Diets Deficient in Rumen Undegraded Protein did not Depress Milk Production[1]

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ABSTRACT

The objective of this study was to evaluate the National Research Council's recommendations for feeding levels of rumen undegraded protein (RUP) for cows fed a one-group total mixed ration. Sixty Holstein cows were paired by parity (1 to 6) and DIM (23 to 315) and were randomly assigned to one of two treatment sequences. Diets contained alfalfa silage (30% diet DM) and corn silage (26% diet DM), and were isonitrogenous (16% CP) and isocaloric (1.71 Mcal/kg). Soybean meal, protected soybean meal (Soy Best), and urea were used to make ration protein fractions that were predicted to be 35 or 29% RUP. The 35% RUP diet was formulated to provide 98 and 105% of the average requirement for RUP and rumen degraded protein (RDP), respectively. The ration containing 29% RUP provided 79 and 117% of average required RUP and RDP, respectively. All cows were group-fed the high RUP diet during a 2-wk pretreatment period, and then were fed one ration for 4 wk followed by the other for 4 wk according to their assigned treatment sequence. Data were collected in the last wk of each period. Mean milk production, milk fat, and milk protein were 32.6 kg/d, 4.35%, and 3.36%, respectively, with no treatment differences. Treatment response was not affected by degree of predicted RUP deficiency. National Research Council requirements for RUP may be too high for cows fed diets similar in energy to a one-group total mixed ration. Alternatively, estimates of RUP content of feedstuffs may be low.
(**Key words:** rumen undegraded protein, group feeding)

Abbreviation key: HRUP = high RUP experimental diet, **LRUP** = low RUP experimental diet, **MUN** = milk urea nitrogen, **PMUN** = predicted MUN.

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INTRODUCTION

Nitrogen emissions from dairy farms to water and air resources are a major public concern. One of the most effective ways to reduce total farm N losses to the environment is to improve the utilization efficiency of protein within dairy herds (9). Because protein is a high-cost ingredient in dairy rations, improving the efficiency of its utilization can also reduce total ration cost per unit of milk produced.

To feed a dairy herd to maximize nitrogen efficiency, it is important to know the protein requirements for that herd. This matter is complicated by the fact that nutrient requirement models such as that of the National Research Council (10) predict requirements for individual cows. Dairy producers do not have the time or resources to formulate a separate diet for each individual cow, but rather they feed groups of cows the same diet. Each cow in the group differs in variables that drive nutrient requirement predictions such as milk production, milk composition, stage of lactation, parity, and BW. If a diet is formulated to meet the average protein requirements for a group of cows, half of the cows in that group would be expected to consume inadequate protein and consequently lose production, while the other half would be expected to consume excess protein. It is therefore suggested that the protein feeding level be targeted to meet the requirements of one of the better cows in the group rather than the average cow. Feeding recommendations used in the industry vary from targeting the 60th to the 83rd percentile cow (16, 17, 19). To determine the optimal amount of protein to feed a group of cows, the production consequences from underfeeding protein need to be determined.

The protein requirements of dairy cows are divided into two fractions. Ruminally degraded protein is needed to synthesize microbial cells that may be an optimal protein source to support milk production (15). In early lactation and at high production levels there is also a need for RUP to meet the protein requirements that cannot be satisfied with microbial protein alone (1, 5). The requirement for RUP is not well established in late lactation and with low producing cows (1, 8, 11, 12). Microbial protein contains a better mix of limiting AA than most sources of RUP (15), and RDP sources are less expensive than RUP sources. Consequently, overfeeding of RUP and underfeeding of RDP is likely to reduce both the N utilization efficiency and economic efficiency of dairy herds.

To determine the optimal level at which to feed protein to a group of dairy cows, the minimum RUP requirement for cows fed the same ration must be ascertained. Our objectives were, 1) to determine whether a diet deficient in RUP would cause milk losses in cows fed a 1-group TMR and, 2) to determine whether RUP underfeeding affects cows differently based on the degree of predicted RUP deficiency.

MATERIALS AND METHODS

Sixty lactating Holstein cows were paired by parity (1 to 6) and DIM (23 to 315) and were randomly assigned to one of two treatment sequences. All cows were fed a high RUP (**HRUP**) TMR during a 2-wk pretreatment period. Following this period, cows assigned to the first treatment sequence were fed the HRUP TMR for a 4-wk period and then were switched to the low RUP (**LRUP**) TMR for the remaining 4-wk period. Cows assigned to the second treatment sequence were fed the same diets in the opposite order. Average BW during the trial was 648 kg.

