Correlation Between Milk and Blood Urea Nitrogen in High and Low Yielding Dairy Cows

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ABSTRACT

A study was carried out in two dairy farms (Olocuilta and Los Conacastes) in the central region of El Salvador. Sixty Holstein cows were grouped according to milk yield and days in milk: high yielding (HY, 30–90 d in milk) and low yielding cows (LY, >180 d in milk). The objective of the study was to evaluate the effect of milk yield and time after feeding on milk and blood urea-nitrogen (BUN) concentration, and to establish a correlation between these two parameters. On Olocuilta, HY cows had the highest BUN and milk urea nitrogen (MUN) concentrations. Blood urea nitrogen least squares concentration was 12.77 mg/dL and 13.98 mg/dL for the LY and HY cows, respectively; while the MUN average concentration was 12.30 mg/dL and 14.82 mg/dL for the LY and HY cows, respectively. BUN and MUN concentrations were similar at 30 min, one and two h post-feeding but by four h post-feeding BUN concentrations had decreased and were significantly lower than those of MUN ($P < 0.05$). On the other hand, in Conacastes the highest values were found for the LY group. BUN least square concentration was 11.22 mg/dL and 9.12 mg/dL for the LY and HY cows, respectively; while the MUN average concentration was 10.18 mg/dL and 8.83 mg/dL for the LY and HY cows, respectively. The reason for these differences seems to be related to protein balance. For instance on the Los Conacastes farm, protein balance was negative in the HY group (-88 g/d) while on Oloculita farm the balance was positive. The correlation between BUN and MUN for the Olocuilta farm had a regression coefficient of 0.84, and a correlation (r^2) of 0.7543. For Los Conacastes these values were 1.04 and 0.9017, respectively. It should be noted that BUN and MUN concentrations were better correlated at 30 min, one h and two h after feeding and that the correlation decreased at four h post-feeding due to a drop in BUN concentration. It is concluded that BUN and MUN concentrations are not related directly to milk yield but with the protein balance. There was a high correlation between BUN and MUN concentrations; hence, either of these parameters can be used to monitor protein nutrition in dairy farms.

Key words: *Holstein cows, milk yield, time of feeding, blood urea nitrogen, milk urea nitrogen, protein nutrition.*

INTRODUCTION

Dairy cows need appropriate quantities of protein for milk production and for this reason diets are supplemented with additional protein.

However, excess protein can negatively affect production and reproduction, and pollute the environment (Sonderegger and Schurch, 1977; Peabody, 2004). When an excess of degradable protein relative to energy is present in the rumen, the concentration of rumen ammonia increases and elevates rumen pH (Gómez and Fernández, 2002). Some of the ammonia liberated in the rumen cannot be fixed by the microorganisms; this excess is absorbed and taken into the blood. The liver converts ammonia to urea which is excreted by the animal in the urine (Garriz and López, 2002). High concentrations of urea reflect an excess of protein in the diet, which can adversely affect fertility (Ropstad and Refsdal, 1987; Melendez et al., 2000; Nousiainen et al, 2004). An urea-nitrogen concentration higher than 20 mg/dL of milk suggests an excess protein supply in the diet which can decrease production and cause fertility problems (Ferguson and Chalupa 1989; Hojman et al., 2004). This also makes the diet more expensive and increases nitrogen excretion to the environment (Jonker et al., 1998). Measurement of urea nitrogen in blood and milk has been proposed as a tool to monitor protein nutrition (Ferguson et al., 1993; Hof et al., 1997).

Blood urea gets transported into milk and therefore urea is a normal constituent of milk (Ferguson, 2002; Acosta and Delucchi, 2002). The relationship between the levels of (BUN) and MUN in dairy cattle depends on the degradability of the different protein sources and nitrogen compounds (Acosta and Delucchi, 2002). Since it is both simpler and less stressful to take milk than blood samples (Acosta and Delucchi, 2002; Acosta et al., 2006), measurement of MUN is an easy method for determining BUN levels and for assessing the protein and energy supply in the diet. By determining MUN levels, milk producers could be advised on the appropriateness of different diets for providing a proper protein to energy ratio.

The main objective of this investigation was to establish correlations between BUN and MUN concentrations in dairy cows at two levels of milk production i.e. high and low, and at different times after feeding.

MATERIALS AND METHODS

Animals and Feeding

The study was carried out from December 2006 to July 2007 on two dairy farms: Ranch Olocuilta and Ranch Los Conacastes located in the central region of El Salvador. The herds had more than 80% of Holstein genetic make up and were producing more than 15 kg milk/cow/d. The cows were kept and managed in free stall barns. The feed offered consisted of forage (silage or green grass) and concentrate. Thirty cows were selected on each farm. Fifty percent of the cows on each farm were between 30–90 d in milk (high production) and the other 50% were > 150 d in milk (low production).

