

# 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

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## ABSTRACT

Six multiparous Holstein cows were used in a  $6 \times 6$  Latin square to investigate the ability of the Cornell Net Carbohydrate and Protein System to predict accurately rumen microbial yield, plasma urea N, and milk urea N. Estimations for microbial protein yield were compared with the measured excretion of purine derivative N in urine. A  $3 \times 2$  factorial arrangement of treatments was adopted. Three concentrations of a rumen-undegradable protein (RUP) supplement (4.5, 14.9, and 29.1% of dry matter intake) and two levels of feed restriction (90 and 80% of ad libitum intake) were the corresponding factors. No effect of concentration of RUP supplement or feed restriction was detected on the excretion of purine derivative N in urine (mean, 18.5 g/d). Conversely, the Cornell system predicted a linear decrease in metabolizable protein from bacteria as the concentration of the RUP supplement increased. The Cornell system also predicted a significant reduction in metabolizable protein of microbial origin as feed restriction was increased. Measured values and values derived from the Cornell system for plasma and milk urea N increased linearly as the concentration of the RUP supplement increased. The Cornell system overpredicted milk urea N for cows consuming the highest RUP concentration. Predictions by the Cornell Net Carbohydrate and Protein System were of limited value because the empirical nature of the model is insufficiently rigorous to yield accurate predictions under the conditions described herein.

(**Key words:** dairy cows, rumen-undegradable protein, purine derivatives, urea N)

**Abbreviation key:** CNCPS = Cornell Net Carbohydrate and Protein System, MUN = milk urea N, PD = purine derivatives, pMUN = MUN predicted by the CNCPS, pPUN = PUN predicted by the CNCPS, PUN = plasma urea N.

## INTRODUCTION

Nutritional strategies for dairy cow nutrition are progressing, and modifications of the AA content of postruminal digesta are useful in the development of such strategies. The use of high quality RUP sources is becoming an increasingly practical way to supply high producing dairy cows with appropriate amounts of AA to promote milk production with a desirable protein content (12). The strategic use of RUP is most efficient when these supplements are coordinated with maximized microbial protein yield. Dietary regimens that reduce microbial protein yield affect the quantity and quality of protein delivered to the small intestine. Therefore, the ability to monitor microbial protein yield is important. Maximal microbial protein yield depends on the synchronous availability of N and energy within the rumen. Measurements such as plasma urea N (PUN) and milk urea N (MUN) have been described as good indicators of the ratio of ruminal N to energy balance (22).

One method available to evaluate the nutrition of dairy cows is the Cornell Net Carbohydrate and Protein System [CNCPS; (9)]. The CNCPS is a deterministic, static model that estimates, among other outputs, metabolizable protein of microbial origin, PUN, and MUN. The CNCPS assumes the rumen microbial yield to be proportional to the rate of carbohydrate digestion, although some adjustment is made for N and forage NDF sources in this calculation (23).

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TABLE 1. Chemical analysis of the basal diet.<sup>1</sup>

Chemical	$\bar{X}$	SE
DM, %	51.17	0.90
	— (% of DM) —	
CP	8.00	0.09
Soluble protein	36.67	1.09
NDF	36.15	1.75
ADF	20.15	1.01
NDF Insoluble protein, % of CP	11.68	0.57
ADF Insoluble protein, % of CP	6.48	0.55
Crude fat	3.08	0.09
NSC <sup>2</sup>	48.08	1.71
NE <sub>L</sub> , Mcal/kg	1.61	0.02
C	0.83	0.08
P	0.34	0.06

<sup>1</sup>Means are based on six analyses (DM).

<sup>2</sup>Nonstructural carbohydrates.

Microbial N flow to the duodenum can also be estimated by measurement of the excretion of purine derivatives (PD) in the urine of ruminants (mainly allantoin and uric acid in cows). The amount of microbial nucleic acids in the rumen has been found to be closely correlated with the quantity of PD excreted in urine (29). Urinary PD N measurements have been validated over a wide range of dietary situations in N balance studies with both dry and lactating cows (28). Given the close relationship between the nucleic acid content of microbial biomass and microbial protein yield (24), the urinary excretion of allantoin or PD has been proposed for use as an indicator of microbial protein synthesis in the rumens of cows (32) and sheep (2, 6).

Plasma urea N and MUN are helpful indicators in the determination of whether dietary protein and energy are effectively used by cows (22). The uptake rates for NPN, AA, and peptides by rumen microorganisms in conjunction with available carbohydrates that provide ATP for microbial growth are determining factors for microbial utilization of N or the production of ammonia and its subsequent absorption into blood (18). Ammonia in blood is converted to urea in the liver. Urea can be recycled to the rumen, excreted in urine, or secreted in milk. The measurement of PUN and MUN is a relatively simple process; however, accuracy is affected by animal factors including parity, days in milk, and technical aspects such as sampling time relative to feeding. The CNCPS provides predictions for PUN and MUN based on a multiple regression of apparent TDN, RDP, and RUP (22).