Cows were housed in tie-stall barns at the University of Maryland Research Farm in Clarksville, Maryland, and they were milked and fed twice daily. Feeding rates were adjusted for each cow to allow for approximately 10% feed refusals, and feed intake and refusals were recorded daily. Body weights and forage DM content were measured weekly. Milk samples from both milkings on the last three days of each treatment period were composited and analyzed at the Lancaster (PA) DHIA laboratory for percentage protein, fat, solids, SCC, and milk urea nitrogen (**MUN**). Composition of the milk samples and the average production and intake during the last week of the pretreatment period and each treatment period were used to test treatment effects. Eight cows showed clinical signs of mastitis during the study and were removed from data analysis. Cows that completed the trial were primarily in midlactation (60 to 179 DIM, n = 31) and in late lactation (180 to 301 DIM, n = 18) at the start of the study. However, three primiparous cows were in early lactation and averaged 21 DIM at the start of the study. Average milk production in the pretreatment period was 35.4, 34.0, and 33.4 kg/d for early-, mid-, and late-lactation cows, respectively. Overall, the standard deviation in milk production was 5.45 kg/d.

Table 1. Diet composition.

	% Diet dry matter	
Ingredient	High RUP diet	Low RUP diet
Alfalfa silage	30.4	30.0
Corn silage	25.5	25.4
Corn grain	33.4	36.3
Soy Best[1]	7.0	0
Soybean meal (48% CP)	0	4.1
Megalac[2]	1.1	1.1
$NaHCO_3$	1.0	1.0
Urea	0.5	0.9
Mineral and vitamin mix[3]	0.2	0.2
NaCl	0.2	0.2
MgO	0.1	0.1
Ca 23%:P 18%	0.6	0.7
Crude protein	16.0	16.1
RUP[4]	5.6	4.6
RUP (% CP)[4]	35.4	28.6
NE_L (Mcal/kg)	1.71	1.71
NSC	41.0	43.8
NDF	31.9	29.9
ADF	17.9	17.3

[1]Grain State Soya, Inc., West Point NE.

[2]Church & Dwight, Co., Inc., Princeton, NJ.

[3]Contained (per kg ration dry matter basis) 4 g of limestone, 6620 IU of vitamin A, 1540 IU of vitamin D, 22 IU of vitamin E, 0.1 mg/kg of Co, 20 mg/kg of Cu, 50 mg/kg of Mn, 50 mg/kg of Zn, 0.6 mg/kg of I, and 0.3 mg/kg of Se.

[4]Calculated using NRC values for all ingredients except for Soy Best, which was reported by the manufacturer to contain 58% RUP.

Diet composition is shown in Table 1. Both diets consisted of 30% alfalfa silage, 25.5% corn silage, and 44.5% concentrate on a DM basis. The HRUP diet contained 7.0% Soy Best (Grain States Soya, Inc., West Point, NE), a rumen protected extruded soybean meal product, and 0.5% urea on a DM basis as supplemental protein sources. The protein supplements for the LRUP diet were 48% CP soybean meal (4.1% diet DM) and urea (0.9% diet DM). Supplemental energy sources for both diets were corn grain (33.4 and 36.3% for the HRUP and LRUP diets, respectively) and Megalac (Dwight & Church Co. Inc., Princeton, NJ) (1.1% diet DM). More corn grain was fed to the cows on the LRUP diet to account for differences between the diets in energy supplied by the protein sources.

Individual feeds were analyzed at the Northeast DHIA laboratory (Ithaca, NY) for CP, NDF, ADF, NSC, and NE_L, and content of the two TMR are shown in Table 1. Samples of soybean meal and Soy Best were also analyzed for acid detergent insoluble CP. Consistent with current industry practices, estimates of the RUP fraction of each diet were calculated based on NRC (10) table values for all diet ingredients, except Soy Best where values listed by the manufacturer were used. The total CP content of the diets was 16.0% for the HRUP diet and 16.1% for the LRUP diet. The predicted RUP fraction of total CP in each diet was 35.4% for the HRUP diet and 28.6% for the LRUP diet. Neutral detergent fiber was 31.9% in the HRUP diet and slightly lower (29.9%) in the LRUP diet. Acid detergent fiber was not different between diets. The average NE_L content was estimated at 1.71 Mcal/kg DM.

