Investigation of different levels of RDP in the rations of lactating cows and their effects on MUN, BUN and urinary N excretion

Ali Moharrery

Animal Science Department. Shahrekord University, Iran

Corresponding author: Dr. Ali Moharrery. Animal Science Department. Agricultural College, Shahrekord University, Iran - Tel. +98 381 4424401 - Fax: +98 381 4424412 - Email: moharrery@yahoo.com

Paper received September 21, 2003; accepted February 05, 2004

ABSTRACT

Twenty-one multiparous Holstein cows in the late stages of their lactation period were used in complete randomized design to investigate the effect of rumen degradable protein on milk urea nitrogen (MUN) and some blood metabolites. Experimental periods were 6 weeks in length, with days 1 to 14 used for adjustment and weeks 3 to week 6 used for sampling (urine, blood, and milk). Three concentrations of a rumen-degradable protein (RDP) supplement according to National Research Council recommendations (9.3, 11.4, and 14% of dry matter intake) were treatments. Dietary RDP content altered both total urinary N and urinary N concentration, leading to increased urinary output. Estimations for microbial protein yield were compared with the measured excretion of purine derivative as yeast RNA equivalent, in urine. No significant effect of concentration of RDP supplement was detected on microbial N production. Plasma cholesterol concentration decreased linearity by increasing RDP concentration in diets (P<.05). In this regard, milk urea nitrogen, as well as triglyceride concentration in plasma, was not associated with dietary RDP concentration. To ensure a correct balance between energy and protein available in the rumen and consequently higher N efficiency for late lactation cows, a MUN content of 15.1mg/dl milk is the upper margin. Milk urea N is a simple and noninvasive measurement that can be used to monitor N efficiency in dairy cows.

Key words: Dairy cattle, Milk urea nitrogen (MUN), Rumen degradable protein (RDP)

RIASSUNTO

EFFETTI DELLA SOMMINISTRAZIONE DI DIFFERENTI LIVELLI DI RDP A BOVINE IN LATTAZIONE SU MUN, BUN ED ESCREZIONE URINARIA DI AZOTO.

In questo lavoro sono stati studiati in ventuno bovine di razza Holstein in lattazione avanzata gli effetti della somministrazione di proteina degradabile a livello ruminale (RDP) sul contenuto di azoto ureico del latte (MUN) e su alcuni metaboliti ematici.

La prova è stata condotta utilizzando un disegno sperimentale randomizzato. Il periodo sperimentale è stato di sei settimane, nel quale le prime due settimane (1-14 d) sono state utilizzate come periodo di aggiustamento, mentre a partire dal termine della seconda settimana sino alla sesta settimana sono stati effettuati i prelievi (urine, sangue e latte). I trattamenti, costituiti da tre differenti concentrazioni di RDP (9,3; 11,4 e 14% sulla sostanza secca) sono stati calcolati in accordo con quanto indicato dal National Research Council. La stima della produzione di proteina microbica è stata comparata con la purina escreta con l’urina, derivata come equivalente dell’RNA nel lievito. Le diverse concentrazioni di proteina degradabile a livello ruminale non hanno avuto effetti significativi sulla produzione di azoto batterico. Dal risultato si evince che la misurazione dell’azoto ureico nel latte è una misurazione semplice e non invasiva e può essere utilizzata per monitorare l’escrescita di azoto in bovine in lattazione.

Parole chiave: Bovine da latte, Azoto ureico nel latte (MUN), Proteina degradabile a livello ruminale (RDP).
Introduction

Interest has been developed in using milk urea nitrogen (MUN) levels as a method of monitoring the dietary efficiency and protein utilization of dairy herds (Wood et al., 2002). Protein eaten by the cow is classified into two components: rumen degradable protein (RDP) or rumen undegradable protein (UDP). Microbes in the rumen metabolize rumen degradable feed proteins into ammonia and volatile fatty acids (VFAs). Ammonia is in turn used by the microbes for growth (microbial protein synthesis). The extent to which ammonia is used is largely determined by the availability of energy provided by readily fermentable carbohydrates. An improper balance between rumen degradable protein and fermentable carbohydrate (energy) results in inefficient rumen microbial growth. Ammonia not used by rumen microbes is absorbed into the bloodstream and is toxic to body tissues in high concentrations. If the amount of rumen degradable protein is too high relative to rapidly fermentable carbohydrate (energy) more ammonia enters the bloodstream since there is insufficient energy available to rumen microbes for its incorporation into microbial protein. The body deals with ammonia primarily in the liver by converting it to urea, a small, non-toxic molecule, composed of nitrogen, oxygen and carbon. This process requires energy. Urea enters the bloodstream and is cycled back into the rumen and removed from the body through the urine. As a small, water soluble molecule it freely diffuses into other body fluids such as saliva and milk. Therefore, milk urea nitrogen (MUN) reflects blood urea nitrogen (BUN) which is an indication of the efficiency of protein metabolism in the rumen. On the other hand, the amount of urea excreted in urine by a cow is directly proportional to the concentration of urea in blood, and this amount is proportional to the concentration of urea in milk (Roseler et al., 1993). Therefore, milk urea N should be a good predictor of urinary N excretion by dairy cows (Ciszuk and Gebregziabher, 1994; Kohn et al., 1997; Jonker et al., 1998).