Because of the empirical nature of the CNCPS, the predictions of microbial N, PUN, and MUN over a wide range of diets are questionable. The objective of this study was to compare PD excreted in urine (as an indicator of microbial protein yield), PUN, and MUN with the predictions of the CNCPS for metabolizable protein from bacteria, PUN, and MUN, respectively. These comparisons were used to test the hypothesis that the CNCPS provides accurate estimates of microbial protein yield, PUN, and MUN for diets that vary in the content of RUP.

## MATERIALS AND METHODS

### Experimental Design

Complete details of the experimental design have been described previously by Wright et al. (33). Briefly, six mature Holstein cows past peak lactation that were housed in stalls equipped for feces and urine collection were fed in equal amounts at 0700 and 1230 h. The cows were milked at 0530 and 1600 h. A 6 × 6 Latin square with 21-d periods and a 3 × 2 factorial arrangement of treatments was used. Two levels of feed restriction (either 90 or 80% of ad libitum intake) of the basal mixed diet (4.04% straw, 60.6% corn silage, 33.6% high moisture corn, 0.83% mineral mix, 0.19% NaCl, 0.67% CaCO<sub>3</sub>, and 0.1% KCl; as-fed basis; Table 1) were imposed on the cows. Three concentrations of a pelleted RUP supplement (25% wheat, 42.7% herring meal, 22.8% feather meal and 9.5% blood meal; as-fed basis; Table 2) and a rumen-protected AA product (52.8 ± 0.43% Met and 18.1 ± 0.21% Lys; Smartamine ML<sup>®</sup>; Rhône-Poulenc, Mississauga, ON, Canada) were top-dressed onto the basal diet. The CP concentration of the diet increased as the RUP and AA replaced the basal diet.

TABLE 2. Chemical analysis of the pelleted RUP supplement.<sup>1</sup>

Chemical	$\bar{X}$	SE
DM, %	92.78	0.17
	— (% of DM) —	
CP	63.72	0.74
Soluble protein	7.17	0.40
NDF Insoluble protein, % of CP	36.43	2.10
ADF Insoluble protein, % of CP	6.73	0.38
Crude fat	7.48	0.22
C	2.05	0.10
P	1.27	0.03
Ash	7.51	0.16

<sup>1</sup>Means are based on six analyses (DM basis).

### Protein and Intake Concentrations

The three overall dietary CP concentrations consumed were 10.5, 17.0, and 23.6% (DM basis) and are defined as low, medium, and high CP diets, respectively. During each period, the cows were offered feed for ad libitum intake for the first 7 d. The basal diet was distributed in amounts to ensure at least 10% orts, and fixed amounts of RUP supplement were fed according to treatment during the 1st wk. On d 8 of each period, mean feed intake was determined from the previous 7 d for each cow. The RUP supplement then constituted a fixed proportion of the DMI according to each diet offered: low CP diet, 4.5%; medium CP diet, 14.9%; and high CP diet, 29.1%. Allocation of the basal component of the diet was restricted by either 10 or 20% of the remainder of the dietary allotment, depending on treatment. Therefore, feed restriction was for the basal component of the diet only.

### Sample Collection

Measurements were taken during the last 5 d of each period. Urine was collected using indwelling bladder catheters (33). A daily sample of urine was taken from the total amount collected from each cow and immediately diluted five times with water to avoid precipitation of PD during storage at  $-20^{\circ}\text{C}$  (7). Milk samples were collected from consecutive morning and afternoon milkings and pooled daily based on milk production. A subsample was frozen at  $-20^{\circ}\text{C}$  for analysis of MUN. Blood was taken on d 21 between 1530 and 1630 h from the coccygeal vein for analysis of urea.

### Analytical Determinations

Urine samples were analyzed for allantoin and uric acid. Allantoin was measured according to the colorimetric method proposed by Fujihara et al. (14). Commercial kits were used to analyze uric acid (Sigma procedure no. 686; Sigma Chemical Co., St. Louis, MO) and MUN (cat. no. 542946; Boehringer, Mannheim, Germany). Blood urea N was measured in the serum fraction using a Coulter Dacos Biochemistry Analyzer (Coulter, Hialeah, FL) with a Dart urea N prepared kit (no. 7546773; Coulter) and was considered to be equivalent to PUN. Creatinine was measured with a kit designed specifically for the analysis of urine samples (cat. no. 839434; Boehringer).

### Feed Analyses and Calculations

Feed samples were collected twice during each collection period, and orts also were collected when

present. Feed samples were pooled equally, and, when necessary, an orts sample from each cow was derived proportionately from each of the 5 collection d. All samples were freeze-dried, ground, and analyzed by a commercial laboratory (Northeast DHI Forage Laboratory, Ithaca, NY) for standard chemical analyses [DM, NDF, lignin, total N, soluble protein, NDIN, ADIN, ash, and solvent-soluble fat; (27)] used as dietary inputs for the CNCPS. The complete animal, management, and environmental variables required by the model were also recorded. All data were used in the CNCPS to yield predictions for the complete Latin square.

