Diurnal Variations in Milk and Blood Urea Nitrogen and Whole Blood Ammonia Nitrogen in Dairy Cows**

Sen-Yuan Hwang*[,1], Mei-Ju Lee[1] and Huo-Cheng Peh[2]
Department of Animal Physiology, Taiwan Livestock Research Institute, 112 Mu-Chang Road
Hsin-Hua, Tainan 712, Taiwan, ROC

ABSTRACT : The levels of urea nitrogen both in blood (BUN) and milk (MUN), and milk protein (MP) reflect protein and energy intake in dairy herd feeding. Blood and milk constituents may be changes rhythmically and influence by different sampling time within a day and after feeding. Trials were conducted using five dietary treatments in both lactating and dry cows to study the effects of sampling time on concentrations of BUN, MUN and whole blood ammonia nitrogen (BAN) in practical dairy cow feeding in Taiwan. The conventional feed ingredients and forages including corn silage, alfalfa hay, timothy or pangola hay and corn grain were used as major source of the diet to follow practical dairy cow feeding. Five different diets were varying in amounts (low=L; standard=S; high=H) of crude protein (P) and energy (E) according to the NRC (1989). The energy to protein ratios in kcal/kg for the PSES, PLES, PHES, PSEH and PSEL were 10.82, 12.54, 9.41, 12.53 and 9.13 in lactating cows, and 11.38, 13.33, 9.78, 13.28 and 9.74 in dry cows, respectively. Results showed that after feeding at 9:30, BUN reached peak at 13:30 and was significantly higher than those to that sampled at 14:30 to 18:30 ($p<0.05$) in dry cows. Therefore the best blood sampling time for urea nitrogen assay in dry cows is 4 hours after morning feeding. In lactating cows, BUN of 13:30 was significantly higher than those of 8:30 to 11:30 ($p<0.05$), but there were no significant difference between the BUN values of other sampling time. Hence the suitable blood sampling time for BUN value in lactating cows was located on 3 to 8 hours after morning feeding, but the best time was 4 hours after morning feeding. MUN content is significantly higher in the afternoon collected bulk milk than the fore-strip morning milk ($p<0.05$), therefore the best sampling time for MUN is from afternoon collected bulk milk. Diurnal BAN changed without traceable rhythmic pattern and was negatively correlated to the BUN ($r = -0.78$). It is suggested that BAN may not be a good indicator for monitoring dairy cow feeding. *(Asian-Aust. J. Anim. Sci. 2001. Vol 14, No. 12 : 1683-1689)*

Key Words : Dairy Cow, Serum, Milk, Urea Nitrogen, Ammonia Nitrogen

INTRODUCTION

Dietary intake protein was degraded to peptides and amino acids, then converted to ammonia in the rumen of cow after ingested. When ammonia level exceeding bacterial growth under limited energy source in the rumen, diffuse via ruminal wall into blood stream, circulate to liver through portal vein system and is dehydrogenated to urea. In dairy cow, urea or urea nitrogen level, especially in the blood or milk, reflects the protein intake (Ide et al., 1966; Roseler et al., 1993; Jonker et al., 1998), thereby it has been recommended as a good indicator to monitor the balance of the dietary protein and carbohydrate intake (Oltner and Wiktorsson, 1983; Kirchgessner et al., 1986; Broderick and Clayton, 1997). Under constant energy intake, blood urea nitrogen (BUN) and degradable intake protein or undegradable intake protein is generally positively correlated (DePeters and Ferguson, 1992; Roseler et al., 1993; Hof et al., 1997). While under constant protein intake, BUN is negatively correlated to the net energy intake (DePeters and Ferguson, 1992; Roseler et al., 1993; Lykos et al., 1997). Urea nitrogen content in the bulk milk also reflects protein intake (Refsdal et al., 1985; Ropstad and Refsdal, 1987; Schepers and Meijer, 1998). Researches also indicated that BUN or MUN value can be an important tool for monitoring protein and energy intake (Harris, 1995; Hutjens and Barmore, 1995; Hof et al., 1997). Although Ide et al. (1966) suggested that BUN and MUN level almost constant under constant diet.

Feeding and sampling time affected BUN and MUN concentration (Gustafsson and Palmquist, 1993; Staples et al., 1993). Feeding dairy cow once daily, Gustafsson and Palmquist (1993) obtained a BUN level peaked at 2 to 4 hours after feeding with about 1.5 to 2 hours behind the maximum ruminal ammonia concentration, whereas MUN changes followed the BUN pattern, but peaked at about 1 to 2 hours behind the blood level. Manston et al. (1981) suggested a peak of BUN at 2 to 4 hours postprandial under twice daily cow feeding. Taking the blood sample 4 hours after feeding a balanced diet according to NRC (1989) nutrients requirement, Roseler et al. (1993) obtained BUN value of 14.8±0.68 mg/dL with MUN value of 11.6±0.63 mg/dL. The sampling times in measure these concentrations

** Contribution No. 957 from Taiwan Livestock Research Institute (TLRI), Council of Agriculture (COA).

* Address reprint request to Sen-Yuan Hwang. Tel: +886-6-5911884, Fax: +886-6-5912581, E-mail: syhwang@mail.tlri.gov.tw

[1] Taiwan Livestock Research Institute (TLRI), Hsin-Hua, Tainan, Taiwan, ROC.

