein (CP), and undegradable intake protein (UIP%CP) (Appendix 6). Milk urea nitrogen data were collected separately as the trial proceeded.

Data base

All 18 files of the original raw data received from P.A.T.L.Q. were first converted to a Microsoft® Excell 97 format and then imported into a Microsoft® Access 97 database. A query was ran to make sure no duplicates were included and confirmed that the data were unique. Data from all three seasons were combined. Test dates and previous test dates were transformed from P.A.T.L.Q. format to day/month/year format. The software Visual Basic was

used to create a program for determining feed codes and ration calculation (Appendix 7). The final database yielded a total of 2,686 records and 114 fields.

3.6. STATISTICS

Using SAS® System for Windows™ Release 6.12, a multiple regression was run where the dependant variable was either MUN-MAC or MUN-P.A.T.L.Q. and the independant variables were the following: RatNEL, Season, Region, MlkPrtHL, SCC, TotalDM, NmpDay, RatUIPC, RatADF, RatNDF, RatNSC, RatAdFat, RatToFat, RatCP, Penratio, Strcrati, RatDIP, parity, and idim. RatNEL was the ration's net energy of lactation expressed in Mcal/kg of TotalDM. Three seasons existed. The first was during March and April 1997, the second July and August and the third November and December of that same year. Region was as previously described in Table 1. MlkPrtHL represented milk protein where 1 was high and 2 was low. SCC was Somatic cell count (x1000). TotalDM was total dry matter of the ration expressed in kg. NmpDay was the number of meals per day offered. RatUIPCP was ration undegradable intake protein as a percent of crude protein in the ration. RatADF was ration ADF as percent Total DM. RatNDF was ration NDF as percent Total DM. RatNSC was ration NSC as percent Total DM. RatAdFat was ration added fat as a percent Total DM. RatToFat was ration total fat as a percent Total DM. RatCP was ration crude protein as a percent Total DM. Penratio was the protein to energy ratio of the ration and was created dividing RatCP by RatNEL. Strcrati was the starch to protein ratio of the ration and was calculated by dividing RatNSC by RatDIP. RatDIP was the ration's degradable intake protein calculated by sustracting RatUIPCP from 100 and multiplying by RatCP over 100. Parity was the one at test day. Days in milk at the test

day was fitted into 16 classes where blocks of 20 dim were created except the last one which included dim above 300 dim.

The random effects included in the models were herd within region and herd within MlkPrtHL. Repeated effects included the effect of cow within herd and parity.

Residuals were plotted against the predicted values for variables NEL, DIM, SCC, TotalDM, RatUIPC, RatADF, RatNDF, RatNSC, RatAdFat, RatToFat, RatCP, Penratio, Strcrati, RatDIP. The points appeared to be randomly scattered and no pattern was apparent. No dependancy was evident in the residual plots which suggested the regression models were adequate. Thus these variables were included. Univariates were also done to ensure normality of the parameters.

Finally, MlkPrtHL, NmpDay, RatUIPCP, RatADF, RatAdFaT, and RatDIP were found to be non significant. These parameters were dropped and the models were run again. The final model used was as follows:

$$Y_{ijklmn} = \mu + Region_i + Herd_{ij} + b_1*RatNEL_{ijklmn} + Season_k + b_2*SCC_{ijklmn} + b_3*TotalDM_{ijklmn} + b_4*RatNDF_{ijklmn} + b_5*RatNSC_{ijklmn} + b_6*RatToFat_{ijklmn} + b_7*RatCP_{ijklmn} + b_8*PEnratio_{ijklmn} + b_9*Strcrati_{ijklmn} + Parity_l + DIM_m + e_{ijklmn}$$

Where Y = MUN

μ = overall mean

Region = the effect of the i^{th} Region, i= 1, 2, 3, 4, 5, 6, 7, 9, 10, 11, 12

Herd = the effect of the j^{th} Herd in the i^{th} Region, $j = 1, \ldots, n_i$

RatNEL = ration Net Energy of Lactation

b_1 = the coefficient of the relationship between RatNEL and milk urea nitrogen

Season = the effect of the k^{th} Season, k=1,2,3

SCC = Sommatic Cell Count

b_2 = the coefficient of the relationship between SCC and milk urea nitrogen

TotalDM = Total Dry Matter

b_3 = the coefficient of the relationship between TotalDM and milk urea nitrogen

RatNDF = ration Neutral Detergent Fiber

b_4 = the coefficient of the relationship between RatNDF and milk urea nitrogen

RatNSC = ration Non Structural Carbohydrates

b_5 = the coefficient of the relationship between RatNSC and milk urea nitrogen

RatToFat = ration Total Fat

b_6 = the coefficient of the relationship between RatToFat and MUN

RatCP = ration Crude Protein

b_7 = the coefficient of the relationship between RatCP and MUN

PEnratio = Protein to energy ratio

b_8 = the coefficient of the relationship between PEnratio and MUN

Strcrati = Starch to protein ratio

b_9 = the coefficient of the relationship between Strcrati and MUN

Parity = the effect of the l^{th} Parity, l= 1,2,3,...,11

DIM = the effect of the m^{th} DIM, m=1,2,3,...,16

e_{ijklmn} = random error

There was a random effect of herd level within region and again repeated effects included the effect of cow within herd and parity.

CHAPTER 4. RESULTS AND DISCUSSION

Methodology

Prior to initiation of the field trial, fifty seven fresh milk samples from the Macdonald Campus Farm were analyzed for MUN. Using results from the wet chemistry as reference, correlations of 0.980 and 0.658 were obtained with MAC and P.A.T.L.Q. methods, respectively. The reason for the unexpected lower correlation between values from the wet chemistry method and the P.A.T.L.Q. method is unknown. To clarify these findings, another set of samples were analyzed. Using thirty milk samples from DQCI and again using wet chemistry results as a reference, correlations of 0.993, 0.974, and 0.967 were achieved for MAC, DQCI, and P.A.T.L.Q. analysis. Correlations between DQCI and P.A.T.L.Q. was 0.970, and between DQCI and MAC was 0.977. Finally a correlation of 0.965 was yielded between P.A.T.L.Q. and MAC analyses.

P.A.T.L.Q. calibrated their IR instrument with 90 milk samples originating from this province's dairy cows. These samples were first sent to be analyzed by a pH based methodology using a CL-10 unit (Guelph, Ontario, Canada). Results from these milk samples were returned and the IR instrument was calibrated accordingly. The calibration was confirmed with another set of samples sent again to Ontario. Samples previously analyzed by the IR were then sent weekly to DQCI and results were compared on a continuous basis. It is important to note that DQCI also uses a CL-10 unit. Similar correlations had also previously been achieved. In fact, results from 1996 and 1997 revealed correlations of 0.897 and 0.970 between an enzymatic analysis (using urease NADPH reduction) and the CL-10 unit. Moreover, there was a correlation of 0.90 and 0.87 between the Infrared and the enzymatic method or the CL-10,

respectively (Ferguson, personal communication, 1997). Therefore the higher correlations obtained in this trial between our enzymatic method and the CL-10 method were as expected.

Contradicting results exist as to the fate of urea in non-preserved milk during storage. No significant differences in MUN concentrations were observed after storage at 4°C for 10 days (Carlsson and Bergström, 1994) nor after refrigeration for 11 days (Godden et al., 1997). However, others have found that conservation of milk samples at 4°C for 1 week (Eicher et al., 1998) and storage of whole milk in a refrigerator for 14 days (Oltner and Sjaunja, 1982) increased MUN significantly. Another case demonstrated that, after 17 days, the milk turned sour and MUN values increased (Carlsson and Bergström, 1994). Evaporation has been suggested as an explanation for the increase in MUN even if the samples have been stored in the sealed containers (Eicher et al., 1998). Due to the enormous number of samples to be analyzed and to technical considerations of the MAC method, an alternative storage condition had to be considered. First, samples routinely analyzed by P.A.T.L.Q. for milk components contain a preservative, bronopol. That same condition was applied for the purpose of this trial. It has previously been documented that adding bronopol did not affect MUN results (Oltner and Sjaunja, 1982). MUN did not change when bronopol was added for 17 days (Carlsson and Bergström, 1994). Another type of preservative (Broad Spectrum Microtabs) equally did not have any effect on MUN (Butler et al., 1996). Even though it was demonstrated that MUN concentrations were significantly higher in samples containing bronopol compared to non-preserved samples (mean difference of 0.25 mg/dl), the authors judged this effect to be unlikely of biological significance (Godden et al., 1997). Others (Oltner and Sjaunja, 1982) found that when preserved samples were kept for 14 days in a refrigerator, the levels of urea increased

slightly. These authors put forward a possible explanation. Ammonium present in the milk may have caused an overestimation of MUN since ammonium ions act as a rate-limiting substrate in the reaction employed and thus, from a practical point of view, since this initial amount is extremely low, this overestimation may be considered negligible. Therefore the use of preservative in this trial was judged not to have influence the outcome of the study. Secondly, to determine if freezing had an impact on MUN determination, samples were first analyzed fresh and then frozen at -18°C until analysis. Two hundred and five samples analyzed by the MAC method yielded a correlation of 0.95 between fresh and frozen samples. Milk samples were kept frozen for periods varying from approximately 1 day to 2 months. Deepfreezing at -20°C did not influence MUN (Carlsson and Bergström, 1994) nor did freezing at -18°C for 1 week (Oltner and Sjaunja, 1982). On the other hand, freezing unpreserved milk at -20°C for 1 month increased MUN significantly with a correlation of 0.55 (Eicher et al., 1998). In light of the above, it is considered that freezing did not influence MUN and thus did not compromise the validity of the study. Some samples from this trial were frozen for a maximum period of approximately one year.

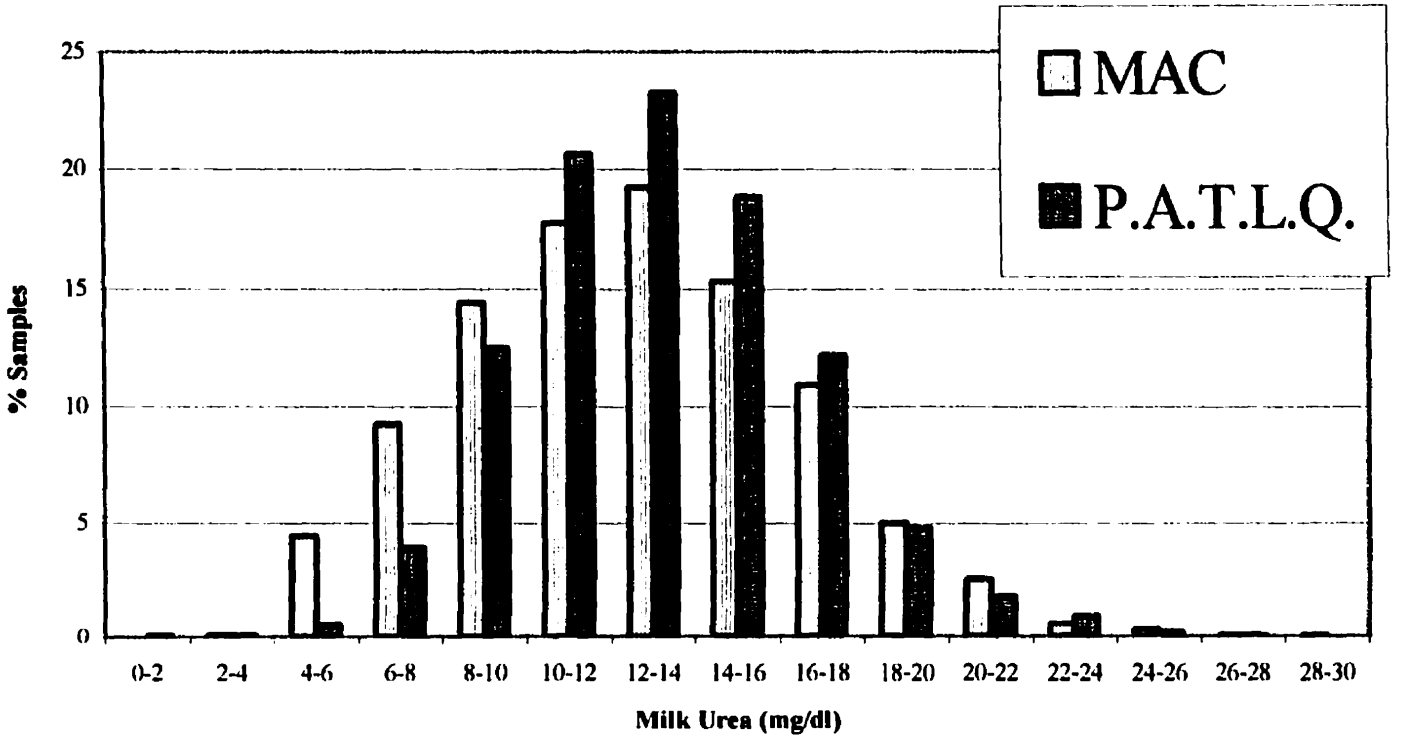
Thirty five milk samples were spiked either with 5, 10 or 15 mg/dl of urea nitrogen. The recovery was 99.10% and the coefficient of variation 2.25%. These results are comparable to what other authors previously reported such as a CV of 2.6% using 20 samples (Oltner and Sjaunja, 1982) and a recovery of 99.94 determined by using only 4 samples (Bentley Instruments, 2000).