### Statistical Analysis

Data were analyzed using the general linear models procedure of SAS (25). The model used for this study was

$$Y_{ijkl} = \pi + \alpha_i + \beta_j + \gamma_k + \tau_l + (\gamma\tau)_{kl} + \epsilon_{ijkl}$$

where

- $\pi$  = overall true mean,
- $\alpha_i$  = effect of cow ( $i = 1, 2, 3, 4, 5,$  or  $6$ ),
- $\beta_j$  = effect of period ( $j = 1, 2, 3, 4, 5,$  or  $6$ ),
- $\gamma_k$  = effect of RUP concentration ( $k = 1, 2,$  or  $3$ ),
- $\tau_l$  = effect of intake level ( $l = 1$  or  $2$ ),
- $(\gamma\tau)_{kl}$  = interaction term, and
- $\epsilon_{ijkl}$  = random residual error.

Five-day means of measurements and analysis were used for statistical purposes. Interactions were not significant ( $P > 0.05$ ) unless indicated.

## RESULTS AND DISCUSSION

### Feed Intake

The mean amounts of RUP supplement, protected AA, and basal diet offered are shown in Table 3. The DMI was not significantly affected by concentration of RUP supplementation but was affected by the level of feed restriction of the basal diet, as was intended.

### Urine Volume, Excretion of PD, and Dietary Purines

The amount of urine increased linearly ( $P < 0.001$ ) as the concentration of RUP supplement in the diet increased (Table 4). No effect of feed restriction on

TABLE 3. Dry matter intake and mean individual dietary components

Item	RUP Concentration			Feed restriction level		SE
	4.5% <sup>1</sup>	14.9%	29.1%	90% <sup>2</sup>	80%	
DMI, <sup>3</sup> kg/d	17.29	17.76	17.78	18.51	16.70	0.26
	Mean components					
Basal diet, kg/d	16.61	15.36	13.19	16.17	13.93	0.20
RUP Supplement, kg/d	0.87	3.02	6.08	3.31	3.33	0.07
AA, g/d	16.9	58.3	117.5	64.1	64.4	1.30

<sup>1</sup>Percentage of DMI.

<sup>2</sup>Percentage of ad libitum intake of basal diet only.

<sup>3</sup>Effect of feed restriction ( $P < 0.025$ ); effect of concentration of RUP supplement ( $P < 0.69$ ).

urine output was detected. The linear increase in urine volume was likely a direct effect of the total CP concentration of the diets because a linear increase in urinary N excretion was also measured in the experiment by Wright et al. (33). This effect of CP on urine volume reportedly results from a progressive increase in N that exceeds the capacity of the kidneys to concentrate urea (21) and is mediated by changes in the glomerular filtration rate (13). Chen et al. (8) reported that if kidney filtration is not constant, the

relationship between plasma PD concentration and daily urinary PD excretion is directly affected. An increase in the glomerular filtration rate reduces the reliability of PD excretion in urine as an indicator of microbial N. The urinary excretion of creatinine N was measured in the present study as an internal standard because its renal clearance approaches that of allantoin (15). Creatinine N excretion was affected ( $P < 0.01$ ) by the concentration of the RUP supplement (Table 4), but the effects of cow, period ( $P <$

TABLE 4. Urine volume, urinary excretion of creatinine and purine derivative (PD) N, and predictions by the Cornell Net Carbohydrate and Protein System (CNCPS) for metabolizable protein and degraded carbohydrate and protein fractions.

Item	RUP Concentration			Feed restriction level		SE	Effect <sup>3</sup>	
	4.5% <sup>1</sup>	14.9%	29.1%	90% <sup>2</sup>	80%		P	FR
Urine, L/d	11.9 <sup>b</sup>	19.3 <sup>a</sup>	25.1 <sup>a</sup>	17.9	19.6	0.148	0.001	NS <sup>4</sup>
	Urinary excretion							
Creatinine N, <sup>5</sup> g/d	3.86 <sup>b</sup>	4.01 <sup>b</sup>	4.21 <sup>a</sup>	4.04	4.02	0.398	0.01	NS
PD:Creatinine N, g/d	4.64	5.16	4.43	4.96	4.52	0.128	NS	NS
PD N, g/d	17.5	19.6	18.3	19.2	17.8	0.064	NS	NS
Uric acid N, <sup>6</sup> g/d	1.3 <sup>b</sup>	1.6 <sup>a</sup>	1.4 <sup>b</sup>	1.5	1.4	0.03	0.01	NS
	Predicted by CNCPS							
Dietary protein, <sup>7</sup> g/d	541 <sup>c</sup>	1186 <sup>b</sup>	1906 <sup>a</sup>	1227	1196	41.7	0.001	NS
Microbial protein, <sup>7</sup> g/d	1420 <sup>a</sup>	1325 <sup>b</sup>	1163 <sup>a</sup>	1375 <sup>a</sup>	1231 <sup>b</sup>	2.3	0.001	0.001
Degradable carbohydrate, g/d	10,343 <sup>a</sup>	9771 <sup>b</sup>	8795 <sup>a</sup>	10,144 <sup>a</sup>	9129 <sup>b</sup>	107.25	0.001	0.001
Degradable protein, g/d	1184 <sup>c</sup>	1528 <sup>b</sup>	1878 <sup>a</sup>	1550	1510	22.14	0.001	NS