[2] Dept. of Animal Science, National Chung-Hsing Univesity, Taichung, Taiwan, ROC.

Received March 23, 2001; Accepted June 8, 2001

was different in literatures, from 2 hours postprandial (Jordan et al., 1983; Oltner and Wiktorsson, 1983; Carroll et al., 1988), 2 to 3 hours postprandial (Holtz et al., 1986), 3 hours postprandial (Garcia-Bojalil et al., 1992), and 4 hours post-feeding (Roseler et al., 1993; Broderick and Clayton, 1997). Some researchers even used fore-strip milk (Gustafsson and Palmquist, 1993), but most did not define their sampling time (Miettinen and Juvonen, 1990; Hof et al., 1997; Rodriguez et al., 1997). The discrepancy of the BUN and MUN values in literature was due to the different sampling time (Gustafsson and Palmquist, 1993), but most of literature did not discuss these differences. In application of these BUN and MUN values as an indicator for monitoring protein and energy intake balance, therefore sampling time of blood and milk is critical. The objective of this study aimed to find out the optimum sampling time from the pattern of diurnal variations in BUN, MUN and BAN.

MATERIALS AND METHODS

Diet formulation

Corn silage, alfalfa hay, timothy or pangola hay and corn grain were used as major source of ingredient in experimental diets. Diets were formulated based on the mean live-weight of 600 kg, 20 kg daily milk yield with 3.5% milk fat for lactating cows, whereas dry cows diets were based on non-lactating and non-pregnant (ruminal fistulated) cows with live-weight of 550 kg according to the NRC (1989) nutrient requirements of dairy cow. Five different diets varied in crude protein (P) and energy (E), approximately 15% of the NRC standard (1989) to make low (L), standard (S), and high (H) levels of P or E. Five different diets were the standard protein and standard net energy lactation (PSES), low protein and standard net energy lactation (PLES), high protein and standard net energy lactation (PHES), standard protein and high net energy lactation (PSEH), and standard protein and low net energy lactation (PSEL) groups. The ratios (kcal/kg) of energy to protein (E/P) were 10.82, 12.54, 9.41, 12.53 and 9.13 for the lactating cows, and were 11.38, 13.33, 9.78, 13.28 and 9.74 for the dry cows, respectively. The diet formulations for lactating cows and dry cows were presented in tables 1 and 2, respectively. To derive information of different energy levels on standard protein and different protein levels under the same energy level, four dietary combinations were excluded from a 3 × 3 factorial arrangement of a 3 levels of proteins by 3 levels of energys in this study. These dietary combinations included low protein and high energy, low protein and low energy, high protein and high energy, and high protein and low energy diets.

Animal management

Two trials involving a lactating cows trial and a dry cows trial were conducted in this study. In the lactating cows trial, thirty lactating cows with mean live-weight of 589±70 kg and daily milk yield of 20 kg were selected into 5 dietary treatments with 3 blocks in a completely randomized block design. Whereas in the dry cows trial, a 5 × 5 Latin square design with 5 dietary treatment and 5 period was applied in this trial since only 5 ruminal fistulated Holstein non-pregnant dry cows were available. Cows fed twice with equal amount of total mixed rations on 9:30 and 14:30 daily. Water was provided through automatic bowl-type drinker *ad libitum*. Feeding the experimental diet was lasting for 3 weeks with the first 2 weeks for adaptation. Blood samples were taken via coccygeal vein at 1 h interval from 8:30 to 18:30 for 3 consecutive days on the third week of the experimental period. Blood samples were separated within 1 hour of blood collection by centrifugation at 3,000×g at 4°C for 15 min. Serum was preserved at -20°C freezer before analysis. Fore-strip milk, mixed milk from volumetric vial and post-strip milk samples were taken at 4 hours before feeding in the morning and 1 hour after feeding in the afternoon, respectively. Milk samples were preserved at 4°C and analyzed within 24 h.

