Estimation of Milk Urea Level as a Potential Tool for Dairy Herd Management

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Introduction

Increased genetic potential for milk production has been associated with a decline in fertility of lactating diary cows. Strategies for meeting the nutritional requirements of high producing cows have necessarily changed in conjunction with genetic gains. Increasing protein concentration of the diet of lactating dairy cows can often increase milk production. However, efficiency of utilization of dietary protein for milk production decreased as more protein is offered. In an effort to sustain milk production during early lactation, dairy producers often increase nutrient density of dairy cows diets to compensate for suboptimal intakes. This situation may lead to protein intakes in excess of recommendation or requirement.

Protein is an expensive ingredient of dairy cattle feeds and thus, overfeeding of protein could be costly to producers. Although, high dietary protein stimulates milk production, increased protein has been found to be detrimental to reproductive performance of the animal (Guo *et al*., 2004). Conception and the establishment of pregnancy are an ordered progression of interrelated events involving all of the various tissues of the reproductive tract, follicular development resulting in ovulation, fertilization of the oocyte, embryo transport and development, maternal recognition and implantation. Ammonia, urea or some other toxic products of protein metabolism may intercede at one or more of these steps to impair reproductive efficiency (Jordan *et al*., 1983).

Another aspects regarding excess protein feeding in dairy cattle is a growing concern for the environment in the last decade. Overfeeding of protein contributes to environmental pollution and higher feed costs (Burgos *et al*., 2007). Vandehaar (1998) predicted that the energy loss from feeding an extra 2% of protein (a diet of 19% CP) would amount to 0.36 Mcal/day. Animal husbandry wastes can contribute to nitrogen (**N**) pollution of the environment as ammonia volatized to the air, nitrate leached to ground water and N that runs off to surface water. Therefore, it is important to reduce manure N output by improving N utilisation by the animal.

Now-a-days, urea and other Non-protein nitrogen (**NPN**) compound has been used extensively for the feeding of ruminants to fulfill their protein requirements, as the conventional protein sources are expensive. The principle behind this approach is to capitalize the ability of the rumen micro-organisms to convert NPN compounds to protein. Urea entering the rumen is rapidly hydrolysed to ammonia by bacterial urease, and the rumen ammonia concentration is therefore liable to rise considerably. For this ammonia to be efficiently incorporated in microbial protein, two conditions must be met. Firstly, the initial ammonia concentration must be below the optimum and secondly, the micro-organism must have a readily available source of energy for protein synthesis. Therefore, urea will not be used efficiently by the ruminants unless ruminal degradable protein (**RDP**) to satisfy the needs of its rumen micro-organisms. Efficiency of protein feeding is a function of nitrogen supply to the rumen as well the cow. Consequently, a system to monitor protein feeding must account for rumen as well as post ruminal supply and efficiency.

How to Monitor Protein Status of the Animals?

Two basic ways to monitor the protein status of the dairy animals are by a) monitoring intake parameter that is crude protein intake of individual animals and b) monitoring output parameter that is potentially useful is milk or blood urea concentration.

What is Milk Urea Nitrogen (MUN)?

Milk protein consists of two major protein fractions- true protein and NPN. True protein accounts for 95% of the nitrogen in milk and it consists of 80% casein and 20% whey. NPN makes up the remaining 5% of the total nitrogen in milk. NPN consists of approximately 30-35% milk urea, 25% creatinine/uric acid, 15% amino acids and 10-30% ammonia.

MUN as an Indicator of Protein Status of Dairy Animals

We need a tool to monitor protein nutrition status of lactating cows and that would be beneficial to reduce losses and maximize efficiency of nitrogen utilization. Suitable input parameters are not available because protein intake or some related trait is difficult to measure in practice, mainly because of the inaccuracies in predicting feed intake. An output parameter that is potentially useful is milk or blood urea concentration. These are mainly three sources of urea in milk; 1) end product of protein and 2) NPN digestion, 3) amino acid catabolism in mammary gland. Results from MUN/BUN measurements can provide valuable information to farmers on the nutritional status and health of their cows. The use of MUN as a fertility marker in dairy cows has received much attention.

Physiological Basis

Rumen ammonia nitrogen in excess of rumen requirement usually is the single largest contributor of urea in the blood; however, catabolism of body protein and deamination of excess dietary protein can contribute to the pool of urea nitrogen in the blood. Rumen ammonia concentration in excess of that utilized by rumen microbes is absorbed across the rumen wall into the portal blood. Rumen ammonia concentration and rumen pH are the two major factors which effect level of ammonia exchange with portal blood. Normal ruminal pH is 6.0 to 6.8 where ammonia exists mostly as

ammonium ion (NH_4^+) and exchange with the blood is uncharged ion (NH_3) through passive diffusion. Therefore, feed or feeding factors elevate rumen ammonia concentration (i.e.-high dietary soluble protein) or pH (i.e.-high roughage diets), increase transfer of ammonia to the blood.