<sup>a,b,c</sup>Means within a row with no common superscript differ according to  $P$  value indicated.

<sup>1</sup>Percentage of DMI.

<sup>2</sup>Percentage of ad libitum intake of basal diet only.

<sup>3</sup>P = Protein effect; FR = feed restriction effect.

<sup>4</sup> $P > 0.05$ .

<sup>5</sup>Interaction between protein and feed restriction effects ( $P < 0.01$ ).

<sup>6</sup>Quadratic effect ( $P < 0.01$ ).

<sup>7</sup>Metabolizable protein as predicted by the CNCPS.

0.001), and the interaction of CP and feed restriction ( $P < 0.01$ ) were also significant. The absence of any significance ( $P > 0.05$ ) for these effects on PD N excretion and on PD N corrected for creatinine N (ratio of PD N to creatinine N; Table 4) indicated that, for the conditions described herein, any possible glomerular filtration rate effect was likely dependent on the cow (thus accounted for by the model) and too small to affect PD N excretion. Vagnoni et al. (30) reported a linear relationship between the ratio of urinary allantoin to creatinine and total daily purine flow, regardless of a significant cow effect on creatinine excretion.

Dietary purine contribution was discounted as a quantitatively negligible contribution to urinary PD N in the present study based on results of a rumen incubation study (data not shown). This decision agreed with the results of Calsamiglia et al. (5) who conducted a continuous culture experiment that indicated that the purines present in a pelleted diet that contained 10.8% fish meal (DM basis) were 95% degraded by rumen microbes.

#### Purine Derivatives and CNCPS Predictions

The urinary excretion of PD N was not significantly affected by the concentration of RUP supplement or by level of feed restriction of the basal diet. The numeric response of urinary excretion of uric acid N to the treatments resembled that of total PD N; however, there was a significant quadratic effect of concentration of RUP supplement on uric acid N ( $P < 0.01$ ; Table 4). Stefanon et al. (28) measured a change in the ratio of allantoin N to PD N that was likely due to a rate-limiting step of the enzyme uricase and suggested that the sum of the two PD was a more reliable index of microbial N yield.

The CNCPS provided predictions of metabolizable protein from both dietary and bacterial sources. The CNCPS predictions for metabolizable protein of dietary origin indicated an almost threefold increase in supply ( $P < 0.001$ ; Table 4) from the low CP diet to the high CP diet. The CNCPS did not predict that feed restriction would cause a significant difference in dietary metabolizable protein (Table 4). Metabolizable protein from bacteria was predicted to decline linearly ( $P < 0.001$ ) as the concentration of the RUP supplement in the diet was increased. The prediction of a linear decline in metabolizable protein from bacteria may be attributable to the higher concentrations of RUP supplement in the diet. This supplementation reduced the amount of fermentable OM in the rumen. This conclusion is supported by the significant main

effect predicted by the CNCPS on metabolizable protein from bacteria caused by restricting intake of the basal diet ( $P < 0.001$ ). The CNCPS predicted a 10% reduction in metabolizable protein from bacteria for cows fed at 80% of ad libitum intake compared with that for cows fed at 90% of ad libitum intake.

The negative effect of the concentration of RUP supplement on metabolizable protein of bacterial origin predicted by the CNCPS did not agree with the measured urinary PD excretion. The measurement of PD in urine has been evaluated as a reliable indicator of rumen microbial yield in cattle using the intragastric infusion technique (32) or using increased concentrations of torula yeast RNA in the rumens of cannulated cows (2). The reasons for the linear decrease predicted by the CNCPS when the concentration of RUP supplement was increased likely originated from some of the assumptions used in the model. The maintenance coefficients (grams of substrate per gram of microbial DM per hour) for bacteria fermenting nonstructural or structural carbohydrate are fixed in the model. This assumption contrasts with the evidence that limitations in the availability of nutrients (ammonia, AA, and peptides) results in higher energetic uncoupling, which increases maintenance coefficients and results in a decreased growth rate of bacteria (11). This explanation was probably true for the low CP diet in the present study and resulted in overprediction of microbial protein yield for cows fed this diet.