Finally, of the 25 herds used in this study, a total of 2,686 samples were analyzed. The

results revealed intra-assay variations of 2.01%, 1.90%, and 2.48% for the Accutrol™ Normal, the standard of 30 mg/dl, and the 2% milk fat UHT milk. Inter-assay coefficients of variation were 10.79%, 5.99%, and 9.46% for the Accutrol™ Normal, the standard of 30 mg/dl, and the 2% milk fat UHT milk. The MUN coefficient of variation (CV) of the 2,686 milk samples analyzed was 1.85%. Although data in the literature were limited, our results were comparable to what others reported. Repeatability expressed as CV was 1.4% and accuracy was 2.6% (Oltner and Sjaunja, 1982). With 108 samples the coefficient of variation was 3% (Carlsson and Bergström, 1994). CV for MUN was 4.82% (Faust et al., 1997a) or between 3.3-7.3% (Faust et al., 1997b).

Milk samples analyzed by the MAC method yielded a MUN average of 12.52 ± 3.99 mg/dl while the P.A.T.L.Q. method 13.22 ± 3.39 mg/dl. These results were in accordance with those reported by Wilson et al. (1998) who found that MUN values were higher when measured by mid infrared reflectance spectroscopy compared to those by the enzymatic assay. However, it has also been observed that when samples were calibrated to the CL-10, lower estimates of MUN were yielded by the Infrared machine (Ferguson, personal communication, 1997). Recommendations as to the desirable range of MUN values are variable. Agsource (2000) recommends values between 12-18 mg/dl. Although not described as a recommendation, Ontario DHI (2000) states that its most common reported range of values lies between 10-18 mg/dl. Pennsylvania DHI, who began MUN testing in September 1996 (Ferguson et al., 1997a,b), recommends 10-14 mg/dl (Center for Animal Health and Productivity, 2000). Others like P.A.T.L.Q. suggest 10-16 mg/dl is more appropriate (P.A.T.L.Q., 2000). The frequency distribution of the samples from this trial is presented in Figure 1. According to the MAC

Figure 1. Frequency distribution of samples according to milk urea concentration obtained by MAC and P.A.T.L.Q. analysis



method, 72% of the herds were within 10-16 mg N/dl compared to 84 % for the P.A.T.L.Q. method. As for individual animals, 52.2% and 63.9% of cows had MUN values within those same limits. Similar results have been described by Lefebvre et al. (1999a), where 75% of the herds and 54% of the cows had MUN values between 10-16 mg N/dl. It is important to note that the general distribution of the curve derived from samples of the MAC method was similar to that reported by Ferguson from the Center for Animal Health and Productivity (Year 2000 report). This may be explained by the fact that Pennsylvania DHIA also calibrated their IR instrument with an enzymatic urease NADPH rate reduction methodology (Ferguson, personal communication, 1997). Our data indicate a lower proportion of samples, representing low MUN values, when measured by the P.A.T.L.Q. method in comparison with the MAC method. It appears that the P.A.T.L.Q. method therefore over estimated MUN values in the low range. The first tentative explanation to support these findings may have been due to the fact that the P.A.T.L.Q. method was calibrated with the CL-10 unit. This method relied on the principle th the differential pH was measured based on the possibility of correlating pH variations, which were measured by two capillary glass electrodes, to the quantity of H^- produced or consumed by the reaction, which was activated by adding the appropriate enzyme. This calculation corrected for the ammonia that may already have been present in the milk and thus eliminated the possibility of a bias. However, the MAC method measured the amount of ammonia produced by the enzymatic reaction but did not correct for the ammonia which may initially have been present in the milk. If this amount was significant, higher MUN values would have been obtained from the MAC method instead of the P.A.T.L.Q. method. Another possible explanation may rely in the way in which the P.A.T.L.Q. method measures urea. Approximately 45 to 50% of the urea estimate comes from the actual optical reading in the sample, while the

other 50 to 55% comes from a mathematical adjustment for concentrations of other interfering components (Hansen, 1997). Some of these components include butterfat and SCC which are known to have a positive or negative effect on MUN, respectively (Godden et al., 2000). The fact that these interfering components may vary considerably between individual cows leads the IR instrument to produce different MUN estimates eventhough the samples may have actually had the same true urea value (Godden et al., 2000). This analytical variabilty may partially explain differences between MUN estimates obtained from the MUN-MAC and MUN-P.A.T.L.Q. method although no evident relationship was established between these interfering compounds and the MUN estimates. Several authors have recommended that this variation may be removed by interpreting MUN values at the group level (Broderick and Clayton, 1997; Cannas et al., 1998; Oltner et al., 1985; Schepers, A.J. and R.G.M. Meijer, 1998). Similarly, Godden et al. (2000) reported a relative lack of agreement between the IR method and the Eurochem test (CL-10 unit) when MUN values were compared on an individual basis. However, when interpreted at the group level their results showed a good overall agreement. This study did not analyze data in such a way. Further research is thus needed in order to determine the exact mechanism which causes differences in estimation of the lower MUN values between the MAC and the P.A.T.L.Q. methods.

Database

Parameters that significantly contributed to the models are summarized in Table 2. The

Table 2. Summary of Parameters That Made Significant Contributions to the Models.

Parameter	Model MUN-MAC	Model MUN-P.A.T.L.Q.
RatNEL	P<0.001	P<0.001
Season	P<0.001	P<0.001
Region	P<0.05	P<0.05
SCC	P<0.01	P<0.001
TotalDM	P<0.01	P<0.05
RatNDF	P<0.001	P<0.001
RatNSC	P<0.001	P<0.001
RatToFat	P<0.001	P<0.001
RatCP	P<0.001	P<0.05
Penratio	P<0.001	P<0.01
Strcrati	P<0.001	P<0.001
parity	P<0.05	P<0.01
idim	P<0.001	P<0.001

results in the present study have demonstrated that the ration's net energy of lactation had a very significant ($P<0.001$) influence on MUN output. The estimated coefficents were -28.07 ± 4.46 ($P<0.001$) for the MUN-MAC model and -15.52 ± 4.16 ($P<0.001$) for the P.A.T.L.Q. model. Although a group of researchers working with lactating ewes recently discovered no effect of energy on MUN, in dairy cows energy Broderick et al. (1997) demonstrated a significantly negative effect between urea nitrogen and energy. Therefore it is not surprising that this relationship is generally accepted in the dairy field. A summary of the regression coefficients of both models is presented in Table 3.

Our results showed that total dry matter intake was significant in both of our models ($P<0.01$ and $P<0.05$ for MUN-MAC and MUN-P.A.T.L.Q., respectively). Estimated coefficients were positively related to MUN. MUN-MAC model had a value of 0.07 ± 0.02 ($P<0.01$) while the MUN-P.A.T.L.Q. had a value of 0.04 ± 0.02 ($P<0.05$). Similarly, Broderick et al. (1997) demonstrated that dry matter intake positively influenced MUN concentrations ($P<0.1$). However, their finding may be questionable due to its low significance.

Significant effect of the ration's crude protein on MUN was confirmed in this study ($P<0.001$, MUN-MAC and $P<0.05$, MUN-P.A.T.L.Q.). Estimated coefficients were positively related to MUN (1.77 ± 0.41, $P<0.001$ and 0.90 ± 0.38, $P<0.05$). Similar results were obtained in a study using data from 35 trials and 482 lactating Holstein cows where crude protein as a percent of dry matter was found to be one of the parameters which made the most significant($P<0.001$) contributions to the model (Broderick et al., 1997).

Table 3. Summary of Regression Coefficients in Both Models

	Model MUN-MAC		Model MUN-P.A.T.L.Q.	
Parameter	**Coefficient**	**Standard Error**	**Coefficient**	**Standard Error**
RatNEL	-28.07	4.46	-15.52	4.16
SCC	-0.0003	0.0001	-0.001	0.0001
TotalDM	0.07	0.02	0.04	0.02
RatNDF	-1.78	0.03	-0.13	0.03
RatNSC	0.12	0.03	0.11	0.3
RatToFat	1.27	0.17	0.74	0.16
RatCP	1.78	0.41	0.90	0.38
Penratio	-2.66	0.63	-1.68	0.60
Strcrati	-1.46	0.28	-1.75	0.26

Surprisingly neither ration's DIP nor UIPCP had a significant effect on MUN. Theoretically, the hypothesis that degradable intake protein or undegradable intake protein may influence MUN concentration seems very plausible. However, the combination of a variety of factors interacting with MUN renders it difficult to demonstrate the isolated influence of these factors. It is therefore postulated that effects of DIP and UIPCP may have been confounded with NSC in our models. It has also previously been reported that when variables were highly correlated, they could not be included together in the final multivariate model (Godden et al., 2001).

The effect of the ration's ADF on MUN was not significant in both of the models but the ration's NDF had very significant effect in both models ($P<0.001$). NDF was negatively related to MUN (-0.18 ± 0.03, $P<0.001$ for MUN-MAC and -0.13 ± 0.03, $P<0.001$ for MUN-P.A.T.L.Q.). NDF is known to be the total cell wall portion of the forage which includes the ADF fraction plus hemicellulose (Aseltine, 1992). It is used to estimate intake because it represents all the fiber components that occupy space in the rumen and are digested slowly (Shaver and Undersander, 1989). This is in agreement with the findings of our trial which found a positive relationship of MUN with DMI. However, these results are in contrast with those by Broderick et al. (1997) who showed no effect of NDF (percentage of DM) in a single factor regression analysis. Furthermore, Cannas et al. (1998) found no association between NDF intake or concentration and MUN.

The effect of non structural carbohydrates of the ration on MUN was significant ($P<0.001$) in each model. Estimated coefficients were 0.12 ± 0.03 ($P<0.001$) and 0.11 ± 0.03 ($P<0.001$). It may be speculated that this parameter is somewhat confounded with effects of NEL.

The effect of the protein to energy ratio on MUN was significant ($P<0.001$ for MUN-MAC and $P<0.01$ for P.A.T.L.Q.). The estimated coefficients were both negative. MUN-MAC had a value of -2.66 ± 0.63 ($P<0.001$) and MUN-P.A.T.L.Q. -1.68 ± 0.60 ($P<0.01$).

The effect of the ration's added fat on MUN was not significant but the total fat was significant ($P<0.001$) in both models. It was positively related to MUN. The estimated coefficients were 1.27 ± 0.17 ($P<0.001$) and 0.74 ± 0.16 ($P<0.001$) for MUN-MAC and MUN-P.A.T.L.Q., respectively.

This study demonstrated that the starch to protein ratio of the ration (RatNSC:RatDIP), had a very significant influence ($P<0.001$) on MUN in both models. These results are similar to those reported by Carruthers et al. (1997). Using a Latin square design, three diets (P: pasture only, PR: 0.85P plus 0.15 NSC/protein mixture, PE: P plus 0.1(trial 1) or 0.15(trial 2) NSC) were offered to twenty-four cows (19 Friesian, 5 Jersey). The three diets were isonitrogeneous while P and PR were isoenergetic. Their findings revealed that increasing the total carbohydrate intake by elevating the NSC:protein ratio was more effective in improving nitrogen utilization in the rumen than increasing the NSC:SC ratio alone. MUN was significantly lower for PE than for P in both trials. This is comparable to our study as this ratio was also found to be negatively associated with MUN (-2.29 $P<0.001$ MUN-MAC; -1.75 $P<0.001$ MUN-P.A.T.L.Q.). Unfortunately not many studies have compared this ratio with MUN, particularly in Holstein cows, and results from the present study definitely indicated that research in this field for further understanding of MUN would be promissing.

Managerial factors such as number of meals per day were investigated in this trial. This parameter was not significant in either model ran.

In the present study, parity was found to be significant in both models ($P<0.05$, MUN-MAC and $P<0.01$, MUN-P.A.T.L.Q.). Least square means are presented in Table 4. These results revealed that Québec Holstein cows in their first lactation yielded MUN values lower than cows of greater parity numbers. These results are in accordance with those reported by Oltner et al. (1985) and (Butler et al., 1995). Carlsson et al. (1995) had previously found in a preliminary trial that multiparous cows had slightly higher MUN concentrations than primiparous cows. However, these same authors discovered that this difference disappeared when other factors were taken into account in the model. Based on results from our trial, particular attention should be made to the proportion of cows in first parity within a herd or a group when interpreting MUN results.

