Responses in Urea and True Protein of Milk to Different Protein Feeding Schemes for Dairy Cows

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ABSTRACT

Four multiparous Holstein cows were used in a 4 x 4 Latin square to investigate the effects of protein concentration, degradability, and quality on plasma urea concentration and milk N constituents. Diets varied in the amount and proportion of RDP and RUP relative to NRC requirements: diet 1, excessive RDP, deficient RUP; diets 2 and 3, balanced for RDP and RUP; and diet 4, excessive RDP, balanced for RUP. Diet 3 was formulated for optimal AA balance as predicted by the Cornell Net Carbohydrate and Protein System. Diets contained 34% corn silage, 19% alfalfa haylage, and 49% concentrate (DM basis). Concentrates varied in amounts of urea and soybean, corn gluten, and fish and blood meals.

Concentrations of urea N and NPN in milk varied among diets: diet 1, 19 and 34 mg/dl; diet 2, 16 and 31 mg/dl; diet 3, 15 and 30 mg/dl; and diet 4, 23 and 39 mg/dl, respectively. Increases in NPN concentration were attributed to increases in the urea fraction of NPN. Intake of RUP and AA balance influenced milk true protein content: diet 1, 2.89%; diet 2, 2.90%; diet 3, 3.01%; and diet 4, 2.95%. The proportions of true protein and urea in milk are influenced by CP concentration, protein type, and protein quality.

INTRODUCTION

Increased cheese consumption and changed market demands have stimulated interest in the protein content of milk. Reports in the literature (6, 27) regarding the impact of dietary protein concentration and type of protein on protein content in milk have not been consistent, and, in many studies, the distinction between milk CP (Kjeldahl total N x 6.38) and true protein (TP; total N minus NPN) has not been made (4, 5). Changes in TP and NPN contents, compared with changes in CP content of milk, are more informative when protein feeding strategies for dairy cows are being evaluated.

Efficiency of protein feeding is maximized when the N supplied in the diet matches the N required by rumen microbes and ruminant tissues. This balance is associated with a baseline concentration of urea in plasma and milk. Excess N supplied to the rumen or to postruminal tissues increases the concentration of urea in plasma and milk above baseline values, increases the excretion of urea in urine, and suggests N wastage and inefficiency of protein feeding. Urea concentrations in milk are closely correlated to those in plasma (13, 18), and urea constitutes a large proportion of the NPN fraction in milk (30). Concentrations of milk urea N (MUN) could be analyzed by DHIA test centers and used routinely on dairy farms as an indicator of protein metabolism and N utilization. Efficient use of dietary N should be reflected in yield responses that

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maximize N in milk as TP and minimize N as MUN. This study was designed to evaluate the sensitivity of MUN to changes in dietary CP, RDP, RUP, and protein quality in lactating dairy cows. A second objective was to use the relative proportions of MUN and TP in milk to assess N balance concurrently with AA supply to postruminal tissues.

**MATERIALS AND METHODS**

**Experimental Design**

Four multiparous Holstein dairy cows, ranging from 86 to 136 DIM and yielding between 31.2 and 32.5 kg/d of 3.5% FCM, were randomized into a 4 x 4 Latin square design with 2-wk periods. Cows were housed in individual tie stalls and had ad libitum access to a TMR fed to 20% orts. Feed was delivered in equal amounts at 1100 and 1600 h.

**Formulation of Diets**

All feed ingredients were analyzed by proximate analysis (1) prior to ration formulation. Neutral detergent fiber was analyzed using a procedure described by Van Soest et al. (29). Nonstructural carbohydrate and NE\textsubscript{T} values were calculated. Degradability estimates used in formulation were taken from NRC (11) values. All diets contained equal OM concentrations of alfalfa haylage (16.9%), corn silage (33.6%), and grain (49.5%) (Table 1). Grain mixes contained similar amounts of ground ear corn, molasses, vitamins, minerals, and additives, but varied in the amounts of soybean meal (48% CP), urea, corn gluten meal, blood meal, and fish meal.