The CNCPS has been reported to overestimate microbial protein when applied to low RUP diets (19). This overestimation can also be explained by an assumption used in the model that considers the growth rate of nonstructural carbohydrate-fermenting bacteria equal to the first-order dissociation rate constant for carbohydrate. This assumption reduces the sensitivity of the model when N supply for bacteria is first-limiting and growth rate can be lower than the dissociation rate constant for carbohydrate (31). The metabolizable protein of microbial origin predicted by the CNCPS was essentially derived from the availability of degradable carbohydrate. Degradable carbohydrate in the diet was determined by the CNCPS to decrease linearly as the concentration of RUP supplement increased (Table 4). The CNCPS might not have sufficiently weighted the limiting nature of N at the low RUP concentration in this study. The RUP supplement was not entirely undegradable in the rumen; therefore, there was a linear increase in degradable protein fed concomitant with the increased concentration of RUP supplement. The importance of degradable protein to improve microbial protein syn-

thesis has been established (16). Hoover and Stokes (16) reported an increase in microbial efficiency (grams of microbial N per kilogram of carbohydrate digested) with an increase in the intake of degradable protein from 6 to 14% of DMI, a range included in the present study (6.8 to 10.5% of DMI) (Tables 3 and 4). The disparity between measured urinary PD N and CNCPS predictions for metabolizable protein from bacteria according to the treatments used herein indicates that a more mechanistic approach to the prediction of microbial protein yield is needed for rigorous predictions of microbial protein yield over a wide range of diets.

### MUN and PUN Concentrations

A significant increase in both PUN and MUN was observed as concentrations of RUP supplement in the diet increased (Table 5). The level of feed restriction of the basal diet did not cause a significant difference in PUN, although MUN was higher ( $P < 0.05$ ) when the level of feed restriction was 80% of ad libitum intake. The CNCPS predicted that values for PUN (**pPUN**) and MUN (**pMUN**; Table 5) increased linearly with the concentration of RUP supplement fed ( $P < 0.001$ ), but neither was significantly affected by level of feed restriction of the basal diet. The concentration of PUN for cows fed the high CP diet

was comparable with that in the study of Botts et al. (3) in which cows were fed similar dietary protein concentrations. The values for pPUN and pMUN differed from the observed values, particularly when the dietary protein concentrations were extreme. Residual analysis (difference between predicted and observed values) indicated that there was a significant effect of the concentration of RUP supplement ( $P < 0.01$  and  $0.001$  for plasma and milk measurements, respectively; Table 5). The CNCPS predictions were evaluated by calculating the mean prediction bias (significance of the mean of residuals,  $t$  test) and linear bias (slope of the regression between residuals and observed values). The evaluation results were different for PUN and MUN. The PUN had a significant mean prediction bias of 3.3 mg/dl ( $P < 0.001$ ) and no linear bias (Figure 1); MUN had no mean prediction bias but a significant ( $P < 0.001$ ) linear bias of 0.31 mg/dl (Figure 2).

The CNCPS predicts PUN and, indirectly, MUN using an equation based on RDP, RUP, and TDN (22). The variability of the relationship between PUN and MUN may account for the significant differences described (Table 5; Figures 1 and 2). Broderick and Clayton (4) showed that a simple linear regression of MUN on PUN yielded a lower degree of correlation than did a mixed effects model because the former could not account for significant interactions of

TABLE 5. Plasma urea N (PUN), and milk urea N (MUN) concentrations as observed and predicted by the Cornell Net Carbohydrate and Protein System (CNCPS), analysis of residuals (predicted values - observed values), and the ratio of milk true protein to urea.

Item	RUP Concentration			Feed restriction level		SE	Effect <sup>3</sup>	
	4.5% <sup>1</sup>	14.9%	29.1%	90% <sup>2</sup>	80%		P	FR
	— $P <$ —							
	Observed							
PUN, mg/dl	1.84 <sup>c</sup>	11.8 <sup>b</sup>	20.1 <sup>a</sup>	10.6	11.9	0.54	0.001	NS <sup>4</sup>
MUN, mg/dl	3.8 <sup>c</sup>	10.9 <sup>b</sup>	18.0 <sup>a</sup>	10.0 <sup>b</sup>	11.8 <sup>a</sup>	0.40	0.001	0.05
	Predicted by CNCPS							
PUN, mg/dl	2.8 <sup>c</sup>	14.1 <sup>b</sup>	26.8 <sup>a</sup>	13.9	15.2	0.46	0.001	NS
MUN, mg/dl	1.2 <sup>c</sup>	11.0 <sup>b</sup>	22.0 <sup>a</sup>	10.8	12.0	0.43	0.001	NS
Residual								
Plasma, mg/dl	0.99 <sup>b</sup>	2.30 <sup>b</sup>	6.61 <sup>a</sup>	3.38	3.22	0.584	0.01	NS
Milk, mg/dl	-2.64 <sup>c</sup>	0.15 <sup>b</sup>	3.97 <sup>a</sup>	0.75	0.23	0.440	0.001	NS
Milk true protein:urea <sup>5</sup>	127.20 <sup>a</sup>	44.64 <sup>b</sup>	25.69 <sup>b</sup>	72.94	58.74	0.699	0.001	NS

a,b,cMeans within a row with no common superscript differ according to  $P$  value indicated.