Excess amino acids and peptides are deaminated in the liver, and N is converted to urea. Ammonia, because it is toxic to the animal, is rapidly converted to urea in the liver (Swenson and Reece, 1993). Normal blood ammonia (0.1 - 0.15 mg %) is low relative to rumen levels (10-50 mg %) due to the rapid detoxification of blood ammonia to urea in the liver. Estimates of the energy costs to synthesize and eliminate excess urea have been calculated to around 12 Kcal of DE per gram of urea synthesized (Van Soest, 1994). The urea enters the circulatory system through the hepatic sinuses, which drain into the hepatic vein (Guyton, 1982) and become part of the pool of blood urea N. The urea is filtered from the blood by the kidney and is excreted from the body in urine. Blood enters the kidneys through the renal artery (Guyton, 1982) and is filtered through the nephrons. This process concentrates the urea for excretion in the urine. Because of counter current flow and differences in membrane permeability in ascending and descending loops of Henle, a concentration gradient for the diffusion of urea into urine is created to remove urea from blood (Swenson and Reece. 1993). Blood flow through the kidney is constant within an animal, which ensures a constant urea filtration rate, regardless of urine volume. With a low volume of urine, urea concentration in the urine would be higher than with a higher volume of urine, but a similar amount of blood would be cleared of urea (Swenson and Reece. 1993). In addition, with high concentrations of urea in blood, more urea would be removed per minute compared with a low concentration of urea in the blood, but the total amount of blood cleared would remain similar. Therefore, urea excretion is proportional to blood urea concentration. Urea, because it is a small neutral molecule, readily diffuses across cellular membranes. As milk is secreted in the mammary gland, urea diffuses into and out of the mammary gland, equilibrating with urea in the blood. Because of this process, MUN equilibrates with and is proportional to blood urea N (Roseler *et al*., 1993). This process allows MUN to be an excellent predictor of blood urea N and Urinary N (Kohn *et al*., 1997).

Milk urea nitrogen (MUN)

I. corresponds to 2.5 to 3.0% of total milk N (De Peters and Cant, 1992)

II. is highly correlated (0.88 to 0.98) with blood urea nitrogen (**BUN**) and is closely related to dietary CP (Dhali, 2001; Roy *et al*., 2005)

III. does not require invasive sampling or restraint of the cow

IV. non-invasive, economical, rapid process

V. easily sampled and easy to perform.

VI. When milk samples are taken as a part of regular testing, sampling involves no extra labor, and it is cheaper than sampling and analyzing blood.

When a group of cows has a mean MUN concentration that is higher than the target MUN values at a given level of production, excess protein for the given level of production was probably consumed. A reformulation of the diet at that production level with a lower protein concentration could reduce feed costs. However, before diet reformulation, the specific cause of high MUN should be identified. High MUN can result from a number of nutritional factors, including but not limited to excess protein, inadequate energy, or excess RDP that decreases production and efficiency of N utilization. Close examination of the current diet helps to elucidate the cause of high MUN and leads to appropriate dietary changes to reduce MUN.

Advantages of Milk Urea Estimation over Blood Urea

While MUN is only indirectly related to ruminal N utilization, depending on the urea clearance rate of the kidneys (Kohn *et al*., 2002), milk is a much more practical fluid to obtain for wide-scale urea testing than urine or blood. As a result, MUN testing can be incorporated as one of the monthly milk tests. Because urea equilibrates within bodily fluids, MUN should be similar to PUN as an indicator of urea nitrogen status in dairy cows and is more conveniently monitored (Roy *et al*., 2005).

Low MUN

Conditions can exist where MUN levels may actually be low indicating a protein deficiency in the diet and potentially lost production. Low MUN levels suggest the cows' diet does not contain adequate available protein. When MUN levels are extremely low, production may be limited because of a protein deficient diet. Suspicious feeds should be analyzed for acid detergent insoluble nitrogen or bound protein by an animal nutrition laboratory.