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Collection and Analysis of Milk

Milk and blood samples were taken at 30 min, one, two, and four h after feeding. Blood samples were taken from the jugular vein into vacutainer tubes without an anticoagulant. Milk samples (50 mL) were obtained directly from the udder.

After clotting, blood samples were centrifuged at 7 000 rpm for 15 min., and sera collected and stored in cryovials at -20 °C for later analysis. BUN concentration was determined by means of the Liquicolor Enzymatic Colorimetric Test (Human ®,Damstad, Germany). A total of 240 samples were analysed.

MUN concentrations were determined in the clear medium obtained after precipitation of proteins using trichloroacetic acid for 15 min and then centrifuged at 4 000 rpm for 10 min and after filtering the supernatant and diluting it to 1:100 with distilled water. Urea nitrogen was analysed using the technique described by Merck® (Darmstat, Germany). Urea standards were prepared at concentrations of 1–5 ppm. A graph relating urea concentration (ppm) and absorbance was drawn to obtain the equation $y = ax + b$; in which x represents the urea concentration and y represents the absorbance. Urea (mg/dL = (*Absorbance-b*) /a. The urea level in mg/dL was multiplied by 4.16 to obtain MUN.

Feed Analyses

Feeds were analysed for crude protein (CP), neutral detergent fibre (NDF) and acid detergent fibre (ADF). Information on the characteristics of the cows and the proportions and composition of ingredients was entered into the NRC Dairy Cattle Program (NRC, 2001) to obtain reports on the protein and energy balances of the high and low producing cows.

Statistical Analyses

The effect of milk yield (high and low) and time after feeding (0.5, one, two and four h) on BUN and MUN concentrations were analysed using repeated measures analysis and the MIXED procedure of SAS (SAS Institute, Version 9.1.3, 2006). Variables for which variance analysis were significant at $P < 0.001$, were subjected to a test of comparison using the Student's t-test. Data were also subjected to linear regression to find the correlation and regression coefficients between BUN and MUN. In this case the GLM procedure of SAS was used.

RESULTS AND DISCUSSION

Protein Balance

On Olocuilta, the protein content in the diet was higher for the high yielding cows than for the low yielding and the same pattern was found for the protein balance (**Table 1**). On Conacastes, the protein

percentage was similar for the two groups, while protein balance was positive in the low yielding cows but negative in the high yielding cows. These results showed that high producing cows are not necessarily those that receive more protein or have a more positive protein balance.

Blood Urea Nitrogen (BUN)

High yielding cows on Olocuilta had higher average values of BUN than low yielding cows (13.94 vs 12.75 mg/dL, P < 0.05). These levels are within the acceptable range of 10–20 mg/dL (Ferguson et al., 1993), and reflect the fact that the protein levels in the diet were adequate. On Conacastes on the other hand, it was found that high producing cows had lower values of BUN than low producing cows (9.09 vs 11.20 mg/dL, P < 0.05); this could be explained by the negative protein balance in high yielding cows.

Milk Urea Nitrogen (MUN)

The pattern of MUN was similar to that of BUN. On Olocuilta, high yielding cows had higher (P < 0.05) values of BUN (average 14.99 mg/dL) than those of low yielding ones (average 12.26 mg/dL) throughout the sampling period (**Figure 1**). However, for both productive states the values were within the normal range (10–20 mg/dL).

On Conacastes, higher mean values for MUN were obtained in the low yielding group (10.15 mg/dL). The MUN for the high yielding group was 8.91 mg/dL. It has been reported that MUN concentra-
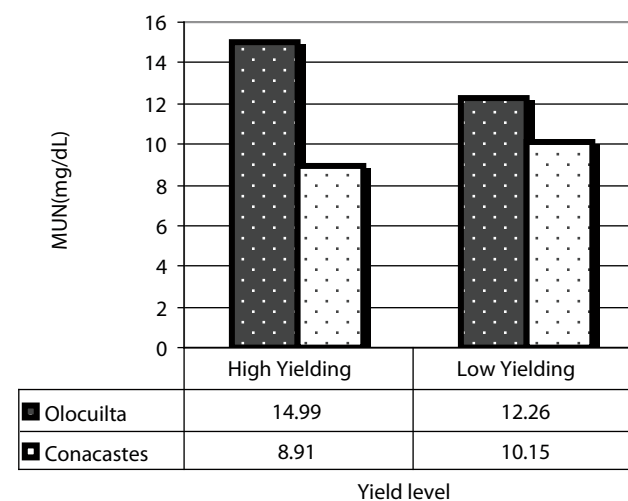