Days in milk were significant ($P<0.001$) in both MUN-MAC and MUN-P.A.T.L.Q. models. Differences in least square means revealed a significant difference ($P<0.05$) between the first 40 days in milk and the remaining of the lactation for the MUN-MAC model. MUN for DIM below or equal to 20 and from 21 to 40 DIM was 11.14 ± 0.58 mg/dl and 11.85 ± 0.56 mg/dl, respectively. Similarly, the MUN-P.A.T.L.Q. model showed a significant difference ($P<0.05$) between the beginning of lactation, especially days 21-60, and the end of lactation. MUN was 13.01 ± 0.47 mg/dl from 21-40 DIM and 13.48 ± 0.48 mg/dl from 41-60 DIM. Milk

Table 4. Parities Expressed as Least Square Means

		Model MUN-MAC		Model MUN-P.A.T.L.Q.	
Parity	**n**	**LSM (mg/dl)**	**Standard Error**	**LSM (mg/dl)**	**Standard Error**
1	914	12.13	0.43	13.09	0.33
2	592	12.62	0.44	13.45	0.34
3	475	12.64	0.44	13.61	0.34
4	324	12.65	0.45	13.36	0.35
5	192	12.73	0.46	13.49	0.38
6	83	12.80	0.51	13.64	0.44
7	65	12.43	0.05	13.86	0.48
8	19	13.20	0.74	15.00	0.73
9	18	12.93	0.76	14.86	0.73
10	3	13.09	1.66	14.60	1.56
11	1	15.22	2.82	15.67	2.67

composition is known to vary in the first days in milk and the possibility of increased interfering components may alter the IR urea estimates as described previously. This may explain the absence of significance between MUN values of DIM below or equal to 20 and the rest of lactation when the P.A.T.L.Q. method is used. Least square means for all classes of DIM were plotted in Figures 2 and 3. Generally, these results are in agreement with those reported by Carlsson et al. (1995) where the concentration of urea was also lower during the first month of lactation compared to later in the lactation. Attempts have been made to try to explain this phenomenon. These authors have speculated that this decrease may be related to the one associated with a reduction of DMI or increase intake in fermentable carbohydrates at that time period. Suboptimal function of the rumen microflora, high risk of metabolic disturbances, and the possibility of a nitrogen conserving mechanism in early lactation have been postulated. There is a lack of direct evidence to support this and thus further investigation is needed. Meanwhile caution must be taken when interpreting MUN values to ensure that this point is taken into account. This will reduce wrongful interpretations of MUN when lower values are found and, for example, the majority of the cows in a herd or in a group have just calved recently. As recommendations of BCS vary according to stage of lactation and stage of growth, this concept could equally be applied for MUN recommendations and warrants further investigation.

The present study showed showed that SCC significantly affected MUN in the MUN-MAC model ($P<0.01$) and in the MUN-P.A.T.L.Q. model ($P<0.001$). The estimated coefficients were -0.0003 ($P<0.01$) for MUN-MAC and -0.0008 ($P<0.001$) for MUN-P.A.T.L.Q.. Although very little studies have been conducted on the effects of somatic cell count on MUN,

Figure 2. The Effect of MUN Analyzed by the MAC Method in Relation to Days in Milk

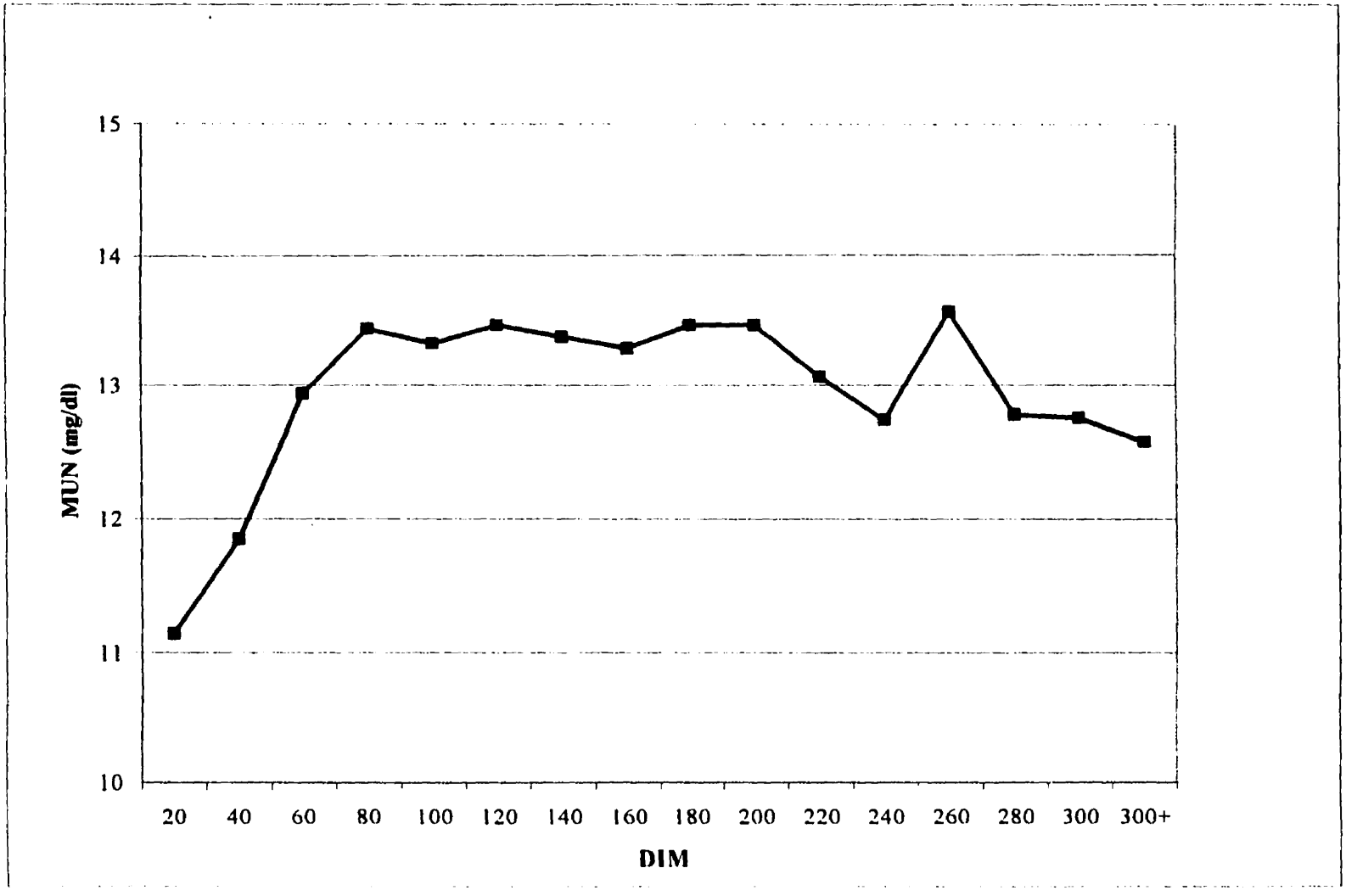
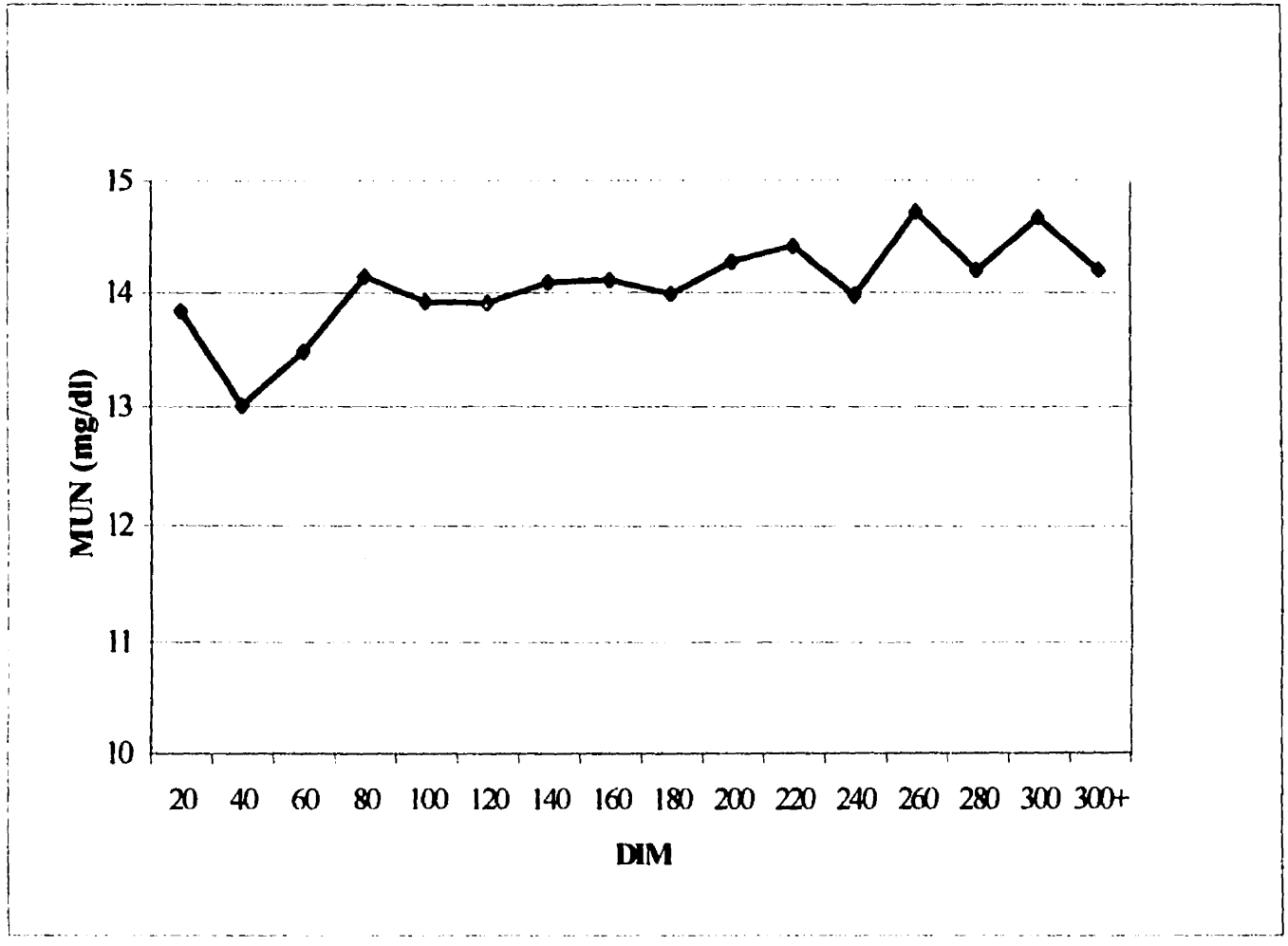


Figure 3. The Effect of MUN Analyzed by the P.A.T.L.Q. Method in Relation to Days in Milk



our results are in agreement with those of Faust and associates (1997b) who reported that MUN values were lowest for samples with the highest SCC.

MUN has been known to increase during the summer when cows are on pasture and decrease during the winter. Although the effect of season on MUN was significant in both models ($P<0.001$), this study revealed an opposite relationship with MUN but the reason is unknown. MUN was lower during the summer sampling. Least square means for MUN-MAC and MUN-P.A.T.L.Q. were 13.45 ± 0.53 mg/dl and 14.47 ± 0.44 mg/dl for March and April 12.14 ± 0.53 mg/dl and 13.35 ± 0.44 mg/dl for July and August, 13.26 ± 0.053 mg/dl and 14.35 ± 0.44 mg/dl for November and December, respectively. Surprisingly, the same drop in MUN during the month of August of 1997 was reported by Ferguson (Center for Animal Health and Productivity, 2000). It can be postulated that this decrease in the summer months may be the result of a decrease in DMI due to certain climatic conditions. However, a lack of direct evidence does not enable us to support or disprove this hypothesis and, therefore, definitely warrants future investigations.

The effect of region on MUN was significant ($P<0.001$) in both models. Least square means for all regions are presented in Table 5. The lowest MUN values (9.59 ± 1.99, 9.31 ± 1.17 mg/dl for MUN-MAC and 11.74 ± 1.50, 11.00 ± 0.90 mg/dl for MUN-P.A.T.L.Q.) were observed in Regions 6 and 7, respectively. These regions comprise Bagot, Missisquoi, Richelieu, Rouville, St-Hyacinthe, Verchères, Beauharnois, Châteauguay, Huntingdon, Iberville, Laprairie, Napierville, Soulanges, St-Jean, and Vaudreuil counties. These are regions where corn is grown extensively and thus is a major component of the ration fed to cows.

Table 5. Regions Expressed as Least Square Means

		Model MUN-MAC		Model MUN-P.A.T.L.Q.	
Region	**n**	**LSM (mg/dl)**	**Standard Error**	**LSM (mg/dl)**	**Standard Error**
1	219	12.36	1.17	13.35	0.90
2	187	12.59	1.42	13.60	1.08
3	160	13.82	1.42	15.00	1.08
4	239	13.58	1.41	14.50	1.07
5	114	15.67	1.98	17.33	1.49
6	120	9.59	1.99	11.74	1.50
7	368	9.31	1.17	11.00	0.90
9	186	15.33	1.42	15.65	1.08
10	242	11.38	1.42	12.96	1.08
11	475	12.18	1.02	13.19	0.78
12	376	16.55	1.17	16.31	0.89

Region 1 = Bonaventure, Kamouraska, Matane, Matapédia, Rimouski, Rivière-du-loup, and Témiscouata; Region 2 = Bellechase, Charlevoix-Est, Lévis, L'islet, Lobinière, Montmagny, Montmorency, Portneuf, and Québec; Region 3 = Beauce, Dorchester, Frontnac, and Mégantic-Sud; Region 4 = Arthabaska, Drummond, Nicolet-Est, Nicolet-Ouest, Yamaska, and Mégantic-Nord; Region 5 = Brôme, Compton, Richmond, Shefford, Sherbrooke, Stanstead, and Wolfe Region 6 = Bagot, Missisquoi, Richelieu, Rouville, St-Hyacinthe, and Verchères Region 7 = Beauharnois, Châteauguay, Huntingdon, Iberville, Laprairie, Napierville, Soulanges, St-Jean, and Vaudreuil. Region 9 = Abitibi-Est, Abitibi-Ouest, Rouyn-Noranda, and Témiscamingue; Region 10 = Berthier, Deux-Montagnes, Jacques-Cartier, Joliette, L'assomption, Montcalm, and Terrebonne; Region 11= Champlain, Maskinongé, and St-Maurice; Region 12 = Chicoutimi, Jonquière, Lac St-Jean, Roberval, and Saguenay

According to previously described results on the starch to protein ratio in this study, it may be speculated that these regions would have the highest NSC ratio. On the other hand, the highest MUN values (16.55 ± 1.17, 15.67 ± 1.98, 15.33 ± 1.42 mg/dl for the MUN-MAC model and 16.31 ± 0.89, 17.33 ± 1.49, 15.65 ± 1.08 mg/dl for the MUN-P.A.T.L.Q. model) were observed in Regions 12, 5, and 9, respectively. Comparable results have been described by Lefebvre and Lacroix (1999b). Abitibi-Est, Abitibi-Ouest, Rouyn-Noranda, Témiscamingue, Chicoutimi, Jonquière, Lac St-Jean, Roberval, and Saguenay are regions deficient in corn silage. Again, it could be speculated that these regions represented the lowest starch to protein ratio. The relationship between each region and the NSC ratio was not investigated in this trial and warrants futher research.