Chemical analysis

The crude protein, calcium and phosphorus of diets were analyzed according to AOAC (1990). Acid detergent fiber of the diet was analyzed according to the method of Van Soest et al. (1991). Serum urea nitrogen was analyzed according to colorimetric diacetyl monoxine precedure (DiGiorgia, 1974) using automatic blood chemistry analyzer (Vitros 750XRC, Johnson & Johnson Co., USA). Milk samples were added 3% trichloroacetic acid (TCA) and centrifuged, supernatants were used for urea nitrogen analysis using enzymatic kits from Sigma (Sigma diagnostic #535, Sigma Chemical Co., USA) and reading the optical density at 540 nm according to the method of Crocker (1967). For whole blood ammonia nitrogen, 20 μl of blood samples pipetted into ammonia test kit II (Kyoto Daiichi Kagaku Co., Japan) by micropipet and assayed according to the micro-diffusion method used in clinical diagnosis using blood ammonia meter (Model AA-4120, Kyoto Daiichi Kagaku Co., Japan).

Statistical analyses

Data were analyzed using the General Linear Model (GLM) procedure of SAS (1989). Means were compared using Duncan's New Multiple Range Test (Steel and Torrie, 1960).

RESULTS AND DISCUSSION

Serum urea nitrogen

Figure 1 presented the diurnal variation of BUN in dry cow. From iso-energetic diets, daily mean BUN value of the

Table 1. Composition of rations for lactating cows

Item	PSES[1]	PLES	PHES	PSEH	PSEL
Calculated (analysed) values					
% DM[2]	71.6	71.5	71.7	72.4	72.5
Crude protein (%)	14.6 (14.8)	12.6 (12.7)	16.8 (16.9)	14.6 (14.5)	14.9 (14.7)
NEL[3] (Mcal/kg)	1.58	1.58	1.58	1.83	1.36
ADF[4] (%)	19.9 (19.4)	19.7 (19.6)	20.3 (19.9)	19.8 (20.1)	26.4 (27.1)
Calcium (%)	0.91 (0.93)	0.90 (0.92)	1.16 (1.13)	1.39 (1.36)	1.23 (1.20)
Phosphorus (%)	0.47 (0.50)	0.42 (0.44)	0.44 (0.43)	0.46 (0.47)	0.41 (0.42)
Forage: Grain	53:47	55:45	53:47	52:48	82:18
E / P[5] (cal/g)	10.82	12.54	9.41	12.53	9.13

[1] A ration containing varying amounts (low = L; standard = S; high = H) of net energy lactation (E) and crude protein (P). Excepting dietary protein and energy ratios of control group (PSES) and NRC (1989) were the same, all diet was adjusted with the various ratios (±15%). PSES: standard crude protein and standard net energy lactation; PLES: Low crude protein and standard net energy lactation; PHES: High crude protein and standard net energy lactation; PSEH: Standard crude protein and high net energy lactation; PSEL: Standard crude protein and low net energy lactation.

[2] DM = dry matter, [3] NEL = net energy lactation, [4] ADF = acid detergent fiber.

[5] E/P = ratio of net energy lactation (E) to crude protein (P).

Table 2. Composition of rations for dry cows

Item	PSES[1]	PLES	PHES	PSEH	PSEL
Calculated (analysed) values					
% DM[2]	69.8	69.8	69.8	70.1	73.2
Crude protein (%)	11.6 (11.5)	9.9 (10.1)	13.5 (13.2)	11.6 (11.5)	11.5 (11.6)
NE_L[3] (Mcal/kg)	1.32	1.32	1.32	1.54	1.12
ADF[4] (%)	25.2 (25.1)	24.9 (25.0)	25.6 (25.4)	24.0 (24.1)	31.8 (31.7)
Calcium (%)	0.75 (0.76)	0.74 (0.75)	0.59 (0.60)	0.94 (0.95)	0.78 (0.79)
Phosphorus (%)	0.34 (0.35)	0.32 (0.35)	0.37 (0.39)	0.35 (0.37)	0.33 (0.35)
Forage: Grain	61:39	61:39	61:39	57:43	76:24
E / P[5] (cal/g)	11.38	13.33	9.78	13.28	9.74

[1 ~ 5] Same as table 1.

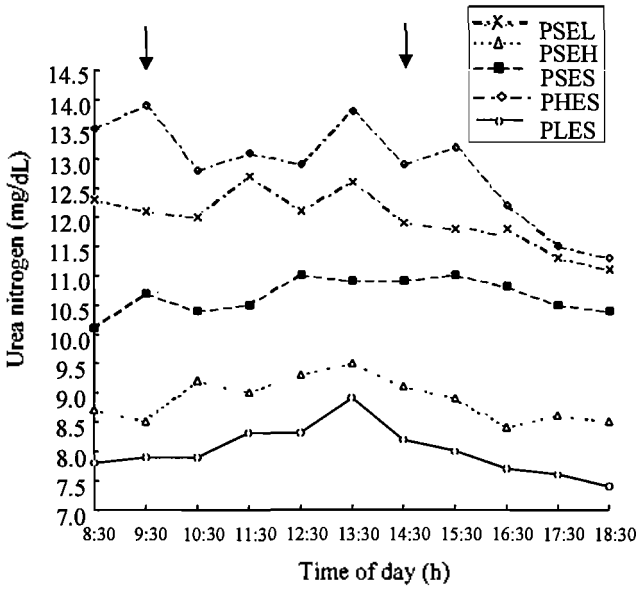

Figure 1. Diurnal variation of blood urea nitrogen (BUN) in dry cows. Each value represents mean of 5 cows. (↓ feeding). PSES, PIES, PHES, PSEH and PSEL are the same as table 1.