MUN in Relation to Reproduction

Fertility is a major contributor to profitability of the dairy herd (Martin, 1992) and is a trait with a very low heritability value (Hoeschele, 1991). Nutritional management plays one of the most important roles in achieving reproductive goals (Ferguson, and Chalupa, 1989). The benefits of feeding excess protein to dairy cattle to maintain peak milk production should be compared with potential negative effects on fertility before such a program is implemented. High protein may affect the ovarian function in several way and in turn the fertility of the animal. Several studies reported the negative effects of BUN or MUN on reproductive performance in dairy cows and suggested that overfeeding crude protein caused reproductive stress (Rajala-Schultz *et al*., 2001). Urea nitrogen concentrations greater than 19 mg/ dL in plasma and milk were associated with decreased pregnancy rate in dairy cattle (Butler *et al*., 1995). Similarly, Ferguson *et al*. (1988, 1993) reported a decrease in conception rate when serum urea nitrogen concentrations were greater than 20 mg/dL. However, others did not find such negative effects of high MUN on fertility of cows (Godden *et al*., 2001)

Three general mechanisms have been proposed to describe how excess dietary protein may negatively influence fertility:

i. nitrogen by-products may alter the uterine pH and mineral balance.

ii. nitrogen by-products or efficiency of energy utilization may alter gonadotropin and (or) progesterone secretion

iii. toxic by-products of nitrogen metabolism from the rumen (ammonia) and liver (urea) may impair sperm, ova, or early embryo survival;

These effects may occur singularly, simultaneously, or synergistically.

i. Alter of Uterine pH and Mineral Balance

Feeding excess crude protein to dairy cows, regardless of protein source and degradability, alters the uterine environment. How this effect is mediated is not completely known at present. The changes in uterine pH observed in cows fed high levels of crude protein may be linked to reductions and fertility, as reported in other studies. Composition of the uterine luminal fluid in cows fed high protein diets has been examined by several workers to elucidate the mechanisms responsible for reduced conception rates.

ii. Hormonal Imbalance

Progesterone is important for follicular development, passage of the fertilized embryo through the oviduct to the uterus, and maintaining pregnancy. The amount and source of protein may influence progesterone (Swanson, 1989). It is possible that decreases in serum progesterone occur at concentrations of dietary crude protein that exceed rumen requirement for rumen degradable protein. However, source of protein, energy status and age of the animal should be considered. Nonetheless, the effect of protein consumption on progesterone concentration needs more study, and factors such as total energy intake and protein source should be examined.

Observations by researchers indicated that gonadotropin secretion was not directly affected by diets with high crude protein, but ovaries and thus steroid secretion were affected. Change in steroid secretion then affected the brain and gonadotropin secretion (Canfield *et al*., 1990). Thus, alteration of the uterine environment as a consequence of high protein may explain the fertility problems.

iii. Gamete and Embryo Survivality

Embryonic mortality is a limiting factor to the success of reproduction in cattle. Most pregnancy wastage occurs early during the pregnancy and the causes are usually unknown. Oviductal fluid provides nutritional environment for the early embryo, the metabolite, facilitates gamete transport and maturation, fertilization and therefore ionic composition of oviductal fluid is clearly important. Early embryonic development requires appropriate oviductal and uterine environments. Variations in the uterine environment caused by high PUN concentrations may therefore create a hostile or suboptimal environment for early embryo development. High PUN concentrations in lactating dairy cows decrease embryo viability through effects exerted on the oocyte or embryo before recovery from the uterus 7 days after insemination (Rhoads *et al*., 2006).

Excess rumen degradable protein has been reported to be deleterious to embryonic development in lactating cows (Blanchard *et al*., 1990), but not in non-lactating cows (Garcia-Bojalil *et al*., 1994). Uterine secretions altered in high-producing cows fed high crude protein diets and resulting in high PUN (Jordan *et al*.. 1983).

Non-nutritional factors affect the MUN/BUN level

Majority of the aforementioned studies were performed on individual animals under experimental conditions using chemical tests to measure MU. The association between MU and both nutritional management and performance should be determined under field conditions using commercial testing procedures (Sackett *et al*., 1991). To investigate these associations, a measure of the non-nutritional factors affecting MU is needed.

Association between Milk Urea and Season, Sampling, and Cow Factors

High MUN may be caused by many factors. Excessive protein intake is a common nutritional factor (Jonker *et al*., 1998). Blood urea nitrogen or plasma urea nitrogen, which is the origin of MUN, may also be affected by diseases or medicines from treatments (Vestweber *et al*., 1989). Any disease or body condition that reduces glomerular filtration such as dehydration, heart disease, and renal disease or any condition that increases protein catabolism can result in increased blood urea nitrogen level (Fraser, 1991).