CHAPTER 5. CONCLUSIONS

This study has demonstrated that the MAC method developed was found to be suitable for MUN analysis and thus is promissing for the needs of the regular calibration of the P.A.T.L.Q. methodology. Differences were obtained between low MUN estimates when measured by the MAC method in comparison to the P.A.T.L.Q. method. The frequency distribution of the samples from the MAC method was found similar to that of a leading U.S.A. MUN laboratory, Pennsylvania DHIA, who calibrated their IR instrument with an enzymatic method. Thus, it may be speculated that these differences observed between the MAC and P.A.T.L.Q. methods were due to different calibrations but unfortunately could not be explained by this study. Further research in this field is warranted.

When using the MAC methodology, the ration's net energy of lactation, NDF, NSC, total fat, crude protein, protein to energy ratio, starch to protein ratio, season, and days in milk made the most significant contributions ($P<0.001$) to the model. As for the P.A.T.L.Q. model, these factors included net energy of lactation, NDF, NSC, total fat, starch to protein ratio, season, SCC, and days in milk. These results demonstrate the importance of the previous factors in relation to MUN in both models although a lack of research is prominent for certain factors such as the starch to protein ratio. Research in this field, for example, for further understanding of MUN would definitely be promissing. However, a number of other parameters were also significant ($P<0.01$) in the MAC model such as total dry matter and SCC as well as protein to energy ratio and parity for the P.A.T.L.Q. model. Other variables such as region and parity in the MAC model as well as region, total dry matter and crude protein in the P.A.T.L.Q.

model were significant at the level $P<0.05$.

Factors such as total dry matter, NSC, total fat, and crude protein were positively related to MUN while others, including net energy of lactation, SCC, NDF, protein to energy ratio, and starch to protein ratio were negatively related. Unexpected results from NSC and protein to energy ratio may have been due to confounding effects in the models. Interestingly, number of meals per day, ADF, and added fat did not make a significant contribution to the overall mixed effects model. Moreover, UIPCP and DIP were equally found to have no influence on MUN but results should be interpreted with caution as they may also have been attributed to confounding effects in the model. The final equations obtained with both models were the following:

1) **MUN-MAC :** $Y_{ijklmn} = \mu + Region_i + Herd_{ij} - 28.07*RatNEL_{ijklmn} + Season_k - 0.0003*SCC_{ijklmn} + 0.07*TotalDM_{ijklmn} - 1.78*RatNDF_{ijklmn} + 0.12*RatNSC_{ijklmn} + 1.27*RatToFat_{ijklmn} + 1.78*RatCP_{ijklmn} - 2.66*PEnratio_{ijklmn} - 1.46*Strcrati_{ijklmn} + Parity_l + DIM_m + e_{ijklmn}$

2) **MUN-P.A.T.L.Q. :** $Y_{ijklmn} = \mu + Region_i + Herd_{ij} - 15.52*RatNEL_{ijklmn} + Season_k - 0.001*SCC_{ijklmn} + 0.04*TotalDM_{ijklmn} - 0.13*RatNDF_{ijklmn} + 0.11*RatNSC_{ijklmn} + 0.74*RatToFat_{ijklmn} + 0.90*RatCP_{ijklmn} - 1.68*PEnratio_{ijklmn} - 1.75*Strcrati_{ijklmn} + Parity_l + DIM_m + e_{ijklmn}$

The overall findings of this study have undoubtedly contributed to a better understanding of nutritional, managerial, physiological, and environmetal factors influencing MUN.

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APPENDICES

APPENDIX 1. Production Raw Data.

Herd number, cow number, test day, milk, fat, protein, lactation number, days in milk, somatic cell count, calving date, days in gestation, herd number, cow number.

00142	00431	35742	0028.9	4.94	3.77	05	00133	00044	35609	053	00142	00431
00142	00434	35742	0027.8	3.66	3.54	05	00159	00570	35583	000	00142	00434
00142	00450	35742	0000.0	0.00	0.00	04	00345	00000	35361	251	00142	00450
00142	00455	35742	0000.0	0.00	0.00	04	00320	00000	35421	160	00142	00455
00142	00457	35742	0027.0	4.45	3.92	04	00254	00610	35488	102	00142	00457
00142	00459	35742	0015.7	3.76	3.48	05	00205	00265	35537	000	00142	00459
00142	00460	35742	0026.0	3.73	3.58	05	00165	01732	35577	072	00142	00460
00142	00461	35742	0041.4	2.99	3.25	04	00111	00050	35631	019	00142	00461
00142	00464	35742	0045.0	4.57	3.51	04	00009	00032	35733	000	00142	00464
00142	00467	35742	0019.2	4.49	3.87	04	00247	00049	35495	108	00142	00467
00142	00469	35742	0018.3	4.55	3.83	03	00300	00097	35442	162	00142	00469
00142	00472	35742	0029.5	3.77	3.48	03	00198	00052	35544	034	00142	00472
00142	00473	35742	0034.4	3.87	3.03	03	00115	00452	35627	000	00142	00473
00142	00475	35742	0039.2	4.09	3.35	03	00095	00079	35647	006	00142	00475
00142	00476	35742	0046.0	3.12	3.23	03	00075	00321	35667	000	00142	00476
00142	00478	35742	0035.9	4.37	3.42	03	00080	00053	35662	000	00142	00478
00142	00480	35742	0045.8	4.06	3.45	04	00023	00007	35719	000	00142	00480
00142	00481	35742	0000.0	0.00	0.00	02	00312	00000	35393	255	00142	00481
00142	00483	35742	0017.8	4.42	4.19	02	00295	00079	35447	195	00142	00483

APPENDIX 2. Supplement Raw Data.

Herd number, cow number, test day, group number, energy supplement 1 individual amount, energy supplement 2 individual amount, protein supplement 1 individual amount, protein supplement 2 individual amount, mineral and vitamin supplement 1 individual amount, mineral and vitamin supplement 2 individual amount.

00142	00431	0019971127	2	09.0	00.0	01.5	00.0	000	000
00142	00434	0019971127	2	08.0	00.0	01.0	00.0	000	000
00142	00450	0019971127	2	00.0	00.0	00.0	00.0	000	000
00142	00455	0019971127	5	00.0	00.0	00.0	00.0	000	000
00142	00457	0019971127	2	10.0	00.0	01.0	00.0	000	000
00142	00459	0019971127	2	07.0	00.0	00.0	00.0	000	000
00142	00460	0019971127	2	09.0	00.0	01.0	00.0	000	000
00142	00461	0019971127	1	11.0	00.0	03.0	00.0	000	000
00142	00464	0019971127	1	06.0	00.0	02.0	00.0	000	000
00142	00467	0019971127	2	08.0	00.0	01.0	00.0	000	000
00142	00469	0019971127	2	10.0	00.0	00.5	00.0	000	000
00142	00472	0019971127	2	09.0	00.0	01.5	00.0	000	000
00142	00473	0019971127	1	10.0	00.0	02.0	00.0	000	000
00142	00475	0019971127	1	11.0	00.0	02.5	00.0	000	000
00142	00476	0019971127	1	11.0	00.0	03.0	00.0	000	000
00142	00478	0019971127	1	10.0	00.0	02.8	00.0	000	000
00142	00480	0019971127	1	11.0	00.0	02.8	00.0	000	000
00142	00481	0019971127	5	00.0	00.0	00.0	00.0	000	000
00142	00483	0019971127	2	07.0	00.0	00.0	00.0	000	000
00142	00484	0019971127	6	00.0	00.0	00.0	00.0	000	000
00142	00486	0019971127	5	00.0	00.0	00.0	00.0	000	000
00142	00488	0019971127	2	07.0	00.0	00.0	00.0	000	000

APPENDIX 3. Forage Raw Data.

Herd number, cow number, previous test day, previous group number, forage 1, forage2, forage3, forage 4, forage 5, stage of lactation associated with body condition score (1=parturition 2=75 days in milk 3=200 days in milk 4=dry), body condition score.

00142	00431	0019971022	1	0020.5	0002.5	0010.0	0000.0	0000.0	2	2.0
00142	00434	0019971022	2	0024.5	0003.0	0010.0	0000.0	0000.0	3	2.5
00142	00450	0019971022	2	0025.2	0003.0	0010.0	0000.0	0000.0	3	2.0
00142	00455	0019971022	2	0021.2	0003.0	0010.0	0000.0	0000.0	3	2.5
00142	00457	0019971022	2	0018.1	0003.0	0010.0	0000.0	0000.0	3	2.0
00142	00459	0019971022	2	0021.2	0003.0	0010.0	0000.0	0000.0	3	1.5
00142	00460	0019971022	2	0015.9	0003.0	0010.0	0000.0	0000.0	3	2.0
00142	00461	0019971022	1	0016.1	0002.5	0010.0	0000.0	0000.0	2	2.0
00142	00464	0019971022	5	0005.0	0006.0	0007.0	0000.0	0000.0	4	2.0
00142	00467	0019971022	2	0018.7	0003.0	0010.0	0000.0	0000.0	3	1.5
00142	00469	0019971022	2	0018.4	0003.0	0010.0	0000.0	0000.0	3	3.0
00142	00472	0019971022	2	0017.1	0003.0	0010.0	0000.0	0000.0	3	2.5
00142	00473	0019971022	1	0014.6	0002.5	0010.0	0000.0	0000.0	2	2.0
00142	00475	0019971022	1	0016.3	0002.5	0010.0	0000.0	0000.0	2	1.5
00142	00476	0019971022	1	0013.2	0002.5	0010.0	0000.0	0000.0	1	2.0
00142	00478	0019971022	1	0013.6	0002.5	0010.0	0000.0	0000.0	1	2.0
00142	00480	0019971022	6	0014.5	0002.0	0007.0	0000.0	0000.0	4	2.0
00142	00481	0019971022	5	0005.0	0007.7	0007.0	0000.0	0000.0	4	2.0
00142	00483	0019971022	2	0022.7	0003.0	0010.0	0000.0	0000.0	3	2.5
00142	00484	0019971022	5	0005.0	0006.9	0007.0	0000.0	0000.0	4	2.0
00142	00486	0019971022	2	0021.4	0003.0	0010.0	0000.0	0000.0	3	2.0
00142	00488	0019971022	2	0018.8	0003.0	0010.0	0000.0	0000.0	3	3.0
00142	00490	0019971022	1	0016.0	0002.5	0010.0	0000.0	0000.0	3	1.5

APPENDIX 4. Feed Group Raw Data.

Herd number, test day, group number, herd feed code, reference feed code, quantity recommended, feed catalogue number.

00142	0019971022	1	0032199002	0032100001	0015.102	1
00142	0019971022	1	0034399001	0034300002	0010.000	3
00142	0019971022	1	0043299001	0043200002	0002.500	2
00142	0019971022	1	0051399001	0051300001	0011.837	1
00142	0019971022	1	0069999002	0069901044	0002.238	1
00142	0019971022	1	0079999012	0079901013	0000.250	3
00142	0019971022	2	0032199002	0032100001	0017.838	1
00142	0019971022	2	0034399001	0034300002	0010.000	3
00142	0019971022	2	0043299001	0043200002	0003.000	2
00142	0019971022	2	0051399001	0051300001	0007.750	1
00142	0019971022	2	0069999002	0069901044	0000.291	1
00142	0019971022	2	0079999012	0079901013	0000.150	3
00142	0019971022	3	0032199002	0032100001	0014.950	1
00142	0019971022	3	0034399001	0034300002	0008.000	3
00142	0019971022	3	0043299001	0043200002	0003.000	2
00142	0019971022	3	0051399001	0051300001	0010.924	1
00142	0019971022	3	0069999002	0069901044	0001.356	1
00142	0019971022	3	0079999012	0079901013	0000.250	3
00142	0019971022	5	0032199002	0032100001	0005.000	1
00142	0019971022	5	0034399001	0034300002	0007.000	3
00142	0019971022	5	0043299001	0043200002	0006.750	2
00142	0019971022	5	0051399001	0051300001	0000.000	1
00142	0019971022	5	0069999002	0069901044	0000.009	1
00142	0019971022	5	0079999001	0079901010	0000.200	3

APPENDIX 5. Feed Identification Raw Data.