three different protein levels (PHES, PSES and PLES) were 12.8±0.8, 10.7±0.3 and 8.0±0.4 mg/dL for high, standard and low protein diets, respectively. This decreasing trend in BUN toward decreasing dietary protein level agreed with the known facts that the level of BUN highly correlated to protein level under the same level of energy intake (DePeters and Ferguson, 1992; Broderick and Clayton, 1997; Hof et al., 1997). Conversely, daily mean BUN value were 8.8±0.4, 10.7±0.3 and 12.0±0.5 mg/dL for high, standard and low energy diets, respectively on different energy levels with iso-nitrogenous diets (PSEH, PSES and PSEL). The trend of negative correlation between dietary energy and BUN also agreed with the previous finding that under same protein level, the BUN decreased as the dietary energy increased (DePeters and Ferguson, 1992; Roseler et al., 1993; Lykos et al., 1997).

Figure 2 presented the diurnal variation of BUN in lactating cow. From iso-energetic diets with three different protein levels (PHES, PSES and PLES), daily mean BUN value were 16.6±0.8, 12.7±0.4 and 9.2±0.6 mg/dL for high, standard and low protein diets, respectively. These BUN values were also higher than those in the dry cows. The trend agreed with the previous finding of positive relation

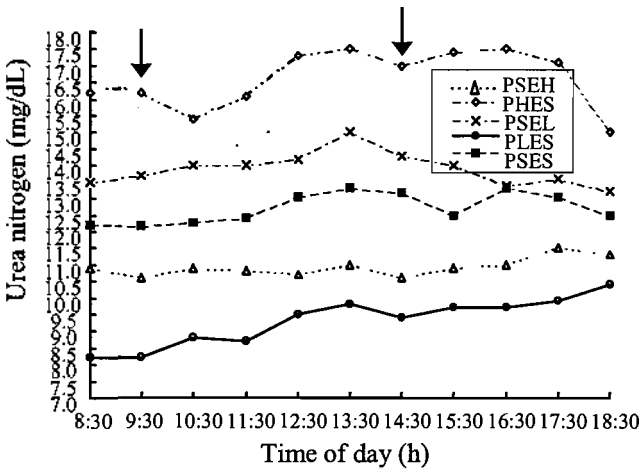

Figure 2. Diurnal variation of blood urea nitrogen (BUN) in lactating cows. Each value represents mean of 6 cows. (↓ feeding). PSES, PLES, PHES, PSEH and PSEL are the same as table 1.

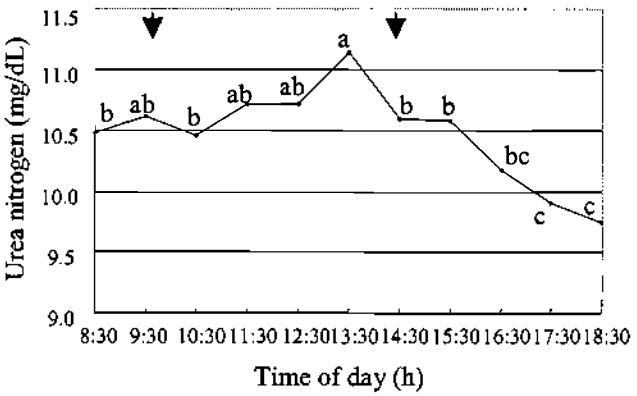

Figure 3. Diurnal variation of average value of blood urea nitrogen (BUN) in dry cows.. Each value represents mean of 25 samples from figure 1. For each sampling time, with different superscripts a, b, and c denote significantly difference ($p<0.05$). (↓ feeding).