Blood urea nitrogen (BUN) concentration is variable and is affected by rumen degradable protein intake, undegradable protein intake, energy intake, water intake, liver function and elimination in the urine. BUN varies throughout the day with levels highest 4-6 hours after feeding and lowest just before feeding. The level of urea in the milk (MUN) reflects (BUN), but is less variable since milk is produced and stored in the mammary gland between milkings. MUN is a convenient way to estimate blood urea nitrogen levels and may be useful in monitoring protein nutrition in the dairy herd.

In general, herd mean milk urea has a positive relationship with levels of dietary CP, RDP, and UDP, and a negative relationship with levels of nonfiber carbohydrates (NFC), the NFC:CP ratio, and the NFC:RDP ratio (Baker et al., 1995; Godden et al., 2001). Housing factors (tie stall vs. free stall), TMR versus component feeding, feeding frequency, and synchrony of offering forages and concentrates are not associated with herd mean milk urea (Godden et al., 2001). Another factor such as stage of lactation (range 100 to 200 DIM) is not a significant factor affecting concentration of plasma or milk N concentration (Roseler et al., 1993). However, controlling for ration nutrient composition showed seasonal change is associated with herds mean milk urea, with highest levels accruing between July and September (Godden et al., 2001).

The main objectives of this study were to determine the MUN concentration and urinary excretion under different levels of RDP in the rations of lactating cows.

Material and methods

Diets and cow management

Twenty-one multiparous Holstein cows with 671 ± 64 (mean ± SD) kg of BW in the late stage of the lactation period (260 ± 50 DIM) and 15 ± 5 kg/d milk yield, were used in complete randomized design to investigate the effect of rumen degradable protein on milk urea nitrogen (MUN) and some blood metabolites. Cows were added to the study individually during the experimental period. The location of the dairy farm was close to Esfahan, which is located in central Iran, between
latitudes 30-35' and 34-45' E. and longitudes 49-31' and 55-27' E.

Experimental periods were 6 weeks in length, with days 1 to 14 used for adjustment and week 3 to week 6 used for sampling (urine, blood, and milk). During the experimental period the mean of relative humidity was 21.5% and Maximum, Minimum and mean temperatures were 35.2, 21.7 and 28.4, respectively. Experimental diets were formulated to meet the requirements according to NRC (1989) for Holstein cows of 671 kg of BW and producing 15 to 20 kg of milk per day. Experimental diets were formulated such that their total CP content was the same (Table 1). Diets differed in the amount of rumen-degradable protein

| Rations |
|---------|
| A       |
| B       |
| C       |
| Alfalfa hay, long | 17 | 20 | 25 |
| Corn silage | 30 | 25 | 20 |
| Barley grain | 3 | 11 | 32 |
| Corn grain | 15.5 | 15.5 | 13.5 |
| Beet pulp | 15 | 1.5 | 2 |
| Cotton seed meal | .5 | 20 | 2 |
| Soybean meal | 10.5 | 1 | .5 |
| Fish meal | 4 | .5 | .2 |
| Wheat bran | 2 | 3 | 1 |
| Urea | - | .2 | 1.5 |
| Dicalcium phosphate | 1.05 | .86 | .85 |
| Limestone | .5 | .5 | .5 |
| Salt | .7 | .7 | .7 |
| Sodium bicarbonate | .25 | .25 | .25 |

### Nutrient composition (% DM):

| CP | RDP | UDP | CP | RDP | SIP |
|----|-----|-----|----|-----|-----|
| 18.4 | 9.4 | 16 | 54 | 22 | 19.2 |
| 18.3 | 11.4 | 16 | 67 | 26 | 19.4 |
| 18.3 | 13.9 | 16.3 | 78 | 45 | 17.5 |
| 18.3 | 18.3 | 16.3 | 78 | 45 | 17.5 |
| 18.3 | 18.3 | 16.3 | 78 | 45 | 17.5 |
| 18.3 | 18.3 | 16.3 | 78 | 45 | 17.5 |

CP = crude protein; RDP = rumen degradable protein; UDP = undegradable protein; SIP = soluble intake protein; ADF = acid detergent fiber; eNDF = effective neutral detergent fiber; NFC = non fiber carbohydrate; TDN = total digestible nutrient; NE = net energy for lactation.