Herd number, test day, herd feed code, reference feed code, long description, short description, comments, feed base: 1=as fed 2=dry matter, cut number, analysis estimate: 0=true 1=estimate.

00142	0019971022	0034399001	0034300002	ENS.MAIS MATURE	ENS.MAIS M NRC89(085)/SILO-MEUL	2	0	0
00142	0019971022	0043299001	0043200002	FOIN 26747NEE 2	197 267472 197EPI/SECHOIR	2	1	0
00142	0019971022	0051399001	0051300001	MAIS-GRAIN HUMIDE	95IS HUMID NRC89 (080)	1	0	0
00142	0019971022	0069999002	0069901044	SYNCHRO 4050 HM	SYNC 4050 COOP	1	0	0
00142	0019971022	0079999001	0079901010	P-7 TARIE CUBE	P-7 TARIE COOP	1	0	0
00142	0019971022	0079999012	0079901013	P-15 SEVIP	P-15 SEVIP COOP	1	0	0
01938	0019971016	0031199001	0031100002	ENS. TREFLE 2C PAS L	ENS LEG 2 DEBFLOR/SILO VERT.	2	2	1
01938	0019971016	0032199004	0032100003	ENS MELMIL LUZ	ENS MEL 3 MIEPI/SILO VERT.	2	1	0
01938	0019971016	0043199001	0043100003	FOIN GRAMINEE 3	FOIN GRA 3 MIEPI/SEC CHAMP	2	1	0
01938	0019971016	0043199002	0043100003	FOIN 1996	FOIN 1996 MIEPI/SEC CHAMP	2	1	0
01938	0019971016	0051199001	0051100001	MAIS CASSE	MAIS CASSE NRC89(077)	1	0	1
01938	0019971016	0052199001	0052100001	ORGE SECHE	ORGE SECHE NRC89 (019)	1	0	1
01938	0019971016	0069199002	0000000000	SUPP PUL 3-1	SUPP PUL 3	1	0	0
01938	0019971016	0079999001	0079901010	P-7 TARIE CUBE	P-7 TARIE COOP	1	0	0
01938	0019971016	0079999002	0079901016	MINERAL C-11	C-11 COOP	1	0	0
03280	0019971022	0032199003	0032100001	ENS MELANGE 1	ENS MEL 1 PREEPI/SILO VERT.	2	3	1
03280	0019971022	0043299001	0043200002	FOIN GRA 1C EST	FOIN EST97 FOIN 1C JUIN	2	1	1
03280	0019971022	0059999002	0059902119	SYNCHRO M10014	SYNC.10014 COOP M10014	1	0	0
03280	0019971022	0069999001	0069901011	SUPPL. PROFIL	PROFIL COOP	1	0	0
03280	0019971022	0079999003	0079901010	P-7 TARIE CUBE	P-7 TARIE COOP	1	0	0
03280	0019971022	0079999004	0079901032	LACTO-CUBE	LACTO-CUBE COOP	1	0	0
03280	0019971022	0083999001	0083901001	FORTIFIANT 6-2	FORT. 6-2 COOP (POUDRE)	1	0	0
03336	0019971014	0033199001	0033100002	ENS 06-96 GRAMINEE	JUIN 96 2 JUIN 96	2	1	0

APPENDIX 6. Feed Analysis Raw Data.

Herd number, test day, pH, dry matter, ADF, NDF, NEL, NSC, added fat, total fat, crude protein, undegradable intake protein.

00142	0019971022	0.0	45	30.0	44.3	1.46	23.7	00.0	03.3	0020.5	15
00142	0019971022	0.0	35	28.0	54.0	1.57	28.1	00.0	03.0	0009.7	25
00142	0019971022	0.0	88	35.5	55.0	1.32	19.5	00.0	03.6	0014.5	34
00142	0019971022	0.0	70	02.3	06.8	1.53	51.4	00.0	03.2	0007.5	45
00142	0019971022	0.0	88	06.4	17.0	1.47	14.9	00.0	02.5	0040.0	50
00142	0019971022	0.0	98	00.0	00.0	0.00	00.0	00.0	00.0	0000.0	00
00142	0019971022	0.0	98	00.0	00.0	0.09	00.0	00.0	00.0	0000.5	50
01138	0019971020	0.0	26	32.0	57.6	1.42	17.3	00.0	03.6	0015.0	28
01138	0019971020	0.0	39	34.0	47.0	1.34	21.8	00.0	03.0	0019.6	18
01138	0019971020	0.0	29	33.0	61.0	1.46	21.8	00.0	03.0	0009.0	25
01138	0019971020	0.0	88	35.0	61.1	1.33	19.3	00.0	03.2	0010.0	33
01138	0019971020	0.0	89	02.6	07.8	1.78	63.1	00.0	03.7	0008.7	58
01138	0019971020	0.0	88	10.1	24.5	1.62	38.8	00.0	02.0	0016.0	34
01138	0019971020	0.0	88	05.3	08.8	1.77	24.8	00.0	00.9	0048.5	28
01138	0019971020	0.0	88	09.5	15.2	2.20	06.7	14.0	14.0	0042.5	60
01138	0019971020	0.0	95	00.0	00.0	0.00	00.0	00.0	00.0	0000.0	00
01138	0019971020	0.0	98	00.0	00.0	0.00	00.0	00.0	00.0	0000.0	00
01138	0019971020	0.0	98	00.0	00.0	0.00	00.0	00.0	00.0	0000.0	00
01938	0019971016	0.0	35	34.0	43.1	1.34	27.6	00.0	03.0	0018.0	18
01938	0019971016	0.0	44	38.8	61.7	1.21	15.6	00.0	02.6	0013.0	26
01938	0019971016	0.0	88	39.2	68.5	1.20	10.7	00.0	03.2	0010.3	40
01938	0019971016	0.0	88	37.0	63.4	1.27	14.7	00.0	03.2	0012.0	37
01938	0019971016	0.0	89	02.7	08.0	1.69	67.0	00.0	03.8	0008.9	56
01938	0019971016	0.0	88	06.2	16.7	1.71	53.7	00.0	01.8	0011.9	28