**Evaluation of Diets**

Diets were isocaloric (1.5 Mcal of NE\textsubscript{T}/kg of DM) and formulated to support 33.3 kg/d of 3.5% FCM according to the schemes outlined in Table 1. Each formulated diet was evaluated for dietary N supply using the Cornell Net Carbohydrate and Protein System (CNCPS) (8, 12, 19, 23). Predictions for dietary N supply relative to CNCPS requirements are listed in Table 2. Diets 1, 2, and 3 were in balance for CP and were isonitrogenous. Diet 1 was formulated to meet the 1978 NRC (9) requirement for CP. The CNCPS confirmed CP balance, but indicated that diet 1 was excessive in RDP and deficient in RUP (Table 2). Diets 2 and 3 were formulated for RDP and RUP according to 1989 NRC (11) guidelines for absorbed protein. Predictions for CP, RDP, and RUP were in balance according to the CNCPS and were in agreement with NRC (11) recommendations. Diet 4 was formulated according to 1989 NRC (11) guidelines for CP. The CNCPS indicated that the RUP requirement was met, but diet 4 was excessive in RDP and CP (Table 2). Diet 3 was formulated using high quality animal proteins to supply adequate amounts of limiting essential AA (EAA) to the small intestine. All other diets were formulated with lower quality protein feeds (Table 1). The CNCPS predicted that diets 1, 2, and 4 would contain a less optimal balance of AA than diet 3 (Table 2).

**Collection and Analysis of Samples**

**Feed.** Feed samples were collected daily from d 8 to 14 of each period, composited, and analyzed for nutrient composition (1, 29). Nonstructural carbohydrate and NE\textsubscript{T} values were calculated. Dietary protein degradabilities were measured following 48 h of incubation with *Streptomycetes griseus* protease solution (16). Feed composition and feed intake from d 12, 13, and 14 were used to calculate nutrient supply for data analysis.

**Milk.** Milk weights were obtained, and a composite milk sample was collected twice daily at regular milking times (0500 and 1700 h) on d 12, 13, and 14. Milk samples were preserved with 2-bromo-2-nitropropane-1,3 diol (Pennsylvania DHIA) and refrigerated at 8°C until all milk samples within a period were collected. Milk samples from a.m. and p.m. were composited for d 12, 13, and 14. A subsample of the composited milk for each day was centrifuged at 3500 x \(g\) for 10 min at 22°C and returned to the refrigerator for 24 h, at which time the fat layer was removed. Defatted milk was analyzed for MUN content using the diacetyl monoxime-binding assay (kit number 535; Sigma Diagnostics, St. Louis, MO). Composited milk samples were also analyzed by Kjeldahl procedures for total N content and for NPN content of the supernatant following deproteination of milk with TCA (1, 3). Milk N in the form of TP was calculated as the difference between Kjeldahl total N and Kjeldahl NPN (3). Composited milk samples were
TABLE 1. Feed ingredients used in dietary formulation to achieve variation in CP concentration, protein degradability, and supply of essential AA.

<table>
<thead>
<tr>
<th>Feed</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>(% of DM)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Forage</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Alfalfa haylage</td>
<td>16.9</td>
<td>16.9</td>
<td>16.9</td>
<td>16.9</td>
<td></td>
</tr>
<tr>
<td>Corn silage</td>
<td>33.6</td>
<td>33.6</td>
<td>33.6</td>
<td>33.6</td>
<td></td>
</tr>
<tr>
<td>Grain mix</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ground ear corn</td>
<td>38.5</td>
<td>35.4</td>
<td>35.8</td>
<td>35.3</td>
<td></td>
</tr>
<tr>
<td>Molasses</td>
<td>1.6</td>
<td>1.6</td>
<td>1.6</td>
<td>1.6</td>
<td></td>
</tr>
<tr>
<td>Vitamins, minerals, and additives</td>
<td>2.4</td>
<td>2.3</td>
<td>1.9</td>
<td>2.3</td>
<td></td>
</tr>
<tr>
<td>Protein supplements</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Soybean meal (48% CP)</td>
<td>6.0</td>
<td>3.9</td>
<td>7.3</td>
<td>1.9</td>
<td></td>
</tr>
<tr>
<td>Urea</td>
<td>1.0</td>
<td></td>
<td></td>
<td></td>
<td>1.2</td>
</tr>
<tr>
<td>Corn gluten meal</td>
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<td>6.3</td>
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<td>7.2</td>
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<tr>
<td>Blood meal</td>
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</tr>
<tr>
<td>Fish meal</td>
<td></td>
<td></td>
<td>1.5</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

1Diets formulated for 635 kg of BW, 33.3 kg/d of 3.5% FCM.
2Formulation based on CP system of 1978 NRC (9) requirements.
3Formulation based on absorbed protein system of 1989 NRC (11) requirements using low quality plant protein sources.
4Formulation based on absorbed protein system of 1989 NRC (11) requirements using high quality animal protein sources.
5Formulation based on CP system of 1989 NRC (11) requirements.
6All diets contained .75% of the DM as sodium bicarbonate, .50% as NaCl, and .09% as vitamin-mineral premix to meet requirements for vitamins A, D, and E and trace minerals; Ca and P were supplemented as needed to meet 1989 NRC (11) requirements.