1 Calculated according to NRC (1989) for diet component.

2 Analyzed values in laboratory (see text).

3 Protein degradability of diets. Based upon 12-h in situ degradability as a percentage of diet total protein.

4 Calculated according to CNCP (2001) for diet component.
(RDP), and UDP (9.3, 11.4, and 14% RDP of dry matter intake). The ratio of RDP/UDP in the rations of A, B and C were 50:50, 65:35 and 80:20, respectively. Diet B was designated to provide 100% of RDP and UDP requirements according to NRC (1989). The A and C diets were designated for low and high RDP, respectively. Concentrate parts were formulated with varying concentrations of urea, soybean, cottonseed and fish meals to achieve the desired levels of RDP and UDP. Urea as a common source of NPN provided the major addition of RDP. Soybean and cottonseed meals provided less RDP but still degradable protein, while fish meal was a source of UDP. The concentrates were top-dressed onto the alfalfa hay and corn silage mixture and completely mixed together before feeding. Experimental diets were fed as a TMR in equal amounts six times per day. During the initial 14 days of diet adaptation, cows were offered the treatment diets on an ad libitum basis to ensure close to 10% orts (as-fed basis). Feed offered from d 15 to the end of each week (week 3 to week 6) was restricted to nearly 95% of the average feed intake, which was calculated from the previous week. Animals had access to clean and fresh water at all times, but the amount of water consumption was not measured.

In situ measurements

Dacron bags containing 2 g of one of the three diets were suspended in the rumen of the fistulated young bull cows at the end of the experiment. Bag size, type, and suspension method were as described by Roe et al., (1991). Duplicate bags were introduced into the rumen after an initial 5-min warm water soaking during h 0, 3, 6, 12, and 24. All bags were removed at the 24-h endpoint, hand rinsed in cold tap water for 5 min, and dried at 60°C for 72 h (Nocek, 1985; Roseler et al., 1993). An estimate of the extent of rumen protein degradation was based upon the 12-h in situ protein residue (Table 1).

Sample collection and analysis

The TMR for each of the three treatment diets were sampled weekly and composited and analyzed for DM, CP, RDP (in situ), and soluble intake protein (SIP). Soluble intake protein was measured as described by Licitra et al. (1996). Cows were milked three times daily at 0700, 1500, and 2300 h, with milk weights recorded at the end of each week. Because the fat content in milk was about 3.2%, milk yield was corrected for 3.2% fat as previously described by Overman and Gaines (1933). Milk samples were collected and analyzed for fat, CP, and MUN. Milk fat was analyzed by the Gerber method and milk CP was analyzed by the Kjeldahl method using a coefficient of 6.38 for converting of nitrogen content to CP.

Blood samples were taken at 2 h after feeding. Blood samples were taken from the coccygeal artery or vein into Vacutainers containing heparin held at 4°C maximum 4 h until plasma was prepared. These samples were centrifuged for 15 min at 2500×g, and plasma was harvested and stored at -25°C.

The testing for urea concentration involved taking a sample of plasma or milk and using a spectrophotometer to measure the change in color in 520 nm wavelength when a reagent was added that acts specifically with urea. Usually this method (Marsh et al., 1957) involves a dye agent, diacetylmonoxime, that reacts with the urea molecule to form a pink color.

Plasma samples were also analyzed for triglyceride using an enzymatic and colorimetric procedure (Kit 10-525, Ziestchem Diagnostic kit, Tehran, Iran) and for cholesterol by an enzymatic procedure (Kit 10-508, Ziestchem Diagnostic kit, Tehran, Iran).

Spot urine samples were also taken on week 3 to week 6 and analyzed for creatinine and purine (yeast RNA equivalent) derivatives. Urine was analyzed for creatinine using a colorimetric procedure (Darman Kave Kit, Isfahan, Iran) and for cholesterol by an enzymatic procedure (Kit 10-508, Ziestchem Diagnostic kit, Tehran, Iran).