<sup>1</sup>Percentage of DMI.

<sup>2</sup>Percentage of ad libitum intake of basal diet only.

<sup>3</sup>P = Protein effect; FR = feed restriction effect.

<sup>4</sup> $P > 0.05$ .

<sup>5</sup>Urea was converted to a CP basis ( $N \times 6.38$ ) for comparison.

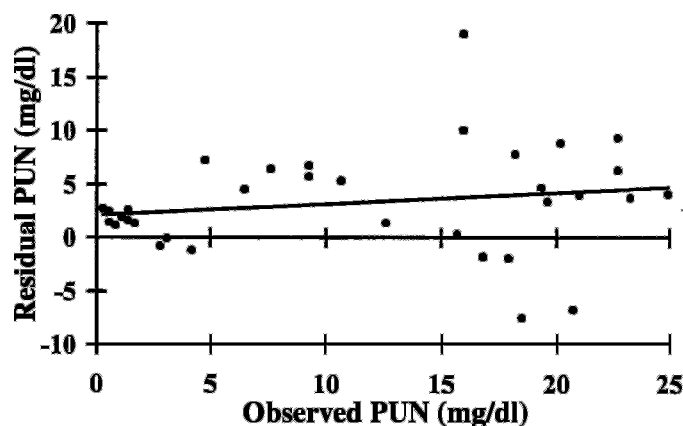


Figure 1. Plot of residual plasma urea N (PUN) (PUN predicted by the Cornell Net Carbohydrate and Protein System—observed PUN) versus observed values of PUN. The regression line shown is described by the equation  $y = 0.105 \times x + 2.12$ . The slope and intercept were not significantly different from 0.

cow and PUN. Moreover, the CNCPS prediction, which was based on regression, was developed with RUP ranging from 4.5 to 7.5% of DMI and RDP from 7.7 to 11.8% of DMI but was not tested on the concentration of RUP supplement in the high CP diet (12.7% DM) in the present study. The RUP content in the present study was composed of animal protein supplements, which usually contribute less than plant supplements to PUN concentrations of lactating dairy cows (17). A significant increase in N digestibility in the present study was observed as the concentration of RUP supplement was increased in the diet (33). This relationship indicates that the protein was digestible in the small intestine. The RUP used in this study was designed to supply essential AA for milk protein synthesis, and the lower observed values for PUN compared with the CNCPS predicted values may indicate that the AA were efficiently used for productive purposes and did not contribute to urea production at the concentrations predicted by the CNCPS. These factors might have contributed to the loss of accuracy in the predictions of the CNCPS for PUN and MUN when the concentration of the RUP supplement was high. The overprediction by the CNCPS was particularly interesting at the high concentration of RUP supplement: 26.8 mg/dl for pPUN and 22 mg/dl for pMUN would be considered excessive, although the corresponding measured PUN and MUN would not be considered extremely high (10).

Baker et al. (1) indicated that the ratio of milk true protein to MUN (compared on a CP basis) could be used to assess dietary protein quality. The medium CP diet was calculated to have a ratio of true protein

to urea of 44.6 (Table 5). This ratio is numerically higher than the ratio of 32.1 obtained by Baker et al. (1), who experimented with diets of 15.1% CP that were balanced for RDP, RUP, and essential AA profile according to the CNCPS. In the present study, the 38.9% improvement in this ratio over that of Baker et al. (1) may be indicative that the model does not strategically balance these variables for optimal efficiency.

Plasma urea N and MUN concentrations are dependent on N and carbohydrate digestion and metabolism in the rumen. Nocek and Russell (18) outlined four parameters that dictate the extent of protein and carbohydrate utilization in the rumen. First, the rate of rumen protein hydrolysis dictates how much NPN, AA, and peptides are available to rumen microorganisms. Second, the uptake rates for NPN, AA, and peptides by rumen microorganisms (18) dictate microbial utilization of N versus ammonia production (26). Third, the availability of carbohydrate to provide ATP for microbial protein synthesis dictates the extent of NPN, AA, and peptide utilization by rumen microorganisms (20). Fourth, the presence of methanogenic bacteria provides a chemical outlet for excess reducing equivalents (18). A model must mechanistically satisfy the above factors to make predictions of MUN or PUN over wide ranges of dietary N and energy degradability and quality to yield accurate predictions across many diets.