TABLE 2. Dietary supply of N and absorbed AA balance as predicted by the Cornell Net Carbohydrate and Protein System for evaluating cattle diets (8, 12, 19, 23).

<table>
<thead>
<tr>
<th>N Allocation</th>
<th>1</th>
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<tbody>
<tr>
<td>CP</td>
<td>=</td>
<td>=</td>
<td>=</td>
<td>+</td>
</tr>
<tr>
<td>RDP</td>
<td>+</td>
<td>=</td>
<td>=</td>
<td>+</td>
</tr>
<tr>
<td>RUP</td>
<td>-</td>
<td>=</td>
<td>=</td>
<td>-</td>
</tr>
<tr>
<td>AA Supply</td>
<td>-</td>
<td>-</td>
<td>=</td>
<td>-</td>
</tr>
</tbody>
</table>

(percentage of difference from 100% of requirement)

<table>
<thead>
<tr>
<th>Essential AA</th>
<th>1</th>
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<th>3</th>
<th>4</th>
</tr>
</thead>
<tbody>
<tr>
<td>Met</td>
<td>-12</td>
<td>-10</td>
<td>2</td>
<td>-12</td>
</tr>
<tr>
<td>Lys</td>
<td>-18</td>
<td>-18</td>
<td>3</td>
<td>-21</td>
</tr>
<tr>
<td>Arg</td>
<td>24</td>
<td>27</td>
<td>50</td>
<td>22</td>
</tr>
<tr>
<td>Thr</td>
<td>1</td>
<td>12</td>
<td>19</td>
<td>11</td>
</tr>
<tr>
<td>Leu</td>
<td>10</td>
<td>47</td>
<td>35</td>
<td>49</td>
</tr>
<tr>
<td>Ile</td>
<td>-14</td>
<td>-4</td>
<td>-6</td>
<td>-6</td>
</tr>
<tr>
<td>Val</td>
<td>-10</td>
<td>3</td>
<td>13</td>
<td>2</td>
</tr>
<tr>
<td>His</td>
<td>0</td>
<td>19</td>
<td>35</td>
<td>18</td>
</tr>
<tr>
<td>Phe</td>
<td>11</td>
<td>32</td>
<td>38</td>
<td>31</td>
</tr>
</tbody>
</table>

1Predictions based on 635 kg of BW, 33.3 kg/d of 3.5% FCM, and 3.2% milk protein. Nitrogen supply in balance with (+), in excess of (+), and deficient of (-) requirement.
2All diets are isocaloric. Diets 1, 2, and 3 are isonitrogenous.
measured for milk fat content by Babcock methods (1).

Blood. Heparinized blood samples were collected from the coccygeal vein of each cow twice daily at 0800 and 1600 h on d 12, 13, and 14. Samples were refrigerated for 1 h and then centrifuged at 3500 \times g for 20 min. The plasma was removed and refrigerated until all samples for the 3 d were collected. Plasma was analyzed for plasma urea N (PUN) content using the diacetyl monoxime-binding assay.

Statistical Analysis

Data were analyzed using the general linear models procedure of SAS (20). The following models were used to determine whether treatment was significant \( P < .05 \) in explaining variation in feed composition, nutrient intake, plasma urea, and milk N constituents. When overall treatment effects were significant, Duncan's multiple range test was used to separate means.

Diet Composition. The following model was used to evaluate nutrient composition for 16 composited feed samples taken during the 2nd wk of each period. The model was

\[
Y_{ij} = \mu + P_i + T_j + C_k + D_t + (PD)_{ij} + (TD)_{ij} + (CD)_{ik} + (PTC)_{ijk} + e_{ijkl}
\]

where

\[
Y_{ijkl} = \text{response of cow } k \text{ in treatment } j \text{ of period } i \text{ on day } l,
\]

\[
\mu = \text{overall sample mean,}
\]

\[
P_i = \text{period } i \text{ effect,}
\]

\[
T_j = \text{treatment } j \text{ effect,}
\]

\[
C_k = \text{cow } k \text{ effect,}
\]

\[
D_t = \text{day } l \text{ effect,}
\]

\[
(PD)_{ij} = \text{interaction of period } i \text{ and day } l,
\]

\[
(TD)_{ij} = \text{interaction of treatment } j \text{ and day } l,
\]

\[
(CD)_{ik} = \text{interaction of cow } k \text{ and day } l,
\]

\[
(PTC)_{ijk} = \text{interaction of period } i \text{ with treatment } j \text{ and cow } k, \text{ and}
\]

\[
e_{ijkl} = \text{ordinary least squares residual error.}
\]