Any number of factors including health or energy balance can affect MUN among individual cows within a herd (Stockham and Scott, 2002).

I. Milk Production Effect

During early lactation, dietary energy intake does not meet energy requirements for increasing milk production. As a result, body fat is mobilized. Reports on the association between MU and milk yield vary between positive (Carlsson *et al*., 1995), no association (Baker *et al*., 1995), and negative (Ismail *et al*., 1996). The positive association between MU and milk yield may be attributed to increased milk production which resulted from increased levels of dietary protein fed (Chalupa, 1984). Supplemental protein may increase milk yield by providing more AA for milk protein synthesis, by increasing the available energy through deamination of AA, or by altering the efficiency of utilization of absorbed nutrients (Chalupa, 1984).

There was a positive association between MU concentration and milk yield (Rajala-Schultz and Saville, 2003) for high-producing herds. Other studies examining the relationship between milk yield and MU level found either no significant correlation between these parameters (Godden *et al*., 2001) or a negative link (Roy, *et al*., 2003).

II. Milk component production.

The negative nonlinear association between MU and both milk fat and total protein percentages, while statistically significant, was numerically very small, posing the question of their biological and economic significance. Other studies have reported no association between MU and either milk fat or true protein percentages (Jaquette *et al*., 1986; Klusmeyer *et al*., 1990). As this study did not measure true protein levels, direct comparisons cannot be made with former studies.

1. Milk fat percentage.

There was a consistent positive association between MU and milk fat content (Rajala- Schultz and Saville, 2003) for high-producing herds. A possible explanation for this association could be that high amounts of NDF may increase milk fat content and at the same time raise MU levels because of the high degradability of its protein. Other studies have reported no association between MU and either milk fat percentages (Klusmeyer *et al*., 1990).

2. Milk total protein percentage.

There was a negative association between MU and milk total protein content (Ferguson *et al*., 1997). The inverse relationship between MU and milk total protein pinpoints the alternative pathways that N can follow: incorporation into milk protein or excretion as urea. However, other studies (Sharma et al., 2009) found no significant relationship between milk total protein and MU.

III. Parity

MU was lower in first-lactation heifers (Rajala-Schultz and Saville, 2003); however other studies reported only minor differences, with no significant association between parity group and MU (Canfield *et al*., 1990).

IV. Stage of lactation

The results of this study are consistent with previous studies that reported MU varied by stage of lactation. MU was lowest immediately after calving, increased to a maximum between 3 and 6 month of lactation, and slowly declined in later lactation. There was significant interaction between parity and DIM. The rate of decline in MU from mid to late lactation was greater in animals in parity two or greater. Changes in ration nutrient composition or feeding programs that occur among parity groups and different stage of lactation could contribute to the variation observed in MU. There may also be physiological or behavioral differences that could affect MU (Carlsson *et al*., 1995).

Lower MU in first-parity animals may be attributable to lean tissue growth and a correspondingly higher efficiency of AA utilization. As a result, deamination of AA and subsequent urea formation in the liver may be reduced (Oltner *et al*., 1985). Also, differences in DMI, rumen microbial adaptation, and rumen absorptive capacity could contribute to differences in MU at different stages of lactation. However, Schepers and Meijer (1998) found no association between parity or stage of lactation and MU when feeding trials were controlled for nutritional factors. This suggests that non-nutritional factors are of minor importance in explaining the association between MU and either parity or stage of lactation.

V. Season

High MU observed during the summer months has been reported by Carlsson *et al*. (1995) and Ferguson *et al*. (1997). Total protein and true protein (mostly casein) in milk are lower during the summer months, while NPN, which includes urea, increases (Ferguson *et al*., 1997). Moller *et al*. (1993) attributed variation in MU to seasonal changes in pasture protein and energy components. Australian spring pasture contained 20 to 30% CP and 5 to 20% soluble carbohydrate, thus, creating a high protein:energy ratio, which could result in elevated MU. Studies are lacking that describe the effects of non-nutritional factors associated with season (climate, water intake, DMI, or stage of lactation) on MU. Because of the absence of data on nutrient balance at the cow-level in this study, the variation in MU across parity groups, stage of lactation, or seasons could not be explained by nutritional management or non-nutritional factors.