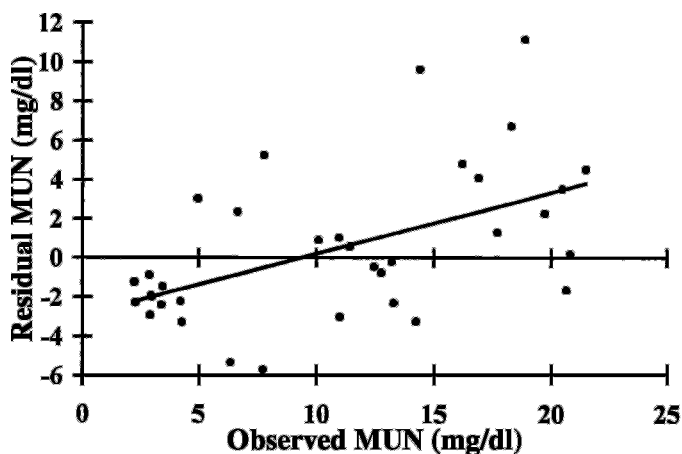


Figure 2. Plot of residual milk urea N (MUN) (MUN predicted by the Cornell Net Carbohydrate and Protein System—observed MUN) versus observed values of MUN. The regression line shown is described by the equation  $y = 0.31 \times x - 2.93$  ( $R^2 = 0.26$ ;  $SE = 0.55$ ). The slope ( $P < 0.001$ ) and the intercept ( $P < 0.05$ ) were different from 0.

## CONCLUSIONS

The measured excretion of PD N in urine suggested that microbial protein yield did not vary enough to affect the AA profile delivered by the RUP supplement to the small intestine. In contrast, the CNCPS predicted differences in microbial protein yield based on the concentration of the RUP supplement fed. In our opinion, the measurements of PD N are more likely to be correct than the predictions of the CNCPS under the conditions described herein. The empirical nature of the model precluded it from accurately predicting microbial protein yield, MUN, and PUN for the range of RUP supplementation in the present study. Results also suggested that the reliability of the pPUN and pMUN values are limited in their scope by the use of a regression equation of best fit rather than modeling our knowledge of the mechanisms in vivo by which plasma urea and milk urea are affected. Thus, we concluded that the CNCPS was not an accurate predictor of microbial protein yield, PUN, and MUN based on diets that varied in RUP at low and high concentrations used in this study.