APPENDIX 7. Determining Feed Codes and Ration Calculation.

```
'*********************************************************************
' Program: DetermineFeedCodesAndRationMUNAccess
' Purpose:
' Author:  Diederik Pietersma
' Date:    19-Mar-1999
'*********************************************************************

Option Explicit
Option Base 1

Private mstrTheDataBase As String
Private mstrTheTable As String
Private mlngHerdID As Long
Private mlngCowID As Long


'*********************************************************************
Private Sub Form_Load()

mstrTheDataBase = "mun.mdb"
mstrTheTable = "tblAllFields"

End Sub

'*********************************************************************
Private Sub cmdDetermineFeedCodes_Click()

Dim dbs As Database
Dim rstAllFields As Recordset
Dim rstPrevForage As Recordset
Dim rstTemp As Recordset
Dim qdfTemp As QueryDef
Dim strSQLStatement As String
Dim blnNoPrevTestDay As Boolean
Dim blnCurrentTDIsFirstTDInLactation As Boolean
Dim intDIMPrevTestDate As Integer
Dim intDIMCurrentTestDate As Integer

Open App.Path + "\FlagsNoFeedCodes.txt" For Output As #1

Set dbs = OpenDatabase(App.Path + "\" + mstrTheDataBase)

Set rstAllFields = dbs.OpenRecordset(mstrTheTable)
Set rstPrevForage = dbs.OpenRecordset("tblPrevForageAmount")
```

```
rstPrevForage.Index = "PrimaryKey"

strSQLStatement = _
 "SELECT " + _
 "FeedCode1, " + _
 "QuantityRec, " + _
 "FeedCatNum, " + _
 "FeedCode " + _
 "From tblFeedGroup " + _
 "WHERE (((HerdID)=[TargetHerdID]) " + _
 "AND ((PrevTestDate)=[TargetPrevTestDate]) " + _
 "AND ((GroupNum)=[TargetFeedGroup]) " + _
 "AND ((FeedCode1)>=[TargetFeedTypeNumLower] " + _
 "AND (FeedCode1)<=[TargetFeedTypeNumUpper])) " + _
 "ORDER BY FeedCatNum"

Set qdfTemp = dbs.CreateQueryDef("", strSQLStatement)

With rstAllFields
 While Not .EOF 'Loop for each record.

  mlngHerdID = !HerdID
  mlngCowID = !CowID

  '*****************************************************************
  ' 0 Determine if CurrentTD is FirstTD in lactation

  blnNoPrevTestDay = False
  blnCurrentTDIsFirstTDInLactation = False

  ' 0.1 Check if PrevTD exists

  rstPrevForage.Seek "=", !HerdID, !CowID, !PrevTestDate
  If rstPrevForage.NoMatch Then
   'This means that there is no previous test day forage
   ' information for this cow. This could be due to an error in
   ' the dataset, the first TD in lactation of a Parity 1 cow,
   ' or the first TD in the herd of a bought cow.
   'When this is the case, use the CurrentFeedGroup and the
   ' general recommendation for that FeedGroup given with the
   ' milk recording data of the revious TD.
   blnNoPrevTestDay = True
  End If

  ' 0.2 Check if DIM of CurrentTD < DIM of PrevTD
  'This procedure cannot be followed since DIM PrevTD is not
```

```
  ' available in tblCowTest.
  'intDIMPrevTestDate = rstPrevForage!Dim
  'rstPrevForage.Seek "=", !HerdID, !CowID, !TestDate
  'intDIMCurrentTestDate = rstPrevForage!Dim
  'If intDIMCurrentTestDate < intDIMPrevTestDate Then
  '  blnCurrentTDIsFirstTDInLactation = True
  'End If

' 0.2 Check if CurrentFeedGroup = 1 and PrevFeedGroup > 2
If !CurrentFeedGroup = 1 And !PrevFeedGroup > 2 Then
  blnCurrentTDIsFirstTDInLactation = True
End If

'Write flags.
.Edit
'Remove flags set during previous runs of the program.
!FlagMissingFeedCode = 0
!FlagGroupRationForageAndSupp = 0
!FlagGroupRationOnlySupp = 0
If blnNoPrevTestDay Or blnCurrentTDIsFirstTDInLactation Then
  !FlagGroupRationForageAndSupp = 1
End If
If (!HerdID = 2938 And !TestDate = #7/18/1997#) _
    Or (!HerdID = 7021 And !TestDate = #3/20/1997#) Then
  !FlagGroupRationOnlySupp = 1
End If
.Update

'If !CurrentFeedGroup = 1 And !PrevFeedGroup = 2 Then
'  Print #1, _
'    Format(!HerdID, "@@@@@@"); _
'    Format(!CowID, " @@@@@ "); _
'    Format(!TestDate, "Medium Date"); _
'    Format(!CurrentFeedGroup, " @"); _
'    Format(!PrevFeedGroup, " @")
'End If

'*****************************************************************
' 1) FeedCodes for forages.
' 1.1) First test after calving.
If blnNoPrevTestDay Or blnCurrentTDIsFirstTDInLactation Then
  'Use previous months recommendation for FeedGroup 1
  ' from tblFeedGroup, update Amount and FeedCode.
  qdfTemp.Parameters("TargetHerdID") = !HerdID
  qdfTemp.Parameters("TargetPrevTestDate") = !PrevTestDate
  qdfTemp.Parameters("TargetFeedGroup") = !CurrentFeedGroup
```

```
qdfTemp.Parameters("TargetFeedTypeNumLower") = 1
qdfTemp.Parameters("TargetFeedTypeNumUpper") = 4
'Open Recordset.
Set rstTemp = qdfTemp.OpenRecordset()
.Edit
!PrevForage1 = 0
!PrevFor1FC = 0
If Not rstTemp.EOF Then
 If rstTemp!FeedCatNum = 1 Then
  !PrevForage1 = rstTemp!QuantityRec
  !PrevFor1FC = rstTemp!FeedCode
  rstTemp.MoveNext
 End If
End If
!PrevForage2 = 0
!PrevFor2FC = 0
If Not rstTemp.EOF Then
 If rstTemp!FeedCatNum = 2 Then
  !PrevForage2 = rstTemp!QuantityRec
  !PrevFor2FC = rstTemp!FeedCode
  rstTemp.MoveNext
 End If
End If
!PrevForage3 = 0
!PrevFor3FC = 0
If Not rstTemp.EOF Then
 If rstTemp!FeedCatNum = 3 Then
  !PrevForage3 = rstTemp!QuantityRec
  !PrevFor3FC = rstTemp!FeedCode
  rstTemp.MoveNext
 End If
End If
!PrevForage4 = 0
!PrevFor4FC = 0
If Not rstTemp.EOF Then
 If rstTemp!FeedCatNum = 4 Then
  !PrevForage4 = rstTemp!QuantityRec
  !PrevFor4FC = rstTemp!FeedCode
  rstTemp.MoveNext
 End If
End If
!PrevForage5 = 0
!PrevFor5FC = 0
If Not rstTemp.EOF Then
 If rstTemp!FeedCatNum = 5 Then
  !PrevForage5 = rstTemp!QuantityRec
```

```
      !PrevFor5FC = rstTemp!FeedCode
      rstTemp.MoveNext
     End If
    End If
    .Update
    rstTemp.Close

' 1.2) Second or later tests after calving.
Else
    ' If the PrevForage(i) amount > 0 then get the FeedCode.
    qdfTemp.Parameters("TargetHerdID") = !HerdID
    qdfTemp.Parameters("TargetPrevTestDate") = !PrevTestDate
    qdfTemp.Parameters("TargetFeedGroup") = !PrevFeedGroup
    qdfTemp.Parameters("TargetFeedTypeNumLower") = 1
    qdfTemp.Parameters("TargetFeedTypeNumUpper") = 4
    'Open Recordset.
    Set rstTemp = qdfTemp.OpenRecordset()
    .Edit
    !PrevFor1FC = 0
    If Not rstTemp.EOF Then
     If rstTemp!FeedCatNum = 1 Then
      !PrevFor1FC = rstTemp!FeedCode
      rstTemp.MoveNext
     End If
    End If
    If !PrevForage1 > 0 And !PrevFor1FC = 0 Then
     !FlagMissingFeedCode = 1
     Call PrintNoFeedCodeFlag(!HerdID, !CowID, !TestDate, _
      !CurrentFeedGroup, !PrevTestDate, !PrevFeedGroup, "For1")
    End If
    !PrevFor2FC = 0
    If Not rstTemp.EOF Then
     If rstTemp!FeedCatNum = 2 Then
      !PrevFor2FC = rstTemp!FeedCode
      rstTemp.MoveNext
     End If
    End If
    If !PrevForage2 > 0 And !PrevFor2FC = 0 Then
     !FlagMissingFeedCode = 1
     Call PrintNoFeedCodeFlag(!HerdID, !CowID, !TestDate, _
      !CurrentFeedGroup, !PrevTestDate, !PrevFeedGroup, "For2")
    End If
    !PrevFor3FC = 0
    If Not rstTemp.EOF Then
     If rstTemp!FeedCatNum = 3 Then
      !PrevFor3FC = rstTemp!FeedCode
```

```
    rstTemp.MoveNext
   End If
  End If
  If !PrevForage3 > 0 And !PrevFor3FC = 0 Then
   !FlagMissingFeedCode = 1
   Call PrintNoFeedCodeFlag(!HerdID, !CowID, !TestDate, _
    !CurrentFeedGroup, !PrevTestDate, !PrevFeedGroup, "For3")
  End If
  !PrevFor4FC = 0
  If Not rstTemp.EOF Then
   If rstTemp!FeedCatNum = 4 Then
    !PrevFor4FC = rstTemp!FeedCode
    rstTemp.MoveNext
   End If
  End If
  If !PrevForage4 > 0 And !PrevFor4FC = 0 Then
   !FlagMissingFeedCode = 1
   Call PrintNoFeedCodeFlag(!HerdID, !CowID, !TestDate, _
    !CurrentFeedGroup, !PrevTestDate, !PrevFeedGroup, "For4")
  End If
  !PrevFor5FC = 0
  If Not rstTemp.EOF Then
   If rstTemp!FeedCatNum = 5 Then
    !PrevFor5FC = rstTemp!FeedCode
    rstTemp.MoveNext
   End If
  End If
  If !PrevForage5 > 0 And !PrevFor5FC = 0 Then
   !FlagMissingFeedCode = 1
   Call PrintNoFeedCodeFlag(!HerdID, !CowID, !TestDate, _
    !CurrentFeedGroup, !PrevTestDate, !PrevFeedGroup, "For5")
  End If
  .Update
  rstTemp.Close
 End If
 '*****************************************************************
 ' 2) FeedCodes for SuppEnergy.
 ' 2.1) First test after calving.
 If blnNoPrevTestDay Or blnCurrentTDIsFirstTDInLactation _
    Or (!HerdID = 2938 And !TestDate = #7/18/1997#) _
    Or (!HerdID = 7021 And !TestDate = #3/20/1997#) Then
  'Daniel Lefebvre: Use group recommendation for
  ' Herd 2938 18-Jul-97 and Herd 7021 20-Mar-97 because
  ' the energy, protein, and minvit data is missing.
  'Use previous months recommendation for FeedGroup 1
  ' from tblFeedGroup, update Amount and FeedCode.
```

```
qdfTemp.Parameters("TargetHerdID") = !HerdID
qdfTemp.Parameters("TargetPrevTestDate") = !PrevTestDate
qdfTemp.Parameters("TargetFeedGroup") = !CurrentFeedGroup
qdfTemp.Parameters("TargetFeedTypeNumLower") = 5
qdfTemp.Parameters("TargetFeedTypeNumUpper") = 5
'Open Recordset.
Set rstTemp = qdfTemp.OpenRecordset()
.Edit
!SuppEn1 = 0
!SuppEn1FC = 0
If Not rstTemp.EOF Then
  If rstTemp!FeedCatNum = 1 Then
    !SuppEn1 = rstTemp!QuantityRec
    !SuppEn1FC = rstTemp!FeedCode
    rstTemp.MoveNext
  End If
End If
!SuppEn2 = 0
!SuppEn2FC = 0
If Not rstTemp.EOF Then
  If rstTemp!FeedCatNum = 2 Then
    !SuppEn2 = rstTemp!QuantityRec
    !SuppEn2FC = rstTemp!FeedCode
    rstTemp.MoveNext
  End If
End If
!SuppEn3 = 0
!SuppEn3FC = 0
If Not rstTemp.EOF Then
  If rstTemp!FeedCatNum = 3 Then
    !SuppEn3 = rstTemp!QuantityRec
    !SuppEn3FC = rstTemp!FeedCode
    rstTemp.MoveNext
  End If
End If
.Update
rstTemp.Close

' 2.2) Second or later tests after calving.
Else
  ' If the SuppEn(i) amount > 0 then get the FeedCode.
  qdfTemp.Parameters("TargetHerdID") = !HerdID
  qdfTemp.Parameters("TargetPrevTestDate") = !PrevTestDate
  qdfTemp.Parameters("TargetFeedGroup") = !PrevFeedGroup
  qdfTemp.Parameters("TargetFeedTypeNumLower") = 5
  qdfTemp.Parameters("TargetFeedTypeNumUpper") = 5
```

```
'Open Recordset.
Set rstTemp = qdfTemp.OpenRecordset()
.Edit
!SuppEn1FC = 0
If Not rstTemp.EOF Then
 If rstTemp!FeedCatNum = 1 Then
  !SuppEn1FC = rstTemp!FeedCode
  rstTemp.MoveNext
 End If
End If
If !SuppEn1 > 0 And !SuppEn1FC = 0 Then
 !FlagMissingFeedCode = 1
 Call PrintNoFeedCodeFlag(!HerdID, !CowID, !TestDate, _
  !CurrentFeedGroup, !PrevTestDate, !PrevFeedGroup, "En1")
End If
!SuppEn2FC = 0
If Not rstTemp.EOF Then
 If rstTemp!FeedCatNum = 2 Then
  !SuppEn2FC = rstTemp!FeedCode
  rstTemp.MoveNext
 End If
End If
If !SuppEn2 > 0 And !SuppEn2FC = 0 Then
 !FlagMissingFeedCode = 1
 Call PrintNoFeedCodeFlag(!HerdID, !CowID, !TestDate, _
  !CurrentFeedGroup, !PrevTestDate, !PrevFeedGroup, "En2")
End If
'Always check if an amount is listed for FeedCat3.
!SuppEn3 = 0
!SuppEn3FC = 0
If Not rstTemp.EOF Then
 If rstTemp!FeedCatNum = 3 Then
  !SuppEn3 = rstTemp!QuantityRec
  !SuppEn3FC = rstTemp!FeedCode
 End If
End If
.Update
rstTemp.Close
End If
'*****************************************************************
' 3) FeedCodes for SuppProtein.
' 3.1) First test after calving.
If blnNoPrevTestDay Or blnCurrentTDIsFirstTDInLactation _
  Or (!HerdID = 2938 And !TestDate = #7/18/1997#) _
  Or (!HerdID = 7021 And !TestDate = #3/20/1997#) Then