between dietary protein and BUN (DePeters and Ferguson, 1992; Broderick and Clayton, 1997; Hof et al., 1997). On the other hand, different energy levels with iso-nitrogenous diets (PSEH, PSES and PSEL), BUN value were 10.9±0.3, 12.7±0.4 and 13.9±0.4 mg/dL for high, standard and low energy diets, respectively. BUN values in lactating cows were also higher than those in the dry cows, but the trend also agreed with the finding that dietary energy negatively correlated to BUN (DePeters and Ferguson, 1992; Roseler et al., 1993; Lykos et al., 1997). BUN value at 4 hours post-feeding from 5 dietary groups was 13.3±2.7 mg/dL and ranged from 9.8 to 17.5 mg/dL in lactating cows. These BUN values in lactating cows were higher than those of the dry cows with averaged of 11.1±1.8 mg/dL and ranged from 8.9 to 13.8 mg/dL. The maximum mean of the BUN concentration showed only 9% higher than that of minimum mean which is different from that 70 to 85% differences obtained from Gustafsson and Palmquist (1993). This may be attributed to the different milk yield and hence different protein intake between trials. Lactating cows also maintained higher BUN level after P.M. feeding than that of dry cows, it may due to higher protein intake in lactating versus dry cows. The findings in lactating cows agreed with Miettinen and Juvonen (1990) that the BUN level gradually increased after the morning feeding and showed a rapid increase 2 to 3 h postprandial, but did not show prominent effects on the BUN level after the afternoon feeding in lactating cows.

Mean BUN values of dry cows in the 5 treatment groups increased after feeding in the morning, reached peak at 4 h after feeding and declined afterward (figure 3). This diurnal pattern agreed with the trend of Gustafsson and Palmquist (1993) and Manston et al. (1981) that BUN reached peak level 2 to 4 h postprandial under feeding once or twice daily. The diurnal variations in BUN, although did not show significantly different among different sampling time of 8:30 to 12:30 ($p>0.05$), but there were significantly different between 13:30 and 14:30 to 18:30 sampling times (figure 3). Therefore the best time for drawing the blood sample was 4 h after morning feeding in dry cows. The mean BUN profiles of lactating cows was slight different from those in dry cows, it slightly increased, and maintained longer after P.M. feeding as compared to the dry cows, and then it also declined (figure 4). There were no significantly differences on BUN values from the blood samples 8:30 to 11:30 and 12:30 to 17:30, respectively. However, the BUN values from the blood samples of 13:30 were significantly higher than those from 8:30 to 11:30

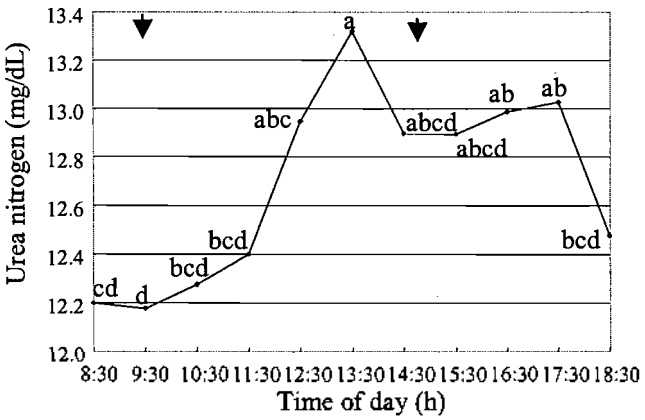

Figure 4. Diurnal variation of average value of blood urea nitrogen (BUN) in lactating cows. Each value represents mean of 30 samples from figure 2. For each sampling time, with different superscripts a, b, c and d denote significantly difference ($p<0.05$). (↓ feeding).

($p<0.05$). Hence the suitable blood sampling time for BUN value in lactating cows was located on 3 to 8 hours after morning feeding, that means from 12:30 to 17:30; but the best time was 4 h after morning feeding.

Milk urea nitrogen

Figure 5 presented the diurnal variation of MUN in lactating cows. The MUN concentrations of morning milk samples are presented in increasing order of fore-strip, mixed and post-strip milk, whereas the pattern of afternoon milk samples is different, which is presented in decreasing order of mixed, post-strip and fore-strip milk. MUN concentration of afternoon mixed milk is 15.2% higher than that of the morning fore-strip milk ($p<0.05$). Reason for peak MUN level seen in the afternoon milk may be attributed to the feeding time between milk sampling. The optimum milk sampling time for urea nitrogen assay appear to be 5 to 6 h post-feeding according to results reviewed by Gustafsson and Palmquist (1993) which indicated that the urea level reflected in blood concentration with 2 h lag period. Since the dairy farmers in Taiwan are practically feeding lactating cows twice daily which are one time 4 h after morning and the other time just before afternoon sampling. In this trial, the level of MUN in the afternoon milk is higher than that of the morning milk which is agreed with the findings of Broderick and Clayton (1997). Gustafsson and Palmquist (1993) also reported that samples took post-strip milk rather than fore-strip milk in the afternoon for urea nitrogen assay, however the mixed milk of afternoon was also available. Therefore, it is suggested that the optimum milk sample for urea nitrogen assay in Taiwan is the mixed milk of the afternoon, and the post-strip milk of the afternoon will be the second choice.