Soybean meal and Soy Best were tested in an in situ analysis to determine approximate rates of digestion of the protein fractions in each product. Bags containing approximately 5 g of soybean product were placed in duplicate in the rumen of a lactating cow not on the trial for 0, 3, 6, 12, 18, 24, or 36 h. Bags were then rinsed thoroughly, dried, and weighed. Kjeldahl analysis was performed on the remaining sample to determine N content. Rumen degradability was determined by the equations:

$$\text{RDP fraction} = S + I * (k_d/[k_d + k_p])$$
$$\text{RUP fraction} = 1 - \text{RDP fraction}$$

where S = fraction of soluble protein at t = 0, I = fraction of insoluble protein at t = 0, k_d = log linear digestion rate, and k_p = log linear passage rate. An estimated k_p of 0.05 per hour (4) was used to calculate protein fractions.

Dietary treatment effects on milk and component yield were tested using the mixed procedure of SAS (14) and the following model:

$$Y = \text{period} + \text{sequence} + \text{cow(sequence)} + \text{diet} + \text{error}$$

where Y represents the parameter tested, sequence was tested against cow within sequence that was included as a random effect, and period and diet effects were tested with residual error. Sequence was included in the model as an alternative to testing for an interaction between diet and period. Specifically, it was used to test whether the LRUP diet fed during the first period would have carryover effects that would reduce production during the second period. Four-percent fat corrected milk was calculated as (18):

$$4\%\ \text{FCM} = \text{milk (kg/d)} * (44.01 * \text{milk fat } \% + 163.56)/339.60.$$

Cows differed in DIM, parity, BW, and production level and thus differed greatly in their expected requirements. Since all cows within a treatment consumed the same diet, cows with greater requirements were expected to be much more deficient in various nutrients than others. Nonetheless, the cows with greater energy requirements may also have higher DMI, thus ameliorating the deficiency somewhat. The actual deficiency for each cow was calculated as the intake of NE_L, RUP, or RDP during each treatment period minus the requirement expected during that period as predicted from the NRC model (10) parameterized with the treatment period data. These data were shown graphically by plotting against the pretreatment period FCM, which is independent of this response.

Having tested the differences between the two dietary treatments and demonstrated variance in the RUP intake relative to the expected requirements, an additional model was used to determine the effect of degree of predicted RUP deficiency on FCM. In this case, the degree of predicted RUP deficiency needs to be the independent variable to test the response in FCM. Therefore, production and intake data from the pretreatment period were used to calculate the expected deficiency in RUP to test the effect on FCM in the treatment periods. Otherwise, a random effect on milk production in the treatment period would affect both milk production and expected deficiency (via NRC requirements which depend on milk production), thus falsely resulting in an apparent significant effect of deficiency on FCM. The model tested was:

$$4\%\ \text{FCM} = \text{period} + \text{sequence} + \text{cow(sequence)} + \text{deficiency} + \text{error}$$

where cow(sequence) was again included as a random effect.

Predicted milk urea nitrogen (**PMUN**) values were calculated from an equation by Jonker et al. (6):

$$\text{PMUN (mg/dl)} = (\text{NI} * 0.83 - \text{milk N} - 97)/12.54$$

where NI = nitrogen intake (g/d) and milk N = milk N content (g/d). Residual MUN was calculated by subtracting PMUN from measured MUN (6). The regression procedure of SAS (14) was used to determine whether there was an effect of pretreatment milk yield on the accuracy of the MUN prediction.

For all statistical analyses, null hypotheses were rejected at $P < 0.05$ and tendencies were noted at $P < 0.10$.

RESULTS AND DISCUSSION

From the in situ analysis, estimated digestion rates were 0.080 and 0.027 per hour for the insoluble protein of soybean meal and Soy Best, respectively (Figure 1). Consequently, rumen undegraded protein fractions were calculated to be 30% for the soybean meal and 56% for the Soy Best. These results concur with the NRC (10) prediction of 35% RUP for soybean meal and with 58% RUP reported by the Soy Best manufacturers. Because the purpose of this trial was to evaluate NRC protein requirement predictions, and because most producers do not have access to in situ analyses, the RUP

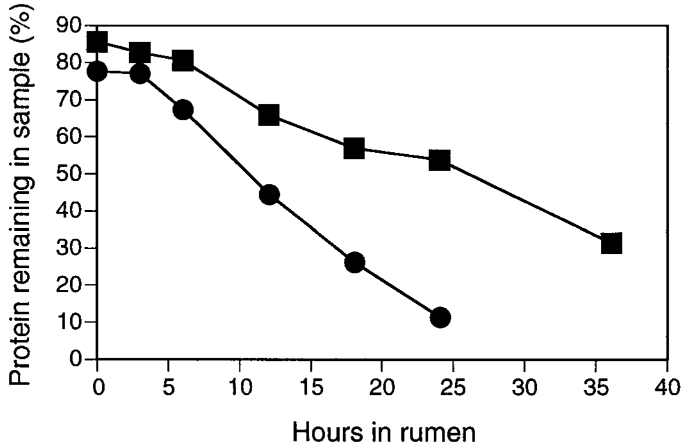

Figure 1. In situ degradation of protein in soybean meal (●) and Soy Best (■).

percentage reported by NRC (10) and Grain States Soya, Inc. were used to estimate RUP content of the rations.