Feed Intake, PUN, MUN, Milk Yield, and Milk Components. Feed intake, plasma, and milk data were collected during d 12, 13, and 14 of each period, resulting in 48 observations for each variable. The following model was used to account for repeated measures in the ANOVA. The error term used to test the significance of overall treatment effects was the interaction of period with treatment and cow. The model was

\[
Y_{ij} = \mu + P_i + T_j + C_k + e_{ij}
\]

where

\[
Y_{ij} = \text{response of treatment } j \text{ in period } i,
\]

\[
\mu = \text{overall sample mean,}
\]

\[
P_i = \text{period } i \text{ effect,}
\]

\[
T_j = \text{treatment } j \text{ effect, and}
\]

\[
e_{ij} = \text{ordinary least squares residual error.}
\]

Parameters from CNCPS. Means for DMI, milk yield, milk fat percentage, and milk TP percentage for each cow from d 12, 13, and 14 of each period were used to estimate RDP, metabolizable protein, and AA supply using the CNCPS. The following model was used to evaluate treatment effects on CNCPS parameters. The model was

\[
Y_{ijk} = \mu + P_i + T_j + C_k + e_{ijk}
\]

where

\[
Y_{ijk} = \text{response of cow } k \text{ in treatment } j \text{ of period } i,
\]

\[
\mu = \text{overall sample mean,}
\]

\[
P_i = \text{period } i \text{ effect,}
\]

\[
T_j = \text{treatment } j \text{ effect,}
\]

\[
C_k = \text{cow } k \text{ effect, and}
\]

\[
e_{ijk} = \text{ordinary least squares residual error.}
\]

Percentage of CP Intake Captured as Milk TP. Efficiency of N utilization was calculated as the N output in milk TP divided by the N intake from feed for d 12, 13, and 14 of each period. Efficiency was analyzed using general linear models of SAS (20) with milk yield as a covariable.
RESULTS

Diet Composition

Analytical compositions for CP were compatible with dietary formulation goals. Diet 4 was significantly higher in CP concentration than were isonitrogenous diets 1, 2, and 3 (Table 3). Protein degradability measurements from S. griseus followed the same trends as NRC (11) values, but were systematically lower on average by 15 percentage units. In addition, S. griseus protein degradability for diet 2 was significantly less than for diet 3, unlike NRC (11) calculations (Table 3). Predictions from the CNCPS, based on actual DMI of the diets, also indicated that diet 2 was less degradable than diet 3 (Table 3). The RDP of diet 2 was 3.2% lower than diet 3 based on CNCPS prediction and 10.8% lower based on S. griseus analysis. The concentrations of ADF, NDF, and nonstructural carbohydrate were not different across treatments (Table 3).

PUN and MUN

Concentrations of PUN and MUN from d 12, 13, and 14 of each period were compared within cow and sample day (n = 48) and were analyzed by linear regression using the model PUN = MUN (Figure 1). Urea concentrations in plasma and milk were closely correlated (r = .96). The relationship between PUN and MUN (in milligrams per deciliter) is described by the following equation:

PUN = 3.20 (.63) + .85 (.03) MUN

where values in parentheses are standard errors.

Treatment significantly affected concentrations of PUN and MUN. Excesses in dietary CP (diet 4) resulted in the highest concentrations of PUN and MUN (Table 4). Diet 1, balanced for CP intake with imbalances in RDP and RUP, produced PUN and MUN concentrations that were lower than those for diet 4, but higher than those produced on isonitrogenous diets balanced for RDP and RUP (diets 2 and 3). Differences in EAA balance between diets balanced for RDP and RUP (diet 3 versus diet 2) tended to reduce PUN and MUN concentrations, but differences were not significant.