Whole blood ammonia nitrogen

In order to monitor protein and energy intake balances, detecting whole blood ammonia nitrogen (BAN) using clinical diagnostic portable device required suitable blood sampling time. The diurnal variation of BAN was determined to define the optimum sampling time. The diurnal variation of BAN in dry and lactating cows was presented in figures 6 and 7, respectively. It showed that BAN profiles did not denote rhythmic diurnal variation both in dry and lactating cows. The result is not consistent with the pattern of BUN and MUN. Mean BAN value in dry cows showed no significantly difference (figure 8); while in lactating cows, mean BAN value at 11:30 was significantly higher than that of 15:30 to 16:30 ($p<0.05$) (fig. 9). Moreover, the mean BAN values were negatively correlated to the BUN values ($r= -0.78$). Results from BAN value profiles in dry and lactating cows of our study, it is implied that BAN value might not be a good indicator for monitoring the protein and energy intake of cows.

In conclusion, for monitoring protein and energy intake in dairy cows, the optimum blood sampling time for urea nitrogen assay is 4 h after morning feeding in dry cows, and 3 to 8 h after morning feeding in lactating cows, but the best time is 4 h after morning feeding. In Taiwan, the optimum time to sample milk is the afternoon mixed milk since the

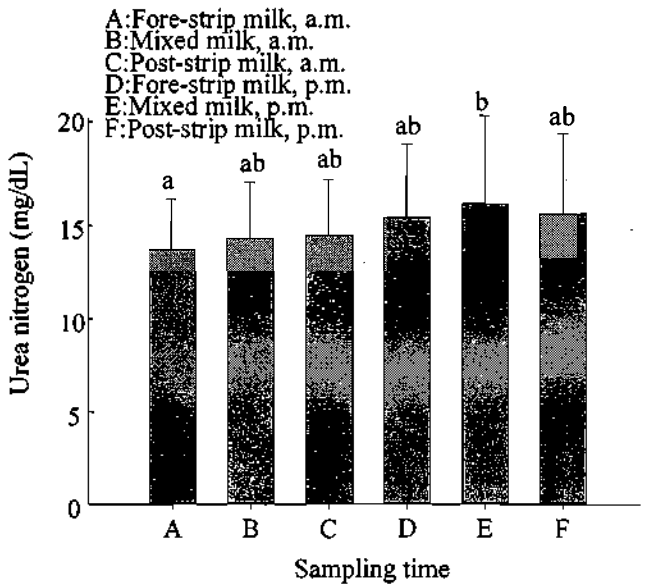

Figure 5. Diurnal variation of milk urea nitrogen (MUN) in lactating cow. Each value represents mean of 18 cows. Two feeding time daily were between C and D. Vertical lines denote the standard error of the mean. For each sampling time, with different superscripts a and b denote significantly differences ($p<0.05$).

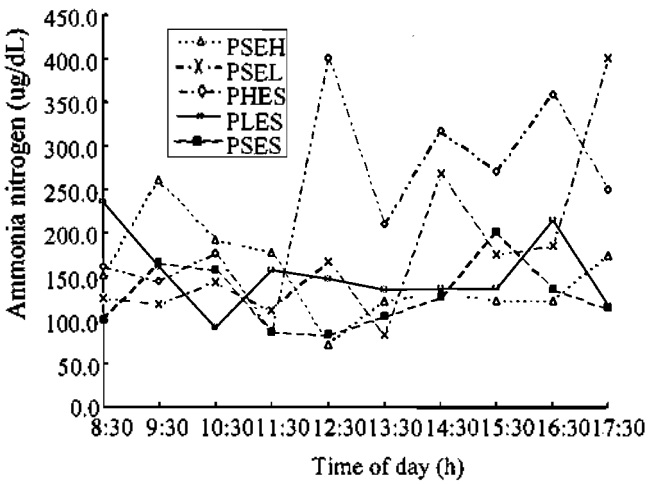

Figure 6. Diurnal variation of whole blood ammonia nitrogen in dry cows. Each value represents mean of 5 cows. Cows were fed once with TMR daily. (↓ feeding). PSES, PLES, PHES, PSEH and PSEL are the same as table 1.