	High Yielding	Low Yielding
◾ Olocuilta	14.99	12.26
◾ Conacastes	8.91	10.15

Yield level

Figure 1. Milk urea nitrogen (mg/dL) for high and low yielding dairy cows on two farms.
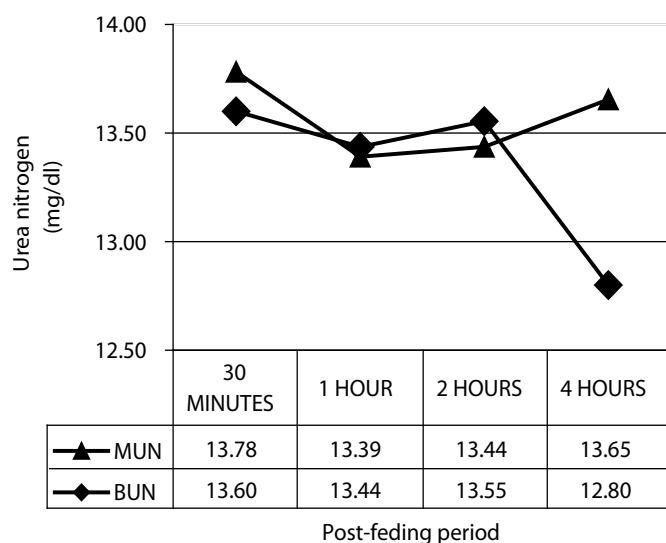
Table 1. Milk production, nutritional composition of the diet and protein balance on two farms.

|	Low yielding group		High yielding group	
	Olocuilta	Los Conacastes	Olocuilta	Los Conacastes
Milk yield, kg/d	16.50	11.40	22.25	19.00
NDF (% DM)	42.30	42.20	41.40	44.30
NE_l (Mcal/kg) DM)	1.48	1.55	1.47	1.57
Crude protein (% DM)	15.90	13.80	16.40	13.70
Crude protein balance (g/d)	428	401	537	–88

tions usually fall at the beginning and the end of lactation rather than in mid lactation. Probably these observed variations respond more to changes in the nutrient demand during the postpartum period than to changes in the diet (Acosta and Delucchi, 2002).

Time after-Feeding

Urea concentrations were compared in blood and milk at different times after feeding. For the Olocuilta farm these values ranged from 12.8 to 13.8 mg/dL (**Figure 2**). BUN and MUN values were similar for high and low producing cows at 30 min, one and two h after feeding. However, by four h post-feeding, BUN concentrations had decreased and were lower than those in milk (P < 0.05). This differ-


	30 MINUTES	1 HOUR	2 HOURS	4 HOURS
MUN	13.78	13.39	13.44	13.65
BUN	13.60	13.44	13.55	12.80

Post-feding period

Figure 2. BUN and MUN concentrations at different times after feeding dairy cows on Olocuilta farm.

	1	2	3	4
MUN	8.62	9.32	9.89	10.30
BUN	9.35	10.31	11.02	9.91

Post-feeding period
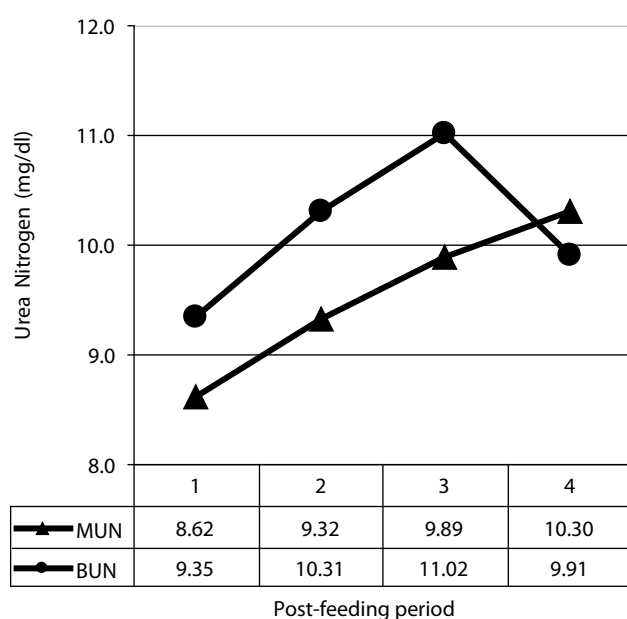
Figure 3. BUN and MUN concentrations at different times after feeding dairy cows on Conacastes farm.