OM', Milk Yield, and Components

The DMI, milk yield, and fat content were not significantly affected by treatment in the overall model. Milk CP and NPN contents were affected by treatment (Table 4). Milk from cows on diets 3 and 4 contained the highest CP content. Concentrations of milk

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**TABLE 3. Means of analytical compositions and comparisons of mean dietary protein degradability estimates by diet.**

<table>
<thead>
<tr>
<th></th>
<th>Diet</th>
<th>(%) of DM</th>
<th>SEM</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>CP</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>15.1 b</td>
<td>14.3 b</td>
<td>15.1 b</td>
</tr>
<tr>
<td>2</td>
<td>23.0</td>
<td>22.5</td>
<td>23.3</td>
</tr>
<tr>
<td>3</td>
<td>36.7</td>
<td>36.4</td>
<td>36.6</td>
</tr>
<tr>
<td>4</td>
<td>39.7</td>
<td>40.8</td>
<td>40.0</td>
</tr>
<tr>
<td>1989 NRC²</td>
<td>73</td>
<td>60</td>
<td>60</td>
</tr>
<tr>
<td>Cornell model³</td>
<td>73⁵</td>
<td>60⁵</td>
<td>62⁶</td>
</tr>
<tr>
<td>Streptomyces griseus⁴</td>
<td>60⁴</td>
<td>41⁴</td>
<td>46⁶</td>
</tr>
</tbody>
</table>

a,b,c,d Means with different superscripts within rows differ (P < .05).

¹Nonstructural carbohydrate.

²Protein degradabilities used in diet formulation (11).

³Dietary protein degradabilities based on actual DMI.

⁴Measured values from 48-h incubation in protease solution.
NPN were highest for cows on diet 4 and lowest for cows on diet 3. The TP content in milk was calculated as CP minus NPN content. The diet balanced for RDP and RUP, containing high quality protein sources (blood and fish meals) and the best balance of EAA (diet 3), resulted in significantly higher concentrations of milk TP (3.01%) than with all other diets (Table 4). Diet 1, which was deficient in RUP, resulted in significantly lower concentrations of milk TP than did diets balanced for RUP (diets 3 and 4).

**Milk N Constituents**

Total N content of milk was significantly different among treatments; diets 3 and 4 resulted in higher total milk N than did diets 1 and 2 (Table 5). Concentrations of NPN and concentrations of NPN as a percentage of total N were significantly different among treatment groups (Table 5). Diet 3 resulted in the lowest milk NPN concentration and percentage of total N as NPN, followed in ascending order by diets 2, 1, and 4. Concentrations of MUN as a percentage of milk NPN followed a similar trend; diets 2 and 3 resulted in the lowest percentage, diet 1 had an intermediate concentration of MUN as a percentage of milk NPN, and diet 4 had the highest percentage of MUN in NPN. Nonurea NPN was calculated as milk NPN minus MUN. Concentrations of nonurea NPN in milk were constant across diets. Differences in NPN content were primarily a result of differences in MUN concentration (Table 5).

**Met and Lys**

All diets had adequate proportions of duodenal EAA as Met (>5%) when evaluated with the CNCPS (Table 6). The proportion of duodenal EAA as Lys was adequate (>15%) only on diet 3, which was formulated with blood and fish meals. Diet 1 resulted in a lower percentage of EAA as Lys than diet 3, but a
TABLE 4. Results of DMI, milk yield, milk components, urea N concentrations, the proportion of true protein (TP) and urea in milk, and milk TP as a percentage of CP intake.

<table>
<thead>
<tr>
<th>Measurement</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>SEM</th>
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</thead>
<tbody>
<tr>
<td>DMI, kg/d</td>
<td>22.7</td>
<td>21.9</td>
<td>21.1</td>
<td>21.3</td>
<td>.3</td>
</tr>
<tr>
<td>Milk yield, kg/d</td>
<td>29.0</td>
<td>31.1</td>
<td>29.6</td>
<td>30.9</td>
<td>.5</td>
</tr>
<tr>
<td>Milk composition, %</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fat</td>
<td>3.80</td>
<td>3.75</td>
<td>3.78</td>
<td>3.92</td>
<td>.05</td>
</tr>
<tr>
<td>CP</td>
<td>3.11b</td>
<td>3.10b</td>
<td>3.20a</td>
<td>3.20a</td>
<td>.01</td>
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<tr>
<td>TP</td>
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<td>2.90c</td>
<td>3.01a</td>
<td>2.95b</td>
<td>.01</td>
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<td>NPN</td>
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<td>.20c</td>
<td>.19d</td>
<td>.25a</td>
<td>&lt;.01</td>
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<td>Urea N, mg/dl</td>
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<td></td>
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<tr>
<td>Plasma</td>
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<td>16.0c</td>
<td>23.4a</td>
<td>.3</td>
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<td>15.6c</td>
<td>15.1c</td>
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<td>.3</td>
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<td>Milk TP:urea</td>
<td>25.0b</td>
<td>29.3b</td>
<td>32.1a</td>
<td>20.4d</td>
<td>.6</td>
</tr>
<tr>
<td>Milk TP:CP intake, %</td>
<td>25.5b</td>
<td>27.4a</td>
<td>28.3a</td>
<td>23.1c</td>
<td>.5</td>
</tr>
</tbody>
</table>