 'Daniel Lefebvre: Use group recommendation for
```

```
' Herd 2938 18-Jul-97 and Herd 7021 20-Mar-97 because
' the energy, protein, and minvit data is missing.
'Use previous months recommendation for FeedGroup 1
' from tblFeedGroup, update Amount and FeedCode.
qdfTemp.Parameters("TargetHerdID") = !HerdID
qdfTemp.Parameters("TargetPrevTestDate") = !PrevTestDate
qdfTemp.Parameters("TargetFeedGroup") = !CurrentFeedGroup
qdfTemp.Parameters("TargetFeedTypeNumLower") = 6
qdfTemp.Parameters("TargetFeedTypeNumUpper") = 6
'Open Recordset.
Set rstTemp = qdfTemp.OpenRecordset()
.Edit
!SuppProt1 = 0
!SuppProt1FC = 0
If Not rstTemp.EOF Then
 If rstTemp!FeedCatNum = 1 Then
  !SuppProt1 = rstTemp!QuantityRec
  !SuppProt1FC = rstTemp!FeedCode
  rstTemp.MoveNext
 End If
End If
!SuppProt2 = 0
!SuppProt2FC = 0
If Not rstTemp.EOF Then
 If rstTemp!FeedCatNum = 2 Then
  !SuppProt2 = rstTemp!QuantityRec
  !SuppProt2FC = rstTemp!FeedCode
  rstTemp.MoveNext
 End If
End If
!SuppProt3 = 0
!SuppProt3FC = 0
If Not rstTemp.EOF Then
 If rstTemp!FeedCatNum = 3 Then
  !SuppProt3 = rstTemp!QuantityRec
  !SuppProt3FC = rstTemp!FeedCode
  rstTemp.MoveNext
 End If
End If
.Update
rstTemp.Close

' 3.2) Second or later tests after calving.
Else
 ' If the SuppProt(i) amount > 0 then get the FeedCode.
 qdfTemp.Parameters("TargetHerdID") = !HerdID
```

```
qdfTemp.Parameters("TargetPrevTestDate") = !PrevTestDate
qdfTemp.Parameters("TargetFeedGroup") = !PrevFeedGroup
qdfTemp.Parameters("TargetFeedTypeNumLower") = 6
qdfTemp.Parameters("TargetFeedTypeNumUpper") = 6
'Open Recordset.
Set rstTemp = qdfTemp.OpenRecordset()
.Edit
!SuppProt1FC = 0
If Not rstTemp.EOF Then
 If rstTemp!FeedCatNum = 1 Then
  !SuppProt1FC = rstTemp!FeedCode
  rstTemp.MoveNext
 End If
End If
If !SuppProt1 > 0 And !SuppProt1FC = 0 Then
 !FlagMissingFeedCode = 1
 Call PrintNoFeedCodeFlag(!HerdID, !CowID, !TestDate, _
  !CurrentFeedGroup, !PrevTestDate, !PrevFeedGroup, "Prot1")
End If
!SuppProt2FC = 0
If Not rstTemp.EOF Then
 If rstTemp!FeedCatNum = 2 Then
  !SuppProt2FC = rstTemp!FeedCode
  rstTemp.MoveNext
 End If
End If
If !SuppProt2 > 0 And !SuppProt2FC = 0 Then
 !FlagMissingFeedCode = 1
 Call PrintNoFeedCodeFlag(!HerdID, !CowID, !TestDate, _
  !CurrentFeedGroup, !PrevTestDate, !PrevFeedGroup, "Prot2")
End If
'Always check if an amount is listed for FeedCat3.
!SuppProt3 = 0
!SuppProt3FC = 0
If Not rstTemp.EOF Then
 If rstTemp!FeedCatNum = 3 Then
  !SuppProt3 = rstTemp!QuantityRec
  !SuppProt3FC = rstTemp!FeedCode
 End If
End If
.Update
rstTemp.Close
End If
'*****************************************************************
' 4) FeedCodes for SuppMinVit.
' 4.1) First test after calving.
```

```
If blnNoPrevTestDay Or blnCurrentTDIsFirstTDInLactation _
  Or (!HerdID = 2938 And !TestDate = #7/18/1997#) _
  Or (!HerdID = 7021 And !TestDate = #3/20/1997#) Then
 'Daniel Lefebvre: Use group recommendation for
 ' Herd 2938 18-Jul-97 and Herd 7021 20-Mar-97 because
 ' the energy, protein, and minvit data is missing.
 'Use previous months recommendation for FeedGroup 1
 ' from tblFeedGroup, update Amount and FeedCode.
 qdfTemp.Parameters("TargetHerdID") = !HerdID
 qdfTemp.Parameters("TargetPrevTestDate") = !PrevTestDate
 qdfTemp.Parameters("TargetFeedGroup") = !CurrentFeedGroup
 qdfTemp.Parameters("TargetFeedTypeNumLower") = 7
 qdfTemp.Parameters("TargetFeedTypeNumUpper") = 7
 'Open Recordset.
 Set rstTemp = qdfTemp.OpenRecordset()
 .Edit
 !SuppMinVit1 = 0
 !SuppMinVit1FC = 0
 If Not rstTemp.EOF Then
  If rstTemp!FeedCatNum = 1 Then
   !SuppMinVit1 = rstTemp!QuantityRec
   !SuppMinVit1FC = rstTemp!FeedCode
   rstTemp.MoveNext
  End If
 End If
 !SuppMinVit2 = 0
 !SuppMinVit2FC = 0
 If Not rstTemp.EOF Then
  If rstTemp!FeedCatNum = 2 Then
   !SuppMinVit2 = rstTemp!QuantityRec
   !SuppMinVit2FC = rstTemp!FeedCode
   rstTemp.MoveNext
  End If
 End If
 !SuppMinVit3 = 0
 !SuppMinVit3FC = 0
 If Not rstTemp.EOF Then
  If rstTemp!FeedCatNum = 3 Then
   !SuppMinVit3 = rstTemp!QuantityRec
   !SuppMinVit3FC = rstTemp!FeedCode
   rstTemp.MoveNext
  End If
 End If
 .Update
 rstTemp.Close
```

```
    ' 4.2) Second or later tests after calving.
    Else
     ' If the SuppMinVit(i) amount > 0 then get the FeedCode.
     qdfTemp.Parameters("TargetHerdID") = !HerdID
     qdfTemp.Parameters("TargetPrevTestDate") = !PrevTestDate
     qdfTemp.Parameters("TargetFeedGroup") = !PrevFeedGroup
     qdfTemp.Parameters("TargetFeedTypeNumLower") = 7
     qdfTemp.Parameters("TargetFeedTypeNumUpper") = 7
     'Open Recordset.
     Set rstTemp = qdfTemp.OpenRecordset()
     .Edit
     !SuppMinVit1FC = 0
     If Not rstTemp.EOF Then
      If rstTemp!FeedCatNum = 1 Then
       !SuppMinVit1FC = rstTemp!FeedCode
       rstTemp.MoveNext
      End If
     End If
     !SuppMinVit2FC = 0
     If Not rstTemp.EOF Then
      If rstTemp!FeedCatNum = 2 Then
       !SuppMinVit2FC = rstTemp!FeedCode
       rstTemp.MoveNext
      End If
     End If
     'Always check if an amount is listed for FeedCat3.
     !SuppMinVit3 = 0
     !SuppMinVit3FC = 0
     If Not rstTemp.EOF Then
      If rstTemp!FeedCatNum = 3 Then
       !SuppMinVit3 = rstTemp!QuantityRec
       !SuppMinVit3FC = rstTemp!FeedCode
      End If
     End If
     .Update
     rstTemp.Close
    End If
    '*****************************************************************
    .MoveNext
  Wend 'rstAllFieldsInHerd.EOF loop.
End With

rstAllFields.Close
dbs.Close

Text1.Text = "Done"
```

```
End Sub

'**********************************************************************
Private Sub cmdCalculateRation_Click()

Dim dbs As Database
Dim rstAllFields As Recordset
Dim rstTemp As Recordset
Dim qdfTemp As QueryDef
Dim strSQLStatement As String
Dim RationIngr() As Single
Dim intIngrIdx As Integer
Dim dblCorrBase As Double
Dim dblTotalKgDM As Double
Dim dblTotalADF As Double
Dim dblTotalNDF As Double
Dim dblTotalNEL As Double
Dim dblTotalNSC As Double
Dim dblTotalAddedFat As Double
Dim dblTotalTotalFat As Double
Dim dblTotalCP As Double
Dim dblTotalUIP As Double
Dim dblSuppTotalDM As Double
Dim dblSuppTotalNDF As Double
Dim dblEstDMI As Double
Dim lngHerdID As Long
Dim lngCowID As Long

Dim I As Integer

Text1.Text = ""

Open App.Path + "\FlagsNoSupplements.txt" For Output As #1

Set dbs = OpenDatabase(App.Path + "\" + mstrTheDataBase)

Set rstAllFields = dbs.OpenRecordset(mstrTheTable)

strSQLStatement = _
 "SELECT " + _
 "Base, " + _
 "DM, " + _
 "ADF, " + _
 "NDF, " + _
 "NEL, " + _
```

```
  "NSC, " + _
  "AddedFatpercentDM, " + _
  "TotFatpercentDM, " + _
  "CP, " + _
  "UIPpercentCP " + _
  "From tblFeedAnalysisPATLQ " + _
  "WHERE (((HerdID)=[TargetHerdID]) " + _
  "AND ((PrevTestDate)=[TargetPrevTestDate]) " + _
  "AND ((FeedCode)=[TargetFeedCode])) "

Set qdfTemp = dbs.CreateQueryDef("", strSQLStatement)


With rstAllFields
  While Not .EOF 'Loop for each record.
    lngHerdID = !HerdID
    lngCowID = !CowID
    'Write flags to file.
    If !CurrentFeedGroup = 1 And _
          (!PrevFeedGroup >= 3 And !PrevFeedGroup <= 4) Then
      Print #1, Format(!HerdID, "@@@@@@"); Format(!CowID, " @@@@@ "); _
      Format(!TestDate, "Medium Date"); _
      Format(!CurrentFeedGroup, " @"); _
      Format(!PrevFeedGroup, " @"); " CurrentFG=1 PrevFG = 3 or 4"
    End If
    If !SuppEn1 = 0 And !SuppEn2 = 0 And !SuppEn3 = 0 _
       And !SuppProt1 = 0 And !SuppProt2 = 0 And !SuppProt3 = 0 _
       And (!CurrentFeedGroup >= 1 And !CurrentFeedGroup < 5) Then
      Print #1, Format(!HerdID, "@@@@@@"); Format(!CowID, " @@@@@ "); _
      Format(!TestDate, "Medium Date"); _
      Format(!CurrentFeedGroup, " @"); _
      Format(!PrevFeedGroup, " @"); _
      Format(!Milk, " @@@@@"); _
      Format(!Dim, " @@@"); _
      " No energy and protein supplements"
    End If
    'Skip cows for which one or more FeedCodes are missing.
    If Not !FlagMissingFeedCode Then
    intIngrIdx = 0

    '*****************************************************************
    ' 1) Forage 1 through 5.
    ' If the PrevForage(i) amount > 0 then add it to Ration array.
    If !PrevForage1 > 0 Then
      qdfTemp.Parameters("TargetHerdID") = !HerdID
      qdfTemp.Parameters("TargetPrevTestDate") = !PrevTestDate
```

```
    qdfTemp.Parameters("TargetFeedCode") = !PrevFor1FC
    Set rstTemp = qdfTemp.OpenRecordset()
    intIngrIdx = intIngrIdx + 1
    ReDim Preserve RationIngr(12, intIngrIdx)
    RationIngr(1, intIngrIdx) = !PrevForage1
    RationIngr(2, intIngrIdx) = rstTemp!Base
    RationIngr(3, intIngrIdx) = rstTemp!DM
    RationIngr(4, intIngrIdx) = rstTemp!ADF
    RationIngr(5, intIngrIdx) = rstTemp!NDF
    RationIngr(6, intIngrIdx) = rstTemp!NEL
    RationIngr(7, intIngrIdx) = rstTemp!NSC
    RationIngr(8, intIngrIdx) = rstTemp!AddedFatpercentDM
    RationIngr(9, intIngrIdx) = rstTemp!TotFatpercentDM
    RationIngr(10, intIngrIdx) = rstTemp!CP
    RationIngr(11, intIngrIdx) = rstTemp!UIPpercentCP
    RationIngr(12, intIngrIdx) = 0 'Forage.
    rstTemp.Close
  End If
  If !PrevForage2 > 0 Then
    qdfTemp.Parameters("TargetHerdID") = !HerdID
    qdfTemp.Parameters("TargetPrevTestDate") = !PrevTestDate
    qdfTemp.Parameters("TargetFeedCode") = !PrevFor2FC
    Set rstTemp = qdfTemp.OpenRecordset()
    intIngrIdx = intIngrIdx + 1
    ReDim Preserve RationIngr(12, intIngrIdx)
    RationIngr(1, intIngrIdx) = !PrevForage2
    RationIngr(2, intIngrIdx) = rstTemp!Base
    RationIngr(3, intIngrIdx) = rstTemp!DM
    RationIngr(4, intIngrIdx) = rstTemp!ADF
    RationIngr(5, intIngrIdx) = rstTemp!NDF
    RationIngr(6, intIngrIdx) = rstTemp!NEL
    RationIngr(7, intIngrIdx) = rstTemp!NSC
    RationIngr(8, intIngrIdx) = rstTemp!AddedFatpercentDM
    RationIngr(9, intIngrIdx) = rstTemp!TotFatpercentDM
    RationIngr(10, intIngrIdx) = rstTemp!CP
    RationIngr(11, intIngrIdx) = rstTemp!UIPpercentCP
    RationIngr(12, intIngrIdx) = 0 'Forage.
    rstTemp.Close
  End If
  If !PrevForage3 > 0 Then
    qdfTemp.Parameters("TargetHerdID") = !HerdID
    qdfTemp.Parameters("TargetPrevTestDate") = !PrevTestDate
    qdfTemp.Parameters("TargetFeedCode") = !PrevFor3FC
    Set rstTemp = qdfTemp.OpenRecordset()
    intIngrIdx = intIngrIdx + 1
    ReDim Preserve RationIngr(12, intIngrIdx)
```

```
  RationIngr(1, intIngrIdx) = !PrevForage3
  RationIngr(2, intIngrIdx) = rstTemp!Base
  RationIngr(3, intIngrIdx) = rstTemp!DM
  RationIngr(4, intIngrIdx) = rstTemp!ADF
  RationIngr(5, intIngrIdx) = rstTemp!NDF
  RationIngr(6, intIngrIdx) = rstTemp!NEL
  RationIngr(7, intIngrIdx) = rstTemp!NSC
  RationIngr(8, intIngrIdx) = rstTemp!AddedFatpercentDM
  RationIngr(9, intIngrIdx) = rstTemp!TotFatpercentDM
  RationIngr(10, intIngrIdx) = rstTemp!CP
  RationIngr(11, intIngrIdx) = rstTemp!UIPpercentCP
  RationIngr(12, intIngrIdx) = 0 'Forage.
  rstTemp.Close
 End If
 If !PrevForage4 > 0 Then
  qdfTemp.Parameters("TargetHerdID") = !HerdID
  qdfTemp.Parameters("TargetPrevTestDate") = !PrevTestDate
  qdfTemp.Parameters("TargetFeedCode") = !PrevFor4FC
  Set rstTemp = qdfTemp.OpenRecordset()
  intIngrIdx = intIngrIdx + 1
  ReDim Preserve RationIngr(12, intIngrIdx)
  RationIngr(1, intIngrIdx) = !PrevForage4