Total N content in urine samples was analyzed by using Kjeldahl method.
Statistical analysis

The data were analyzed using a general linear model procedure of SAS (1988). The complete randomized model was used to analyze milk yield, milk constituent, urine parameter and blood component data on the mean of the samples taken during the experimental period. Feed intake, BW, and DIM were used as a covariant in the model, but because those effects were not significant (P > .05) these variables were omitted from the model. Duncan's multiple range test (SAS, 1988) (P < .05) was used to test the significance of difference between means. The stepwise procedure was used to choose the best regression equation to select the best explanatory model with the lowest number of variables.

Results and discussion

The results of the experiment are presented in Table 2. As planned, dry matter intake and total N intake were not different among dietary treatments. The increase in dietary RDP as a percentage of CP was accompanied by an increase in N% in urine and N% in milk (P<0.05). In this regard, the increase in RDP percentage is a main cause for the reduction in milk production, N-efficiency, as well as blood cholesterol, (P<0.05). Other parameters such as, MUN, plasma urea nitrogen (PUN), triglyceride (TG), urine volume and total N excretion as a g/d were not linearly affected by RDP percentage. In this regard, additional variables such as dietary NFC are affected by the rumen metabolism. In the C ration, higher RDP concentration is accompanied by higher NFC; for this reason the availability of fermentable carbohydrate provided better conditions to capture N by microorganisms in the rumen to compare with the B ration. These results agree with Godden et al. (2001), who reported that MUN had a positive relationship with CP and RDP and had a negative relationship with NFC. On the contrary, Kauffman and St-Pierre (2001) reported that NFC concentration is accompanied by higher NFC; for this reason the availability of fermentable carbohydrate provided better conditions to capture N by microorganisms in the rumen to compare with the B ration. These results agree with Godden et al. (2001), who reported that MUN had a positive relationship with CP and RDP and had a negative relationship with NFC. On the contrary, Kauffman and St-Pierre (2001) reported that NFC concentrations are affected by the rumen metabolism.

Table 2. Effect of different levels of RDP in the ration of lactating cows and their effects on nitrogen metabolism, blood metabolite and milk components.

| Item                | Rations | SEM 1 | P>F 2 |
|---------------------|---------|-------|-------|
| Milk yield kg/d     | A       | 17.27 | 1.0974 | .0001 |
| N efficiency (%)    | B       | 26.58 | 1.6102 | .0001 |
| 3.2% FCM kg/d      | C       | 19.07 | 1.3219 | .0001 |
| Feed intake kg DM/d |         | 16.00 | 2.038  | .4986 |
| Fat in milk %       |         | 3.63  | .0949  | .0081 |
| N in milk %         |         | .57   | .0142  | .0036 |
| Total N intake g/d  |         | 397.8 | 3.0034 | .5813 |
| MUN mg/dl           |         | 15.29 | .7250  | .0131 |
| PUN                 |         | 16.23 | .7426  | .0001 |
| TG                  |         | 14.27 | .6305  | .0011 |
| Chol                |         | 126.37| .7324  | .0001 |
| Urinary N g/d       |         | 147.43| 2.1866 | .0001 |
| Urine volume L/d    |         | 18.839| 1.1399 | .0004 |
| Urine N %           |         | .67   | .0136  | .0001 |
| Microbial N g/d     |         | 345.8 | 60.532 | .8812 |

MUN= milk urea nitrogen, PUN= plasma urea nitrogen, TG= triglyceride, Chol= cholesterol.

1 SEM= Standard error of the mean.
2 Probability of a significant effect of diet.
Urinary volume parallel with total urinary N excretion increased \((P<0.01)\). This effect on urine volume results from a progressive increase in urea in the blood that exceeds the capacity of the kidneys to concentrate the urea for excretion in the urine (Moscardini et al., 1998; Sannes et al., 2002). These results agree with Ciszuk and Gebregziabher (1994), who mentioned that the amount of urea excreted in urine by a cow is directly proportional to the concentration of urea in blood. In this regard, Kauffman and St-Pierre (2001) reported which UN excretion is related linearly to MUN over the range of MUN concentration of 5 to 14 mg/dl, but this range of MUN is insufficient to adequately challenge the assumption of a linear relationship between UN and MUN. Nevertheless, milk urea N should be a good predictor of urinary N excretion (UN) by dairy cows (Broderick, 1995; Ciszuk and Gebregziabher, 1994; Kohn et al., 1997; Jonker et al., 1998).