$$y = 0.8406x + 1.9456$$
$$R^2 = 0.7543$$
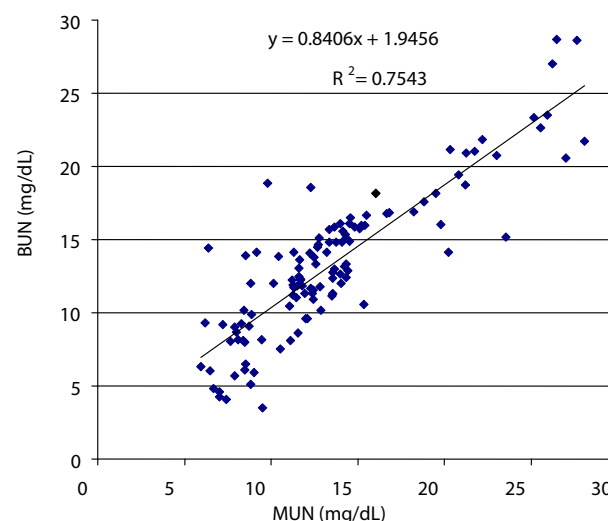
Figure 4. Correlation between BUN and MUN concentrations for dairy cows in Olocuilta farm.

$$y = 1.0467x + 0.1715$$
$$R^2 = 0.9017$$
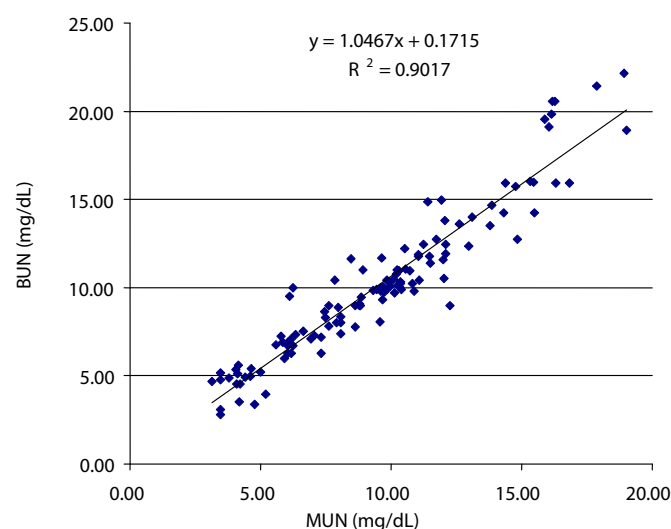
Figure 5. Correlation between BUN and MUN concentrations for dairy cows on Los Conacastes farm.

ence could be due to changes in absorption or production of urea that are quickly reflected in blood levels but not in previously synthe-sised and stored milk.

On Los Conacastes farm, BUN concentrations tended to be higher than those of MUN (**Figure 3**) and as observed on Olocuilta farm, a decrease was observed in concentration at four h post-feeding. However, BUN and MUN concentrations were statistically similar at all times after feeding. Butler (1998) reported elevations of BUN at four and eight h after feeding a 19% CP total mixed ration. However, the CP content in the present study was lower (**Table 1**).

Noticeable was how BUN values increased up to three h post-feeding and then started to decrease while MUN values increased steadily. These results suggest that MUN measurements have an advantage over BUN since milk samples can be taken at any time

after feeding. In other words, MUN values are better for predicting the nutritional status of the animals.

Correlation between BUN and MUN

Figure 4 shows the correlation between BUN and MUN concentration considering the values at all time periods after feeding. For Olocuilta farm, the regression coefficient between BUN and MUN was 0.84 (slope of the curve), while the correlation was 0.7543 (r2) (**Figure 4**). It should be noted that BUN and MUN concentrations were better correlated at 30 min, one, and two h after feeding.

The relationship between BUN and MUN for animals on Conacastes farm is shown in **Figure 5**. In this case the correlation was 0.90 (r^2) and the regression coefficient was 1.04. The values for MUN and BUN were almost identical (9.53 mg/dL and 10.15 mg/dL, respectively).

When all blood and milk determinations were compared using the Student's test the association between both methods was high (P < 0.05), and when averages were compared they are almost the same for MUN (13.53 mg/dL) and BUN (13.35 mg/dL). Therefore, either measurement can be used to determine protein status using urea nitrogen although milk sampling is both simpler and causes no stress on the animals. Nonetheless, BUN values tend to decrease when sampling four h post-feeding, while that variation was not seen when determining MUN concentrations.

It has been established that urea balances quickly with other body fluids including milk, and that a relationship between BUN and MUN can be calculated (Broderick and Clayton, 1997). MUN values represent 83–98% of BUN values and hence by dividing MUN by 0.85 a good estimate of BUN can be obtained (Arias and Nesti de Alonso, 1999). The results obtained in this study are in accordance with this statement.

When comparing BUN concentration for the two farms, the values differed significantly (P < 0.05) with means being higher in farm Olocuilta (13.34 mg/dL vs. 10.14 mg/dL).

CONCLUSIONS

The determination of urea in milk is a useful tool for monitoring protein nutrition in dairy cows since is a reliable technique and highly correlated with BUN. In the nutritional management of dairy cows diets should be balanced based on the analysis of the feedstuff to have a good approach of the balance of the nutrients in the animal. For determinations of BUN, it is better to carry out the samplings before two h after feeding; for MUN, sampling can be carried out until four h after feeding without significantly altering the values.

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