Preliminary Results from a Study Evaluating the Test Characteristics of a Milk Urea Nitrogen Assay and the Influence of Factors Related to Sample Collection and Handling

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Measurement of urea levels in milk has been promoted as a potentially useful diagnostic aid in monitoring the nutritional management of dairy herds. However, considerable research is required in order to develop criteria to accurately interpret Milk Urea Nitrogen (MUN) measurements. As part of a larger research project designed to investigate the relationship of MUN measurements to nutritional management and reproductive performance in dairy herds, a series of preliminary experiments were performed. The objectives were to evaluate the test characteristics of the Fossomatic 4000 Milk Analyser MUN test and to investigate how various factors relating to the sample type,(composite vs stripping, use of Bronopol preservative and storage over time) may affect MUN measurements.

Milk and blood samples were collected at time of morning milking from 26 Holstein cows of mixed parity and stage of lactation. For each cow, milk samples included a single small volume pre-milking stripping (collected 3 to 5 minutes prior to milking), a composite milk sample that was split into 10 identical smaller samples, and a single small volume post-milking stripping (collected 3 to 5 minutes after milking). A single blood sample was also collected from each cow at time of milking, for the determination of serum urea nitrogen (SUN), using the DART Urea Nitrogen test (Modified Talke and Schubert Method). Of the 10 identical composite samples, 5 samples had Bronopol preservative added while the other 5 did not. Bronopol preservative was also added to the pre- and post-milking stripping samples. All milk samples were stored at 4 degrees Celsius until time of submission for analysis of MUN, SCC, % protein and % fat, using the Fossomatic 4000 Milk Analyser. For each cow, samples submitted on the first day included the pre- and post-milking stripping samples, and a preserved and non-preserved composite sample. A preserved and non-preserved composite sample, for each cow, was also submitted for analysis on days 3, 4, 7 and 11 of the experiment.

MUN measurements obtained from the composite preserved samples analysed on day 1 were compared to SUN measurements, using a Pearson's Correlation Coefficient. An r value of 0.64 was obtained ($p<0.05$). An agreement test between MUN and SUN resulted in a Kappa value of 0.43 with a 95% Confidence Interval of (0.06, 0.79). These preliminary results suggest that MUN values from composite milk samples are reasonably well correlated to SUN values, although the level of agreement between the two tests is only moderate.

A paired t-test was performed comparing both the pre- and post-milking stripping samples to the composite samples analysed on day one. The mean MUN value from the composite samples were 2.15 mg/dl higher than that of the pre-milking stripping samples ($p<0.05$ and Std. Error = 0.33) The mean MUN value from composite samples were not significantly different from that of the post-milking stripping samples ($p>0.05$). However, there was a very large standard error (1.08). A Pearson's Correlation Coefficient between the pre-milking stripping MUN values and the SUN values was 0.5102 ($p<0.05$), meaning these samples were less strongly correlated to SUN levels than were the MUN values from the composite samples. A Pearson's Correlation Coefficient between the post-milking stripping MUN values and the SUN values was 0.05 ($p>0.05$), meaning there was no correlation between the post-milking stripping MUN levels and SUN levels. A significant positive correlation ($r=0.70$) existed between the MUN measurements and the % fat measurements for the post-stripping samples. Also, the mean and standard error (8.83% and 0.5003, respectively) for % fat in the post-milking stripping samples were considerably greater than for the % fat in the composite milk samples (3.37% and 0.13, respectively). Thus, the % fat in the post-milking stripping samples may be associated with the large standard error of the MUN mean measurement for these samples. These preliminary results suggest that caution should be used in interpreting MUN results obtained from stripping samples. Pre-milking stripping samples should be avoided as they will result in significantly lower MUN measurements than those of composite samples and are not as representative of SUN levels and there is no correlation between post-milking stripping samples and SUN levels. Future work is needed to address the effect of % fat on MUN measurements from stripping samples collected over several hours post-milking in order to calculate a 'fat-corrected' MUN value. Preliminary results from this experiment suggest that a composite sample, and not a stripping sample, should be collected.

An ANOVA was performed on the MUN results

from the group of preserved composite samples that were analysed on days 1, 3, 4, 7 and 11. No significant difference in was found ($p>0.05$) between any samples stored up to and including 11 days. Similarly an ANOVA was performed on the MUN results from the group of non-preserved composite samples that were analysed on days 1, 3, 4, 7 and 11. Again, there was no significant difference found ($p>0.05$) between samples, although several unpreserved samples were sour by day 11. These preliminary results suggest that both preserved and non-preserved milk samples may be stored up to 11 days with no significant effect on MUN measurements. However, eventual souring of non-preserved samples means that storage beyond 7 days is not recommended.

A paired t-test was performed to compare the MUN measurements from preserved composite samples analysed on day 1 to non-preserved composite samples analysed on day 1. MUN levels from preserved samples averaged 0.25 mg/dl higher than the non-preserved samples ($p<0.05$ and Std. Error = 0.10). However, the biological significance of a mean difference of 0.25 mg/dl could be questioned.

In conclusion, preliminary results from these experiments suggest that milk samples collected regularly by milk testing laboratories will provide MUN measurements that are reasonably well correlated with SUN levels in the cow. However, there is only moderate agreement between the MUN and SUN tests. Thus, caution should be used in assuming that the relationship between MUN levels and nutritional management or reproductive performance in dairy herds will automatically be consistent with findings from research on SUN levels. Thus, new research looking at the nature of potential associations between MUN measurements and the nutritional management and reproductive performance in dairy herds is warranted.

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Potassium, Not Calcium Induces Milk Fever: Addition of Potassium or Sodium, but not Calcium, to Prepartum Rations Induces Milk Fever in Dairy Cows

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The effects of prepartal dietary potassium, sodium, and calcium concentration on the incidence of periparturient hypocalcemia or milk fever was determined in older (≥ 4th lactation) Jersey cows. Cows were fed one of six diets differing in potassium and calcium content. In addition, the effect of dietary sodium (tested only at the high dietary calcium level) was also examined. Treatments were arranged in an incomplete 2 X 4 factorial design with dietary calcium (.5 or 1.5%) and dietary strong cations (1.1, 2.1 and 3.1% potassium or 1.3% sodium) as the main effects. There were no significant effects of dietary calcium on the incidence of milk fever or the degree of hypocalcemia experienced by the cows. Milk fever occurred in 2 of 20 cows fed a 1.1% potassium, .12% sodium diet prepartum. Increasing dietary potassium to 2.1 or 3.1% increased the incidence of milk fever to 10 of 20 cows and 11 of 23 cows, respectively. Increasing dietary sodium to 1.3% in a diet containing 1.5% calcium induced milk fever in 5 of 8 cows. Addition of strong cations to the prepartal diet increased blood and urine pH values and reduced plasma hydroxyproline concentrations, suggesting an inhibition of bone calcium resorption in cows fed high potassium or high sodium diets as a result of metabolic alkalosis. These data demonstrate that dietary calcium concentration is not a major risk factor for milk fever, and that dietary strong cations, especially potassium, induce a metabolic alkalosis in the prepartum dairy cow which reduces the ability of the cow to maintain calcium homeostasis.