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## REFERENCES

- Baker, L. D., J. D. Ferguson, and W. Chalupa. 1995. Responses in urea and true protein of milk to different protein feeding schemes for dairy cows. *J. Dairy Sci.* 78:2424-2434.
- Balcells, J., J. A. Guada, C. Castrillo, and J. Gasa. 1991. Urinary excretion of allantoin and allantoin precursors by sheep after different rates of purine infusion into the duodenum. *J. Agric. Sci. (Camb.)* 116:309-317.
- Botts, R. L., R. W. Hemken, and L. S. Bull. 1979. Protein reserves in the lactating dairy cow. *J. Dairy Sci.* 62:433-440.
- 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.
- Calsamiglia, S., M. D. Stern, and J. L. Firkins. 1996. Comparison of nitrogen-15 and purines as microbial markers in continuous culture. *J. Anim. Sci.* 74:1375-1381.
- Chen, X. B., F. D. Hovell, E. R. Ørskov, and D. S. Brown. 1990. Excretion of purine derivatives by ruminants: effect of exogenous nucleic acid supply on purine derivative excretion by sheep. *Br. J. Nutr.* 63:131-142.
- Chen, X. B., G. Grubic, E. R. Ørskov, and P. Osuji. 1992. Effect of feeding frequency on diurnal variation in plasma and urinary purine derivatives in steers. *Anim. Prod.* 55:185-191.
- Chen, X. B., P. Susmel, B. Stefanon, and E. R. Ørskov. 1996. On the use of purine derivatives in spot urine, plasma and milk samples as indicators of microbial protein supply in sheep and cattle. Pages 325-329 in *Proc. 7th Int. Symp. Protein Metab. Nutr.*, Santarem, Portugal. EAAP Publ. No. 81. Estacao Zootecnica Nacional, Santarem, Portugal.
- Cornell Net Protein and Carbohydrate System. 1994. A Manual for Using the Cornell Net Protein and Carbohydrate System for Evaluating Cattle Diets. Rev. CNCPS Release 3. Cornell Univ., Ithaca, NY.
- DePeters, E. J., and J. D. Ferguson. 1992. Nonprotein nitrogen and protein distribution in the milk of cows. *J. Dairy Sci.* 75:3192-3209.
- Dijkstra, J., and J. France. 1996. A comparative evaluation of models of whole rumen function. *Ann. Zootech. (Paris)* 45(Suppl.):175-192.
- Erasmus, L. J., P. M. Botha, C. W. Cruywagen, and H. H. Meissner. 1994. Amino acid profile and intestinal digestibility in dairy cows of rumen-undegradable protein from various feedstuffs. *J. Dairy Sci.* 77:541-551.
- Eriksson, L., and M. Valtonen. 1982. Renal urea handling in goats fed high and low protein diets. *J. Dairy Sci.* 65:385-389.
- Fujihara, T., E. R. Ørskov, P. J. Reeds, and D. J. Kyle. 1987. The effect of protein infusion on urinary excretion of purine derivatives in ruminants nourished by intragastric infusion. *J. Agric. Sci. (Camb.)* 109:7-12.
- Greger, R., F. Lang, and P. Deetjen. 1976. Renal excretion of purine metabolites, urate and allantoin, by the mammalian kidney. Pages 257-281 in *International Review of Physiology: Kidney and Urinary Tract Physiology II*. Vol. 11. K. Thurau, ed. Univ. Park Press, Baltimore, IL.
- Hoover, W. H., and S. R. Stokes. 1991. Balancing carbohydrates and proteins for optimum rumen microbial yield. *J. Dairy Sci.* 74:3630-3644.
- Mabjeesh, S. J., A. Arieli, I. Bruckental, S. Zamwell, and H. Tagari. 1996. Effect of type of protein supplementation on duodenal amino acid flow and absorption in lactating cows. *J. Dairy Sci.* 79:1792-1801.
- Nocek, J. E., and J. B. Russell. 1988. Protein and energy as an integrated system. Relationship of ruminal protein and carbohydrate availability to microbial synthesis and milk production. *J. Dairy Sci.* 71:2070-2107.
- O'Connor, J. D., C. J. Sniffen, D. G. Fox, and W. Chalupa. 1993. A net carbohydrate and protein system for evaluating cattle diets: IV. Predicting amino acid adequacy. *J. Dairy Sci.* 76:1298-1311.
- Oldham, J. D. 1984. Protein-energy interrelationships in dairy cows. *J. Dairy Sci.* 67:1090-1114.
- Rabinowitz, L., and R. A. Gunther. 1972. Excretion and digestion. Pages 118-120 in *Digestive Physiology and Nutrition of Ruminants*. Vol. 1—Digestive Physiology. 2nd ed. D. C. Church, ed. Oxford Press Inc., Portland, OR.
- 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 and protein nitrogen in Holstein cows. *J. Dairy Sci.* 76:525-534.
- Russell, J. B., J. D. O'Connor, D. G. Fox, P. J. Van Soest, and C. J. Sniffen. 1992. A net carbohydrate and protein system for evaluating cattle diets: I. Ruminal fermentation. *J. Anim. Sci.* 70:3551-3561.
- Rys, R., A. Antoniewicz, and J. Maciejewicz. 1975. Allantoin in the urine as an index of microbial protein in the rumen. Pages 95-98 in *Tracer Studies of Non-Protein Nitrogen for Ruminants*. Int. Atomic Energy Agency, Vienna, Austria.
- SAS® User's Guide: Statistics, Version 6.03 Edition. 1988. SAS Inst., Inc., Cary, NC.



- 26 Satter, L. D., and R. E. Roffler. 1975. Nitrogen requirements and utilization in dairy cattle. *J. Dairy Sci.* 58:1219–1237.
- 27 Sniffen, C. J., J. D. O'Connor, P. J. Van Soest, D. G. Fox, and J. B. Russell. 1992. A net carbohydrate and protein system for evaluating cattle diets: II. Carbohydrate and protein availability. *J. Anim. Sci.* 70:3562–3577.
- 28 Stefanon, B., C. R. Mills, M. Spanghero, and P. Susmel. 1996. An evaluation of purine derivatives as indicators of rumen microbial protein synthesis in dry and lactating cows. Pages 147–152 in *Proc. 7th Int. Symp. Protein Metab. Nutr.*, Santarem, Portugal. EAAP Publ. No. 81. Estacao Zootechnica Nacional, Santarem, Portugal.
- 29 Topps, J. H., and R. C. Elliott. 1965. Relationships between concentration of ruminal nucleic acids and excretion of purine derivatives by sheep. *Nature (Lond.)* 205:498–499.
- 30 Vagnoni, D. B., G. A. Broderick, M. K. Clayton, and R. D. Hatfield. 1997. Excretion of purine derivatives by Holstein cows abomasally infused with incremental amounts of purines. *J. Dairy Sci.* 80:1695–1702.
- 31 Van Kessel, J. S., and J. B. Russell. 1996. The effect of amino nitrogen on the energetics of ruminal bacteria and its impact on energy spilling. *J. Dairy Sci.* 79:1237–1243.
- 32 Verbic, J., X. B. Chen, N. A. MacLeod, and E. R. Ørskov. 1990. Excretion of purine derivatives by ruminants. Effect of microbial nucleic acid infusion on purine derivative excretion by steers. *J. Agric. Sci. (Camb.)* 114:243–248.
- 33 Wright, T. C., S. Moscardini, P. H. Luimes, P. Susmel, and B. W. McBride. 1998. Effects of rumen-undegradable protein and feed intake on milk protein production in dairy cows. *J. Dairy Sci.* 81:784–793.