No significant effect of concentration of RDP supplement was detected on the excretion of purine derivative as yeast RNA equivalent in urine. Therefore, microbial N, which corresponds to purine derivative (Moharrery and Das, 2002), was not affected by RDP concentration in the rations \((P>0.05)\). These results agree with Moscardini et al. (1998) who has reported that the urinary excretion of purine derivatives was not significantly affected by the concentration of RDP in the ration. The correlation of MUN to BUN is high, \((r=0.70)\) (Figure 1). MUN reflects the level of PUN even across differences in degradable and undegradable protein because milk urea nitrogen arises from the passive transfer of blood urea into milk. In the present study, when MUN was regressed against PUN, a linear relationship was determined, with an intercept of 8.83 mg/dl (Figure 1). These results agree with other studies (Broderick and DeLeon Gatti, 1998; Lyatuu and Eastridge, 1998) and confirms that urea in the blood system is the major source of urea nitrogen in milk. Several factors should be considered when interpreting urea nitrogen data. These include such factors as diurnal variations and the lag time between the peak level of the PUN and that of MUN (Baker et al., 1995; Lyatuu and Eastridge, 1998), and the difference in specific gravity between various components of milk solids that could alter the relative concentration of urea in milk (Roseler et al., 1993).

In the present study, dietary CP content was not changed, but manipulated dietary RDP content altered both total urinary N and urinary N

**Figure 1.** MUN is related to plasma urea nitrogen

\[
\text{MUN} = 0.4512 \times \text{PUN} + 8.8307 \quad (R^2 = 0.4573)
\]
concentration, leading to increased urinary output (Table 2). This increase in urinary N (UN) from cows fed diets with increasing levels of RDP is indicative of inefficient capture of ruminal NH3-N for microbial protein production, resulting in increased portal uptake of NH3-N, which is detoxified to urea in the liver and excreted in urine (Haig et al., 2002). The relationship of urinary nitrogen to MUN has been plotted and is shown in Figure 2. The correlation between total urea excretion in urine with the urea content in milk was 0.75. Urea, because it is a small neutral molecule, readily diffuses across cellular membranes (Jonker et al., 1998). 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 UN (Ciszuk and Gebregziabher, 1994; Kohn et al., 1997). The results confirm that PUN and MUN represent the urea pool in the body and urea excretion and may, therefore, have the potential to serve as a parameter for the nitrogen losses in dairy cows.

Concentration of MUN was negatively correlated to N-efficiency (milk N: N intake) and explained about 70% of the variation in MUN concentration (Figure 2). When N-efficiency was regressed against MUN, a linear relationship was determined with a negative of coefficient (-1.73) (Figure 3). Solving the regression equation (Figure 3) for the MUN corresponding to the average N-efficiency from all treatments (mean efficiency in three rations is 22.5%) yielded a MUN estimate of 15.1 mg N/dl (26.16/1.73=15.1); MUN greater than this would imply an N-efficiency of less than 22.5%. This MUN value which is margin for higher N-efficiency is lose to the value of 16.1 mg/dl observed by Broderick and De Leon Gatti (1998).

Recent studies have shown that high concentrations of milk urea nitrogen have negative effects on the cheese making processes. In particular, high concentrations of urea are the direct or indirect cause of numerous problems, such as an increase in coagulation time, the formation of a more fragile and less structured curd, premature development of irregular fermentation, and a more intense proteolysis. With the analysis of MUN, it is possible to detect possible problems in the cheese making process (Miles and Jacob, 1999). An analyzer capable of carrying out the urea test on milk is used with considerable profit in dairies.

As producers are encouraged to increase efficiency of nitrogen utilization from the perspective of minimizing the release of excess nitrogen into the environment, measuring average herd MUN

**Figure 2. Urinary-N excretion is related to MUN**

![Graph showing the relationship between urinary-N excretion and milk urea N](image-url)
by sampling from the bulk tank may be a way of monitoring efficiency of nitrogen utilization on a whole farm basis.

Conclusions

Results from this experiment indicate that milk volume, altering dietary protein degradability, and NFC intake influenced MUN concentration. Urea in body fluids including milk reflects N inefficiency due to both excess protein degradation and deficiency of fermentable organic matter in the rumen. As dietary RDP content increased, urinary N concentration was increased, and it was the primary route of waste N excretion. Plasma cholesterol concentration decreased linearity by increasing RDP concentration in diets (P < .05). In this regard, milk urea nitrogen as well as triglyceride concentration in plasma was not associated with dietary RDP concentration. To ensure a correct balance between energy and protein available in the rumen and consequently higher N efficiency for late lactation cows, a MUN content of 15.1 mg/dl milk is the upper margin.