a,b,c,dMeans with different superscripts within rows differ (P < .05).

1Diet did not explain variation in DMI, milk yield, or fat content (P > .05).
2Calculated by difference (CP minus NPN).
3NPN and milk urea converted to a CP basis (N x 6.38) for comparison.
4Values are least squares means analyzed using general linear models procedure of SAS (20) with milk yield as a covariate.

The ratio of milk TP to milk urea (CP basis) was significantly different among treatment groups (Table 4). Milk from cows on diet 3 contained 32.1 times the amount of TP as MUN and resulted in the highest ratio of TP to urea. Milk from cows on diet 2 had a ratio of 29.3, milk from cows on diet 1 had a ratio of 25.0, and milk from cows on diet 4 resulted in the lowest ratio of TP to urea in milk, 20.4

Ration of TP to Urea in Milk

The percentage of CP intake captured as milk TP was higher for cows on diets 2 and 3, which were balanced for RDP and RUP, than for cows on diets 1 and 4, which were balanced for CP (Table 4). Cows on diet 4 had

TABLE 5. Mean concentration of milk N constituents by diet.

<table>
<thead>
<tr>
<th>Milk N component</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>SEM</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total N, g/L</td>
<td>4.87b</td>
<td>4.86b</td>
<td>5.02a</td>
<td>5.01a</td>
<td>.02</td>
</tr>
<tr>
<td>NPN, g/L</td>
<td>.34b</td>
<td>.31c</td>
<td>.30d</td>
<td>.39a</td>
<td>.01</td>
</tr>
<tr>
<td>Urea N, g/L</td>
<td>.19b</td>
<td>.16c</td>
<td>.15c</td>
<td>.23a</td>
<td>&lt;.01</td>
</tr>
<tr>
<td>Nonurea NPN, g/L</td>
<td>.15</td>
<td>.16</td>
<td>.15</td>
<td>.16</td>
<td>&lt;.01</td>
</tr>
<tr>
<td>NPN, % of total N</td>
<td>6.9b</td>
<td>6.4c</td>
<td>6.0c</td>
<td>7.8a</td>
<td>.07</td>
</tr>
<tr>
<td>Urea N, % of NPN</td>
<td>55.1b</td>
<td>49.9c</td>
<td>49.5c</td>
<td>59.3a</td>
<td>.88</td>
</tr>
</tbody>
</table>

a,b,c,dMeans with different superscripts within rows differ (P < .05).

1Calculated by difference (NPN minus urea N).
TABLE 6. Mean duodenal AA proportions estimated by the Cornell Net Carbohydrate and Protein System (8, 12, 19, 23).  

<table>
<thead>
<tr>
<th>Diet</th>
<th>Met</th>
<th>Lys</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>5.26&lt;sup&gt;b&lt;/sup&gt;</td>
<td>14.86&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>2</td>
<td>5.26&lt;sup&gt;b&lt;/sup&gt;</td>
<td>13.00&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>3</td>
<td>5.00&lt;sup&gt;c&lt;/sup&gt;</td>
<td>15.13&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>4</td>
<td>5.32&lt;sup&gt;a&lt;/sup&gt;</td>
<td>12.71&lt;sup&gt;d&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

<sup>a,b,c,d</sup>Means with different superscripts within rows differ (P < .05).

<sup>1</sup>Calculated as a percentage of essential AA based on DMI, milk yield, and milk fat and true protein percentages from each cow in each period.

<sup>2</sup>Schwab et al. (21, 22) recommend 5% of duodenal essential AA as Met.

<sup>3</sup>Schwab et al. (21, 22) recommend 15% of duodenal essential AA as Lys.

DISCUSSION

This study revealed that MUN was sensitive to changes in CP, RDP, and RUP, but insensitive to differences in AA balance. The TP content of milk was influenced by RUP and AA patterns. The diet that was balanced for CP, RDP, and RUP with high quality protein sources was effective in supplying first-limiting EAA; cows fed that diet had the most efficient milk yield with the highest ratio of TP to MUN and the most desirable milk N composition for the manufacturing of cheese products.