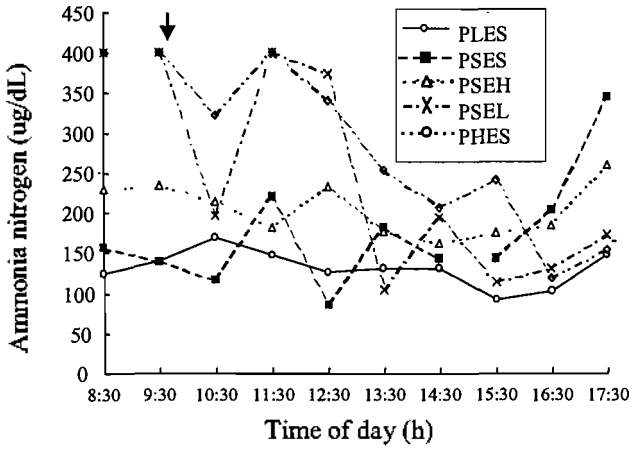

Figure 7. Diurnal variation of whole blood ammonia nitrogen in lactating cows. Each value represents mean of 6 cows. Cows were fed once with TMR daily. (↓ feeding). PSES, PLES, PHES, PSEH and PSEL are the same as table 1.

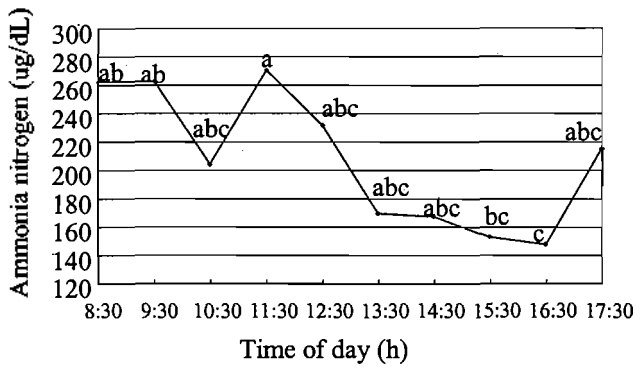

Figure 9. Diurnal variation of average value of whole blood ammonia nitrogen in lactating cows. Each value represents mean of 30 samples from figure 7. Cows were fed once with TMR daily. For each sampling time, with different superscripts a, b and c denote significantly difference ($p<0.05$). (↓ feeding).

feeding regime of dairy farmer is practically feeding twice between the two times daily milking. Taking post-strip milk rather than fore-strip milk in the afternoon sampling in the farm that did not install volumetric vial and the mixed milk is not available. Therefore, it is suggested that the BAN value is not a good indicator for monitoring the protein and energy intake.

ACKNOWLEDGEMENTS

The authors would like to express appreciation to Dr. Yuh-Jia Huang, of the Taiwan Livestock Research Institute, Department of Animal Breeding, for his assistances and suggestions in statistical analysis of this paper.

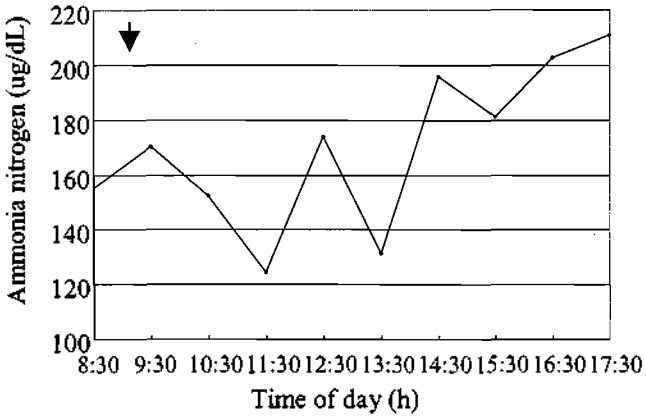

Figure 8. Diurnal variation of average value of whole blood ammonia nitrogen in dry cows. Each value represents mean of 25 samples from figure 6. Cows were fed once with TMR daily. For each sampling time, it denotes no significantly difference. (↓ feeding).

REFERENCES

AOAC. 1990. Official Methods of Analysis. 15th Ed. Association of Official Analytical Chemists. Arlington, VA. U.S.A.

Broderick, G. A. and M. K. Clayton. 1997. A statistical evaluation of animal and nutritional factors influencing concentrations of milk urea nitrogen. J. Dairy Sci. 80:2964-2971.

Carroll, D. J., B. A. Barton, G. W. Anderson and R. D. Smith. 1988. Influence of protein intake and feeding strategy on reproductive performance of dairy cows. J. Dairy Sci. 71:3470-3481.

Chen, K. H., J. T. Huber, C. B. Theurer, D. V. Armstrong, R. C. Wanderley, J. M. Simas, S. C. Chan and J. L. Sullivan. 1993. Effect of protein quality and evaporative cooling on lactational performance of Holstein cows in hot weather. J. Dairy Sci. 76:819-825.

Crocker, C. L. 1967. Rapid determination of urea nitrogen in serum or plasma without deproteinization. Am. J. Med. Technol. 33:361.

DePeters, E. L. and J. D. Ferguson. 1992. Nonprotein nitrogen and protein distribution in the milk of cows. J. Dairy Sci. 75:3192-3209.