REFERENCES

Baker, L.D., Ferguson, J. D., Chalupa, W., 1995. Responses in urea and true protein of milk to different protein feeding scheme for dairy cows. J. Dairy Sci. 78:2424-2434.

Broderick, G.A., 1995. Use of milk urea as an indicator of nitrogen utilization in the lactating dairy cow. In: US Dairy Forage Research Center (ed.) Research Summaries. USDA-ARS, University of Wisconsin-Madison, USA, pp 1-3.

Broderick, G.A., DeLeon Gatti, N., 1998. Potential of Biochlor and fermented for improving nitrogen utilization in lactating dairy cows. In: US Dairy Forage Research Center (ed.) Research Summaries. USDA-ARS, University of Wisconsin-Madison, USA, pp 83-86.

Ciszuk, A.U., Gebregziabher, T.,1994. Milk urea as an estimate of urine nitrogen of dairy cows and goats. Acta Agric. Scand. A. 44:87-95.

Cornell Net Carbohydrate and Protein System, 2001. A Manual for Using the Cornell Net Carbohydrate and Protein System for Evaluating Cattle Diets. CNCP Ver 5. Cornell Univ., Ithaca, NY, USA.

Godden, S.M., Lissemore, K.D., Kelton, D.F., Lesli, K.E., Walton, J.S., Lumsden, J.H., 2001. Relationship between milk urea concentration and nutritional management, production, and economic variables in Ontario dairy herds. J. Dairy Sci. 84:1128-1139.

Figure 3. N-Efficiency in the cow is closely related to MUN
Haig, P.A., Mutsvangwa, T., Spratt, R., McBride, B.W., 2002. Effects of dietary protein solubility on nitrogen losses from lactating dairy cows and comparison with predictions from the Cornell net carbohydrate and protein system. J. Dairy Sci. 85:1208-1217.

Kohn, R.A., Janker, J.S., Erdman, R.E., High, J., 1997. Milk urea nitrogen: theory and practice. pp 83-90 in Proc. Maryland Nutr. Conf. Feed Manuf., Maryland University, College Park, Baltimore, USA.

Kuffman, A.J., St-Pierre, N.R., 2001. The relationship of milk urea nitrogen to urine nitrogen excretion in Holstein and Jersey cows. J. Dairy Sci. 84:2284-2294.

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

Licitra, G., Hernandez, T.M., Van Soest, P.J., 1996. Standardization of procedures for nitrogen fractionation of ruminant feeds. Anim. Feed Sci. Technol. 57:347-358.

Lyatuu, E.T., M.L. Eastridge, M.L., 1998. Nutritional factors affecting milk production, milk composition, milk urea nitrogen and plasma urea nitrogen. In: The Ohio State Univ. (ed.) Research and Reviews: Dairy, Special Circular. Extension Research Bulletin, Columbus, USA, pp 163-199.

Marsh, W.H., Fingerhut, B., Kirsch, E., 1957. Determination of urea N with the diacetyl method and an automatic dialyzing apparatus. Am. J. Clin. Path. 28:681-688.

Miles, R., Jacob, J., 1999. Milk urea nitrogen. SPES- FEED NEWS, Autumn 1999. Home page address: http://www.spesfeed.co.za/news.htm

Moharrery, A., Das, T.K., 2002. Correlation between microbial enzyme activities in the rumen fluid of sheep under different treatments. Reprod. Nutr. Dev. 41:513-529.

Moscardini, S., Wright, T.C., Luimes, P.H., McBride, B.W., Susmel, P., 1998. Effects of rumen-undegradable protein and feed intake on purine derivative and urea nitrogen: Comparison with predictions from the Cornell Net Carbohydrate and Protein System. J. Dairy Sci. 81:2421-2329.

Nocek, J.E., 1985. Evaluation of specific variables affecting in situ estimates of ruminal dry matter and protein digestion. J. Anim. Sci. 60:1347-1352.

National Research Council, 1989. Nutrient Requirements of Dairy Cattle. 6th rev. ed. National Academy Press, Washington, DC, USA.

Overman, O.R., Gaines, W.L., 1933. Milk-energy formulas for various breeds of cattle. J. Agri. Res. 46:1109-1116.

Roe, N.H., Chase, L.E., Sniffen, C.J., 1991. Comparison on in vitro techniques to the in situ technique for estimation of ruminal degradation of protein. J. Dairy Sci. 74:1632-1637.

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