VI. Diurnal Variation

Differences between a.m. and p.m. MU concentrations have been reported (Broderick and Clayton, 1997). Others found that a.m./p.m. MU differences may be influenced by differences in feeding to milking intervals between a.m. and p.m. milkings (Godden, 1998). This supports a previous finding that MU was highest when the cow had eaten within 5 or 6 h before sample collection and began to fall as the feeding-to-sampling interval increased (Gustafsson and Palmquist, 1993). The short feeding-to-milking interval (0 to 6 h) typical of the p.m. sampling period in dairy herds and the longer feeding-to-milking interval of the a.m. sampling period could explain the lower MU in the a.m. milk samples in many herds (Godden, 1998).

Because urea equilibrates across the mammary epithelium (Gustafsson and Palmquist, 1993), little variation was found in MUN concentrations in different milk fractions collected during milking (Roy *et al*., 2004).

Thus, it should make little difference whether a milk sample intended for MUN analysis comes from a composite milk sample or from a quarter strip sample (Roy *et al*., 2003), before or after milking (Carlsson and Bergstrom, 1994).

Gustafsson and Palmquist (1993) and Elrod and Butler (1993) reported that PUN concentrations fluctuate throughout the day. Generally, the minimum PUN concentration is before feeding and the maximum is approximately 4 to 6 h after feeding. Because there is a lag of approximately 1 to 2 h between PUN and MUN peaks (Gustafsson and Palmquist, 1993), sampling time relative to feeding can be important in the interpretation of PUN and MUN measurements, especially when cows are fed dietary forages and concentrates separately rather than a total mixed ration.

VII. Somatic Cell Count (SCC)

A strong negative relationship was found between MU and SCC. SCC followed MU concentrations (examined monthly) in an inverse order (Hojman *et al*., 2004). Milk urea is related to protein and NPN supply and their utilization rate in the rumen; SCC reflects the degree of irritation in the udder.

One study of Quebec herds reported a positive association between cow-level SCC and true protein content. They reported a small but significant positive association between SCC and milk NPN levels (which includes urea) (Ng-Kwai-Hang, *et al*., 1985). DePeters and Ferguson (1992), in a review reported that milk from mastitic glands was lower in casein and higher in non-casein protein. They suggested that casein breakdown products contributed to the whey protein fraction of mastitic milk.

Although a negative relationship between cow-level SCC and MU was observed, MU data should be interpreted at the group-level, and not the individual cow level (Schepers and Meijer, 1998). Therefore, unless a large proportion of cows in the group or herd have very high SCC, then the herd average MU should not be greatly affected by SCC. In this study, herd average LS was not associated with herd average MU.

Godden *et al*. (2001) reported that MUN values were associated with log SCC values, and MU values were lowest for samples with largest SCC and log SCC. Licata (1985) reported that milk from quarters positive to the California mastitis test was 0.45 mM lower in MU content than that from healthy quarters. Roy *et al*. (2001) indicated that as the intensity of infection increased from mild to moderate, the MU concentrations decreased. This may be due to the intramammary infection that alters the permeability of the udder tissues.

Preservation of milk sample for urea estimation

In azide preserved cow milk or cow milk storage at 4^0C, the milk urea was unchanged for one week, but urea gradually decreased in milk at room temperature as reported by Miettinen and Juvonen (1990). Carlsson and Bergstrom (1994) reported that without preservative the urea concentrations in cow milk were not changed significantly after storage during 10 days at 4^0C. But when a preservative (bronopol) was added, the urea concentrations remain unchanged till 17 days. Deep freezing did not influence the urea concentration. Urea concentrations in deproteinized cow milk (Dhali, 2001) and buffalo milk (Roy *et al*., 2004) were not changed upto 30 days when preserved at 4°C. Being able to use preservative is beneficial in situations, such as field trials, when there may be a long period between the time of milk sampling and MUN analysis. Field trials or on-farm MUN analysis would be benefited by an easy and reliable test system.

CONCLUSIONS

Maintaining and monitoring MUN in a dairy herd provides an opportunity to formulate the dietary protein constituency that optimizes nitrogen utilization for milk production and avoids possible negative effects on herd fertility. MUN is economical, easy to monitor on a monthly basis, and could be performed on all animals in a herd. Maintaining and monitoring MUN in a dairy herd provides an opportunity to formulate the dietary protein constituency that optimizes nitrogen utilization for milk production and avoids possible negative effects on herd fertility. Milk urea varied by season, month, parity group, stage of lactation, and sample type. Researchers should consider controlling for these variables as potential confounders when exploring the relationship between MU and nutritional management or measures of performance such as production or reproduction. Because of the apparent effect of a.m. and p.m. sampling on MU concentration, producers on an alternating a.m./p.m. test schedule should test routinely to establish a herd pattern for MU and/or submit the same sampling time consistently.

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