DiGiorgia, J. 1974. Nonprotein nitrogenous constituents. In Henry, J. R., D. C. Canon and J. W. Winkelman : Clinical chemistry. 2nd ed. Hagerstown, Harper and Row. pp. 511-514.

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

Garcia-Bojalil, C. M., C. R. Staples, W. W. Thatcher, J. D. Savio and C. Risco. 1992. Effect of dietary protein degradability and calcium salts of long chain fatty acids (CaLCFA) on reproductive performance of lactating Holstein cows. J. Dairy Sci. 75 (Suppl. 1):203.

Gustafsson, A. H. and D. L. Palmquist. 1993. Diurnal variation of rumen ammonia, serum urea, and milk urea in dairy cows at high and low yields. J. Dairy Sci. 76:475-484.

Harris, B., Jr. 1995. MUN and BUN values can be valuable management tools. Feedstuffs. 67(42):14.

Hof, G., M. D. Vervoorn, P. J. Lenaers and S. Tamminga. 1997. Milk urea nitrogen as a tool to monitor the protein nutrition of dairy cows. J. Dairy Sci. 80:3333-3340.

Holtz, C. R., R. D. Smith and C. J. Sniffen. 1986. Reproductive and metabolic responses of dairy cattle to the level and degradability of dietary protein. J. Dairy Sci. 69(Suppl.1):243.

Hutjens, M. F. and J. A. Barmore. 1995. Milk urea test gives us another tool. Hoard's Dairyman. May (25):401.

Ide, Y., K. Shimbayashi and T. Yonemura. 1966. Effect of dietary conditions upon serum and milk urea nitrogen in cows. I. Serum and milk urea nitrogen as affected by protein intake. Jpn. J. Vet. Sci. 28:321-327.

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

Jordan, E. R., T. E. Chapman, D. W. Holtan and L. V. Swanson. 1983. Relationship of dietary crude protein to composition of uterine secretions and blood in high-producing postpartum dairy cows. J. Dairy Sci. 66:1854-1862.

Kirchgessner, M., M. Kreutzer and D. Roth-Maier. 1986. Milk urea and protein content to diagnose energy and protein malmutrition of dairy cows. Arch. Anim. Nutr. 36:192-197.

Lykos, T., G. A. Varga and D. Casper. 1997. Varying degradation rates of total nonstructural carbohydrates: Effects on ruminal fermentation, blood metabolites, and milk porduction and composition in high producing Holstein cows. J. Dairy Sci. 80:3341-3355.

Manston, R., G. J. Rowlands, W. Little and K. A. Collis. 1981. Variability of the blood composition of dairy cows in relation to time of day. J. Agric. Sci. (Camb.) 96:593-598.

Miettinen, P. V. A. and R. O. Juvonen. 1990. Diurnal variations of serum and milk urea levels in dairy cows. Acta Agric. Scand. 40:289-296.

National Research Council. 1989. Nutrient Requirements of Dairy Cattle. 6th rev. Ed. Natl. Acad. Sci. Washington, DC. U.S.A.

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

Refsdal, A. O., L. Baevre and R. Bruflot. 1985. Urea concentration in bulk milk as an indicator of the protein supply at the herd. Acta Vet. Scand. 26:153-163.

Rodriguez, L. A., C. C. Stallings, J. H. Herbein and M. L. McGilliard. 1997. Diurnal variation in milk and plasma urea nitrogen in Holstein and Jersey cows in response to degradable dietary protein and added fat. J. Dairy Sci. 80:3368-3376.

Ropstad, E. and A. O. Refsdal. 1987. Herd reproductive performance related to urea concentration in bulk milk. Acta Vet. Scand. 28:55-63.

Roseler, D. K., J. D. Ferguson, C. J. Sniffen and J. Herrema. 1993. Dietary protein degradability effects on plasma and milk urea nitrogen and milk nonprotein nitrogen in Holstein cows. J. Dairy Sci. 76:525-534.

SAS Institute Inc. 1989. SAS/STAT User's Guide: Version 6. 4th Ed. SAS Institute Inc., Cary, North Carolina.

Schepers, A. J. and R. G. M. Meijer. 1998. Evaluation of the utilization of dietary nitrogen by dairy cows based on urea concentration in milk. 81:579-584.

Staples, C. R., C. Garcia-Bojalil, B. S. Oldick, W. W. Thatcher and C. A. Risco. 1993. Protein intake and reproductive performance of dairy cows: A Review, Proc. Florida Ruminant Nutrition Symposium.

Steel, R. G. D. and J. H. Torrie. 1960. Principles and Procedures of Statistics. McGraw-Hill, New York.

Van Soest, P. J., J. B. Robertson and B. A. Lewis. 1991. Methods for dietary fiber, neutral detergent fiber, and nonstarch polysaccharides in relation to animal nutrition. J. Dairy Sci. 74:3583-3597.