  RationIngr(2, intIngrIdx) = rstTemp!Base
  RationIngr(3, intIngrIdx) = rstTemp!DM
  RationIngr(4, intIngrIdx) = rstTemp!ADF
  RationIngr(5, intIngrIdx) = rstTemp!NDF
  RationIngr(6, intIngrIdx) = rstTemp!NEL
  RationIngr(7, intIngrIdx) = rstTemp!NSC
  RationIngr(8, intIngrIdx) = rstTemp!AddedFatpercentDM
  RationIngr(9, intIngrIdx) = rstTemp!TotFatpercentDM
  RationIngr(10, intIngrIdx) = rstTemp!CP
  RationIngr(11, intIngrIdx) = rstTemp!UIPpercentCP
  RationIngr(12, intIngrIdx) = 0 'Forage.
  rstTemp.Close
 End If
 If !PrevForage5 > 0 Then
  qdfTemp.Parameters("TargetHerdID") = !HerdID
  qdfTemp.Parameters("TargetPrevTestDate") = !PrevTestDate
  qdfTemp.Parameters("TargetFeedCode") = !PrevFor5FC
  Set rstTemp = qdfTemp.OpenRecordset()
  intIngrIdx = intIngrIdx + 1
  ReDim Preserve RationIngr(12, intIngrIdx)
  RationIngr(1, intIngrIdx) = !PrevForage5
  RationIngr(2, intIngrIdx) = rstTemp!Base
  RationIngr(3, intIngrIdx) = rstTemp!DM
  RationIngr(4, intIngrIdx) = rstTemp!ADF
```

```
  RationIngr(5, intIngrIdx) = rstTemp!NDF
  RationIngr(6, intIngrIdx) = rstTemp!NEL
  RationIngr(7, intIngrIdx) = rstTemp!NSC
  RationIngr(8, intIngrIdx) = rstTemp!AddedFatpercentDM
  RationIngr(9, intIngrIdx) = rstTemp!TotFatpercentDM
  RationIngr(10, intIngrIdx) = rstTemp!CP
  RationIngr(11, intIngrIdx) = rstTemp!UIPpercentCP
  RationIngr(12, intIngrIdx) = 0 'Forage.
  rstTemp.Close
 End If
 '*****************************************************************
 ' 2) SuppEnergy 1 through 3.
 ' If the SuppEn(i) amount > 0 then add it to Ration array.
 If !SuppEn1 > 0 Then
  qdfTemp.Parameters("TargetHerdID") = !HerdID
  qdfTemp.Parameters("TargetPrevTestDate") = !PrevTestDate
  qdfTemp.Parameters("TargetFeedCode") = !SuppEn1FC
  Set rstTemp = qdfTemp.OpenRecordset()
  intIngrIdx = intIngrIdx + 1
  ReDim Preserve RationIngr(12, intIngrIdx)
  RationIngr(1, intIngrIdx) = !SuppEn1
  RationIngr(2, intIngrIdx) = rstTemp!Base
  RationIngr(3, intIngrIdx) = rstTemp!DM
  RationIngr(4, intIngrIdx) = rstTemp!ADF
  RationIngr(5, intIngrIdx) = rstTemp!NDF
  RationIngr(6, intIngrIdx) = rstTemp!NEL
  RationIngr(7, intIngrIdx) = rstTemp!NSC
  RationIngr(8, intIngrIdx) = rstTemp!AddedFatpercentDM
  RationIngr(9, intIngrIdx) = rstTemp!TotFatpercentDM
  RationIngr(10, intIngrIdx) = rstTemp!CP
  RationIngr(11, intIngrIdx) = rstTemp!UIPpercentCP
  RationIngr(12, intIngrIdx) = 1 'Supplement.
  rstTemp.Close
 End If
 If !SuppEn2 > 0 Then
  qdfTemp.Parameters("TargetHerdID") = !HerdID
  qdfTemp.Parameters("TargetPrevTestDate") = !PrevTestDate
  qdfTemp.Parameters("TargetFeedCode") = !SuppEn2FC
  Set rstTemp = qdfTemp.OpenRecordset()
  intIngrIdx = intIngrIdx + 1
  ReDim Preserve RationIngr(12, intIngrIdx)
  RationIngr(1, intIngrIdx) = !SuppEn2
  RationIngr(2, intIngrIdx) = rstTemp!Base
  RationIngr(3, intIngrIdx) = rstTemp!DM
  RationIngr(4, intIngrIdx) = rstTemp!ADF
  RationIngr(5, intIngrIdx) = rstTemp!NDF
```

```
  RationIngr(6, intIngrIdx) = rstTemp!NEL
  RationIngr(7, intIngrIdx) = rstTemp!NSC
  RationIngr(8, intIngrIdx) = rstTemp!AddedFatpercentDM
  RationIngr(9, intIngrIdx) = rstTemp!TotFatpercentDM
  RationIngr(10, intIngrIdx) = rstTemp!CP
  RationIngr(11, intIngrIdx) = rstTemp!UIPpercentCP
  RationIngr(12, intIngrIdx) = 1 'Supplement.
  rstTemp.Close
 End If
 If !SuppEn3 > 0 Then
  qdfTemp.Parameters("TargetHerdID") = !HerdID
  qdfTemp.Parameters("TargetPrevTestDate") = !PrevTestDate
  qdfTemp.Parameters("TargetFeedCode") = !SuppEn3FC
  Set rstTemp = qdfTemp.OpenRecordset()
  intIngrIdx = intIngrIdx + 1
  ReDim Preserve RationIngr(12, intIngrIdx)
  RationIngr(1, intIngrIdx) = !SuppEn3
  RationIngr(2, intIngrIdx) = rstTemp!Base
  RationIngr(3, intIngrIdx) = rstTemp!DM
  RationIngr(4, intIngrIdx) = rstTemp!ADF
  RationIngr(5, intIngrIdx) = rstTemp!NDF
  RationIngr(6, intIngrIdx) = rstTemp!NEL
  RationIngr(7, intIngrIdx) = rstTemp!NSC
  RationIngr(8, intIngrIdx) = rstTemp!AddedFatpercentDM
  RationIngr(9, intIngrIdx) = rstTemp!TotFatpercentDM
  RationIngr(10, intIngrIdx) = rstTemp!CP
  RationIngr(11, intIngrIdx) = rstTemp!UIPpercentCP
  RationIngr(12, intIngrIdx) = 1 'Supplement.
  rstTemp.Close
 End If
 '*****************************************************************
 ' 3) SuppProt 1 through 3.
 ' If the SuppProt(i) amount > 0 then add it to Ration array.
 If !SuppProt1 > 0 Then
  qdfTemp.Parameters("TargetHerdID") = !HerdID
  qdfTemp.Parameters("TargetPrevTestDate") = !PrevTestDate
  qdfTemp.Parameters("TargetFeedCode") = !SuppProt1FC
  Set rstTemp = qdfTemp.OpenRecordset()
  intIngrIdx = intIngrIdx + 1
  ReDim Preserve RationIngr(12, intIngrIdx)
  RationIngr(1, intIngrIdx) = !SuppProt1
  RationIngr(2, intIngrIdx) = rstTemp!Base
  RationIngr(3, intIngrIdx) = rstTemp!DM
  RationIngr(4, intIngrIdx) = rstTemp!ADF
  RationIngr(5, intIngrIdx) = rstTemp!NDF
  RationIngr(6, intIngrIdx) = rstTemp!NEL
```

```
  RationIngr(7, intIngrIdx) = rstTemp!NSC
  RationIngr(8, intIngrIdx) = rstTemp!AddedFatpercentDM
  RationIngr(9, intIngrIdx) = rstTemp!TotFatpercentDM
  RationIngr(10, intIngrIdx) = rstTemp!CP
  RationIngr(11, intIngrIdx) = rstTemp!UIPpercentCP
  RationIngr(12, intIngrIdx) = 1 'Supplement.
  rstTemp.Close
 End If
 If !SuppProt2 > 0 Then
  qdfTemp.Parameters("TargetHerdID") = !HerdID
  qdfTemp.Parameters("TargetPrevTestDate") = !PrevTestDate
  qdfTemp.Parameters("TargetFeedCode") = !SuppProt2FC
  Set rstTemp = qdfTemp.OpenRecordset()
  intIngrIdx = intIngrIdx + 1
  ReDim Preserve RationIngr(12, intIngrIdx)
  RationIngr(1, intIngrIdx) = !SuppProt2
  RationIngr(2, intIngrIdx) = rstTemp!Base
  RationIngr(3, intIngrIdx) = rstTemp!DM
  RationIngr(4, intIngrIdx) = rstTemp!ADF
  RationIngr(5, intIngrIdx) = rstTemp!NDF
  RationIngr(6, intIngrIdx) = rstTemp!NEL
  RationIngr(7, intIngrIdx) = rstTemp!NSC
  RationIngr(8, intIngrIdx) = rstTemp!AddedFatpercentDM
  RationIngr(9, intIngrIdx) = rstTemp!TotFatpercentDM
  RationIngr(10, intIngrIdx) = rstTemp!CP
  RationIngr(11, intIngrIdx) = rstTemp!UIPpercentCP
  RationIngr(12, intIngrIdx) = 1 'Supplement.
  rstTemp.Close
 End If
 If !SuppProt3 > 0 Then
  qdfTemp.Parameters("TargetHerdID") = !HerdID
  qdfTemp.Parameters("TargetPrevTestDate") = !PrevTestDate
  qdfTemp.Parameters("TargetFeedCode") = !SuppProt3FC
  Set rstTemp = qdfTemp.OpenRecordset()
  intIngrIdx = intIngrIdx + 1
  ReDim Preserve RationIngr(12, intIngrIdx)
  RationIngr(1, intIngrIdx) = !SuppProt3
  RationIngr(2, intIngrIdx) = rstTemp!Base
  RationIngr(3, intIngrIdx) = rstTemp!DM
  RationIngr(4, intIngrIdx) = rstTemp!ADF
  RationIngr(5, intIngrIdx) = rstTemp!NDF
  RationIngr(6, intIngrIdx) = rstTemp!NEL
  RationIngr(7, intIngrIdx) = rstTemp!NSC
  RationIngr(8, intIngrIdx) = rstTemp!AddedFatpercentDM
  RationIngr(9, intIngrIdx) = rstTemp!TotFatpercentDM
  RationIngr(10, intIngrIdx) = rstTemp!CP
```

```
  RationIngr(11, intIngrIdx) = rstTemp!UIPpercentCP
  RationIngr(12, intIngrIdx) = 1 'Supplement.
  rstTemp.Close
End If
'*****************************************************************
' 4) SuppMinVit 1 through 3.
' Assume effect of MinVit on total kg DM negligable.
' MinVit have no ADF NEL CP etc.
'*****************************************************************
' 5) Calculate the total DM in the ration and the ration
'   composition per kg DM.
dblTotalKgDM = 0
dblTotalADF = 0
dblTotalNDF = 0
dblTotalNEL = 0
dblTotalNSC = 0
dblTotalAddedFat = 0
dblTotalTotalFat = 0
dblTotalCP = 0
dblTotalUIP = 0
dblSuppTotalDM = 0
dblSuppTotalNDF = 0
For I = 1 To intIngrIdx
 If RationIngr(2, I) = 1 Then 'As Fed.
  dblCorrBase = 100 / RationIngr(3, I)
 Else 'Base = 2 which means DM.
  dblCorrBase = 1
 End If
 'All Amounts in table are expressed in Kg As Fed.
 dblTotalKgDM = dblTotalKgDM _
   + RationIngr(1, I) * (RationIngr(3, I) / 100)
 'Amount fed of ingredient in KgDM * Analysis * CorrBase to
 ' adjust for analyses expressed per Kg As Fed.
 dblTotalADF = dblTotalADF _
   + RationIngr(1, I) * (RationIngr(3, I) / 100) _
   * dblCorrBase * (RationIngr(4, I) / 100)
 dblTotalNDF = dblTotalNDF _
   + RationIngr(1, I) * (RationIngr(3, I) / 100) _
   * dblCorrBase * (RationIngr(5, I) / 100)
 dblTotalNEL = dblTotalNEL _
   + RationIngr(1, I) * (RationIngr(3, I) / 100) _
   * dblCorrBase * RationIngr(6, I)
 dblTotalNSC = dblTotalNSC _
   + RationIngr(1, I) * (RationIngr(3, I) / 100) _
   * dblCorrBase * (RationIngr(7, I) / 100)
 dblTotalAddedFat = dblTotalAddedFat _
```

```
    + RationIngr(1, I) * (RationIngr(3, I) / 100) _
    * dblCorrBase * (RationIngr(8, I) / 100)
  dblTotalTotalFat = dblTotalTotalFat _
    + RationIngr(1, I) * (RationIngr(3, I) / 100) _
    * dblCorrBase * (RationIngr(9, I) / 100)
  dblTotalCP = dblTotalCP _
    + RationIngr(1, I) * (RationIngr(3, I) / 100) _
    * dblCorrBase * (RationIngr(10, I) / 100)
  'UIP is expressed as percentage of CP.
  dblTotalUIP = dblTotalUIP _
    + RationIngr(1, I) * (RationIngr(3, I) / 100) _
    * dblCorrBase * (RationIngr(10, I) / 100) _
    * (RationIngr(11, I) / 100)
  'Determine total kg DM and kg NDF fed as supplements.
  If RationIngr(12, I) = 1 Then
   dblSuppTotalDM = dblSuppTotalDM _
    + RationIngr(1, I) * (RationIngr(3, I) / 100)
   dblSuppTotalNDF = dblSuppTotalNDF _
    + RationIngr(1, I) * (RationIngr(3, I) / 100) _
    * dblCorrBase * (RationIngr(5, I) / 100)
  End If
 Next I
 .Edit
 !TotalDM = dblTotalKgDM
 !RationADF = 100 * dblTotalADF / dblTotalKgDM
 !RationNDF = 100 * dblTotalNDF / dblTotalKgDM
 !RationNEL = dblTotalNEL / dblTotalKgDM
 !RationNSC = 100 * dblTotalNSC / dblTotalKgDM
 !RationAddedFat = 100 * dblTotalAddedFat / dblTotalKgDM
 !RationTotalFat = 100 * dblTotalTotalFat / dblTotalKgDM
 !RationCP = 100 * dblTotalCP / dblTotalKgDM
 !RationUIPpercentCP = 100 * dblTotalUIP / dblTotalCP
 !PATLQForageTotalDM = dblTotalKgDM - dblSuppTotalDM
 !PATLQForageTotalNDF = dblTotalNDF - dblSuppTotalNDF
 !SuppTotalDM = dblSuppTotalDM
 !SuppTotalNDF = dblSuppTotalNDF
 '*****************************************************************
 'Calculate estimate of DMI.
 'Based on equation from Fox et al., 1992. J.Anim Sci. 70:3578.
 'EstDMI = (0.0185*BW + 0.305*4%FCM)*TempCorr*MudCorr
 'Assume TempCorr = 1 and MudCorr = 1
 If !CurrentBodyWeight > 0 Then
  dblEstDMI = (0.0185 * !CurrentBodyWeight _
        + 0.305 * (0.4 + 0.15 * !Fat) * !Milk) * 1
  !EstDMI = dblEstDMI
  !DiffTotalDMIEstDMI = dblTotalKgDM - dblEstDMI
```

```
    End If
    .Update
    End If
    .MoveNext
  Wend 'rstAllFieldsInHerd.EOF loop.
End With

rstAllFields.Close
dbs.Close

Text1.Text = "Done"


'*********************************************************************
Sub PrintNoFeedCodeFlag(ByRef HerdID As Long, ByRef CowID As Long, _
    ByRef TestDate As Date, ByRef CurrentFeedGroup As Integer, _
    ByRef PrevTestDate As Date, PrevFeedGroup As Integer, _
    ByRef Comment As String)

Print #1, _
Format(HerdID, "@@@@@@"); _
Format(CowID, " @@@@@ "); _
Format(TestDate, "Medium Date"); _
Format(CurrentFeedGroup, " @"); _
Format(PrevTestDate, "Medium Date"); _
Format(PrevFeedGroup, " @"); _
Format(Comment, " @@@@@@")

End Sub
```