PUN and MUN

Urea is synthesized in the liver as an end product of protein metabolism. Urea equilibrates in body water, and kinetic analysis suggests the passive transfer of urea from plasma to milk along with water (2). No partitioning of urea from water is evident in the mammary gland (2). In this study, concentrations of PUN and MUN were closely correlated (r = .96) and indicated that 93% of the variation in MUN was accounted for by variation in PUN. These results supported the findings of other investigators (13, 18).

Concentrations of PUN and MUN are a function of dietary protein supply relative to requirements. Protein supply is partitioned into N necessary for rumen ammonia production and N in the form of AA for tissue metabolism. In this study, concentrations of PUN and MUN were most sensitive to dietary concentrations of CP (diet 4) and imbalances in RDP and RUP (diets 1 and 4). This result was consistent with those of other reports that demonstrated increases in PUN with increases in CP (13, 15) and with imbalances in RDP and RUP (18, 24).

Supply of RDP for ammonia utilization is a function of fermentable OM in the rumen (10). Diets in this study were formulated to be isocaloric, and nonstructural carbohydrate was not different among diets; however, RDP as a percentage of CP was higher for cows on diets 1 and 4 than for cows on diets 2 and 3 (Table 3). Dietary N supplied to the rumen in excess of microbial needs is wasted as urea and reduces the efficiency of N utilization for product formation. Diets formulated according to NRC (9, 11) requirements for CP (diets 1 and 4) were excessive in RDP and were relatively inefficient, as indicated by higher PUN and MUN concentrations and lower efficiencies of N utilization for milk TP compared with diets formulated for RDP and RUP (diets 2 and 3).

Excesses in AA supplied to tissues can result in deamination of unused AA and conversion to urea. A previous study (18) has shown increases in PUN and MUN concentrations with excesses in RUP. Cows on diets 1 and 4 had similar intakes of RDP but different intakes of RUP. Differences in concentrations of PUN and MUN between diets 1 and 4 were probably a result of differences in total AA supply to postruminal tissues.

Imbalances in AA pattern can also result in deamination of unused AA and conversion to
urea in the cow. The effect of AA balance on concentrations of PUN and MUN in dairy cows has not been fully described. In our study, MUN concentrations were not sensitive to differences in AA balance. Differences in the urea and nonurea fractions of NPN between diets 2 and 3 were not large enough to be significant; however, the combined effect of urea and nonurea NPN resulted in significantly higher total NPN and NPN as a percentage of total N on diet 2 than on diet 3 (Table 5). Thus, diet 3, formulated with high quality protein sources to provide more optimal balance of EAA, generated milk with less NPN than diet 2.

Milk urea N constitutes a large proportion of the NPN fraction in milk (30). Diets balanced for CP, RDP, and RUP (diets 2 and 3) had 50% of the NPN as urea N and 50% as nonurea N (Table 5). Imbalances in RDP and RUP (diets 1 and 4) increased the percentage of MUN in NPN because the nonurea component of NPN remained constant across all diets. Concentrations of MUN and milk NPN varied similarly with imbalances in RDP and RUP; thus, measurement of NPN content in milk is a reflection of MUN concentration. This result is consistent with those of other reports (4, 18) regarding MUN and milk NPN contents.

Milk Protein

The CP concentration in milk is determined by NPN and TP content. Diets excessive in CP or imbalanced in RDP and RUP may increase NPN and CP contents in milk, but not TP content. In our study, true protein content of milk was influenced by supply of RUP and EAA balance. Diet 1 was deficient in RUP and resulted in significantly lower concentrations of milk TP than did diets 3 and 4, which were adequate in the supply of RUP. Diet 3 was balanced for RUP with fish and blood meals to supply adequate amounts of Met and Lys to the mammary gland. Milk from cows on diet 3 contained the highest concentration of milk TP. Diets 2 and 4 were formulated with corn gluten meal to meet the RUP requirement, and TP percentages in milk from cows fed diets 2 and 4 were significantly lower than those from cows fed diet 3. The Schwab ratio (21, 22) for Met and Lys as a percentage of duodenal EAA was ideal for milk protein synthesis on diet 3 (Table 6). Methionine was 5% and Lys was >15% of duodenal EAA. Diets 2 and 4 were both adequate in Met (>5%), but both diets contained <15% of duodenal EAA as Lys (Table 6). Lysine appeared to limit protein synthesis of cows on diets 2 and 4, unlike its effect on cows fed diet 3. Diet 1, although low in RUP, was only marginally low in the proportion of EAA as Lys (14.86%). The TP percentages in milk from cows on diet 1 were not different from TP percentages in milk from cows on diet 2. Diet 2 should have resulted in a significantly higher concentration of milk TP than diet 1 because diet 2 contained more RUP. However, cows on diet 2 were limited in milk protein synthesis because of the limitations of Lys availability at the duodenum. This limitation is supported by results from another study (25) that showed reduced amounts of Lys at the duodenum when cows were fed corn gluten meal. If diets 2 and 4 had been supplemented with rumen-protected Lys, they probably would have responded with an increase in milk TP percentage. However, supplementing diet 1 with rumen-protected Lys probably would not result in an increase in milk TP percentage because this diet was limited by the supply of RUP. Both the quantity and quality of RUP influence the synthesis of milk protein, as indicated by our data and by the work of others (24, 25).

Efficiency in Dietary Protein Supply

Milk CP encompasses all N fractions in milk and is too general to assess the efficiency of diet formulation and protein supply. To evaluate efficiency in dietary protein supply, TP and urea content in milk must be known. Efficient use of dietary N for lactating dairy cows should be reflected in milk N composition in which TP is maximized and urea is minimized.

Diet formulated for absorbed protein (diets 2 and 3) were more efficient at converting dietary N into milk TP than diets formulated for CP requirements (diets 1 and 4) (Table 4). This would suggest that diets balanced for RDP and RUP are more efficient at N utilization in both the rumen and tissues. The diet with the highest concentration of CP (diet 4) was the least efficient diet at converting intake N into milk TP.
The ratio of TP to urea in milk varied with dietary protein formulation schemes and established relative efficiencies among diets. Diet 3 formulated for CP, RDP, RUP, and EAA balance was the most efficient feeding strategy for generating milk yields with high concentrations of TP and low concentrations of urea (Table 4). Diet 4, balanced for RUP by supplying excessive CP, was the least efficient feeding strategy and resulted in high milk CP, the lowest ratio of TP to urea, and the least desirable milk N composition for the manufacturing of cheese products. To achieve high concentrations of TP and low concentrations of urea in milk, diets should be balanced for RDP and RUP and formulated with protein quality in mind, not just quantity.

**MUN as an Indicator of Protein Metabolism**

Urea is a metabolic indicator of N wastage, and concentrations of PUN and MUN reflect protein metabolism in the cow. Kinetic analysis has suggested that MUN concentration is a reasonable indicator of mean plasma urea concentration (2). Because milk samples are routinely collected on dairy farms, MUN concentration could be measured to monitor protein utilization. Milk urea concentration may be a better indicator of mean plasma urea concentration than PUN because milk integrates variation in plasma concentration (2). Diets balanced for CP, RDP, and RUP with high quality protein sources resulted in MUN concentrations of 15.1 mg/dl (SE = .3). Excesses in CP or imbalances of RDP and RUP can elevate MUN above this value and indicate excess N supply to rumen microbes, ruminant tissues, or both. High concentrations of urea in body fluids of dairy cows reduce metabolic efficiency of milk yield (28), have negative impacts on health (14) and reproduction (7, 17), and contribute to environmental contamination (26) because >95% of endogenous urea is excreted in urine (2). A system to monitor excess protein feeding is needed. Routine analysis of MUN content could be useful to assess efficiency of protein feeding on dairy farms.

**CONCLUSIONS**

Percentages of CP in milk are not adequate to assess the efficiency of diet formulation. The dairy industry needs consistent methodology to analyze milk for urea and TP contents. The MUN concentration is sensitive to CP, RDP, and RUP; elevations in MUN concentration indicate dietary N excess to rumen microbes, ruminant tissues, or both.

The TP content in milk is influenced by supply of RUP and AA balance. The relative proportions of TP and urea in milk help in the assessment of N utilization. This study has shown that diets can be balanced efficiently so that cows produce milk with relatively high TP content and low concentrations of urea without sacrificing milk yield. Economics associated with the manufacturing of cheese products will encourage the industry to formulate diets that maximize the N captured in milk as TP. This result can be achieved by balancing diets for CP concentration, protein degradability, and AA supply.

**ACKNOWLEDGMENTS**

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**REFERENCES**