The differences in nutrient intake and milk production between the two dietary treatments are shown in Table 2. Average milk and 4% FCM for the HRUP diet were 32.6 and 33.9 kg/d, respectively, and these were not different from 32.2 and 33.8 kg/d milk and 4% FCM for the LRUP diet. Total milk fat production was not different between diets (1.40 kg/d for the HRUP diet and 1.41 kg/d for the LRUP diet). Milk fat percentage was not significantly different for the LRUP diet (4.39%) compared with the HRUP diet (4.31%). Dry matter intake was the same for both diets (21.4 kg/d) and intake of energy was not different and was 36.2 Mcal/d for the HRUP diet and 36.6 Mcal/d for the LRUP

Table 2. Effect of high (HRUP) or low (LRUP) ruminally undegraded protein on production parameters.

	HRUP diet	LRUP diet	*P* <
Milk (kg/d)	32.6	32.2	NS
3.5% FCM (kg/d)	33.9	33.8	NS
Milk fat (%)	4.31	4.39	NS
Milk fat (kg/d)	1.40	1.41	NS
Milk protein (%)	3.35	3.34	NS
Milk protein (kg/d)	1.09	1.07	NS
DMI (kg/d)	21.3	21.4	NS
NE_L (Mcal/d)	36.2	36.6	NS
RDP (g/d)	2313	2570	0.001
RDP (% NRC)[1]	105	117	0.001
RUP (g/d)	1105	867	0.001
RUP (% NRC)[2]	98	79	0.001

[1]Rumen degraded protein calculated as percent of NRC predicted RDP requirements: (RDP actual/NRC predicted RDP requirement) * 100.

[2]Rumen undegraded protein calculated as a percent of NRC predicted RUP requirement: (RUP actual/NRC predicted RUP requirement) * 100.

diet. Concordant with treatment design, there was a significant difference in RDP intake (2313 vs. 2570 g/d for the HRUP and LRUP diets, respectively) and in RUP intake (1105 vs. 867 g/d) for cows on the two diets. Consequently, there was also a difference in the fraction of RDP and RUP fed as a percentage of NRC (10) predicted requirements. Cows consuming the HRUP diet were predicted to consume 105% and 98% of their NRC (10) requirements for RDP and RUP, respectively. For cows on the LRUP diet, RDP and RUP intake was at 117 and 79% of requirements, respectively. For most variables, the effects of period and cow (sequence) were highly significant ($P < 0.001$) and the effect of sequence tended toward significance ($P < 0.09$) when using the model to test the difference between dietary treatments. The effect of diet was not significant (Table 2, $P > 0.85$). The tendency for significance of a sequence effect was due to a slightly greater pretreatment milk production (2.5 kg/d) for the cows fed the HRUP diet first than for the cows fed the LRUP diet first, and it was not due to any carryover effects.

The mean NE_L, RUP, and RDP intakes relative to NRC requirements during treatment periods and the variation among cows are shown in Figure 2. On the average, the energy intake of cows from both diets was not different, and it tended to be below NRC (10) recommendations at high levels of production and above NRC recommendations at low levels of production ($P < 0.001$). There was no mean bias, indicating that on the average cows ate to their predicted energy requirements. The plot of residual RDP versus milk production shows that most cows ate more than their NRC (10) requirements for RDP. Cows consuming the HRUP diet ate about 118 g/d more than required and cows on the LRUP treatment consumed 379 g/d more RDP than required. The maximum RDP deficiency was 13% below predicted requirements for cows on the HRUP diet, and only one cow on the LRUP diet was predicted to consume less RDP than required (10% less). About half of the cows on the HRUP treatment were predicted to consume adequate RUP while the remaining half were predicted to be deficient by a maximum of 32% of total requirements. On average, cows on the HRUP diet consumed 22 g/d less RUP than thought to be required. Cows on the LRUP diet consumed an average of 230 g/d (21%) below their NRC (10) requirements of about 1098 g of RUP/d. Only four cows on this diet were predicted to consume more RUP than required, and levels of RUP deficiency for the remaining cows were predicted to range from 4 to 39% below NRC (10) requirements. For cows on both the HRUP and LRUP diets, predicted RUP deficiencies increased as production increased. At low levels of production, RUP intake was adequate or only moderately deficient, but at high production, RUP

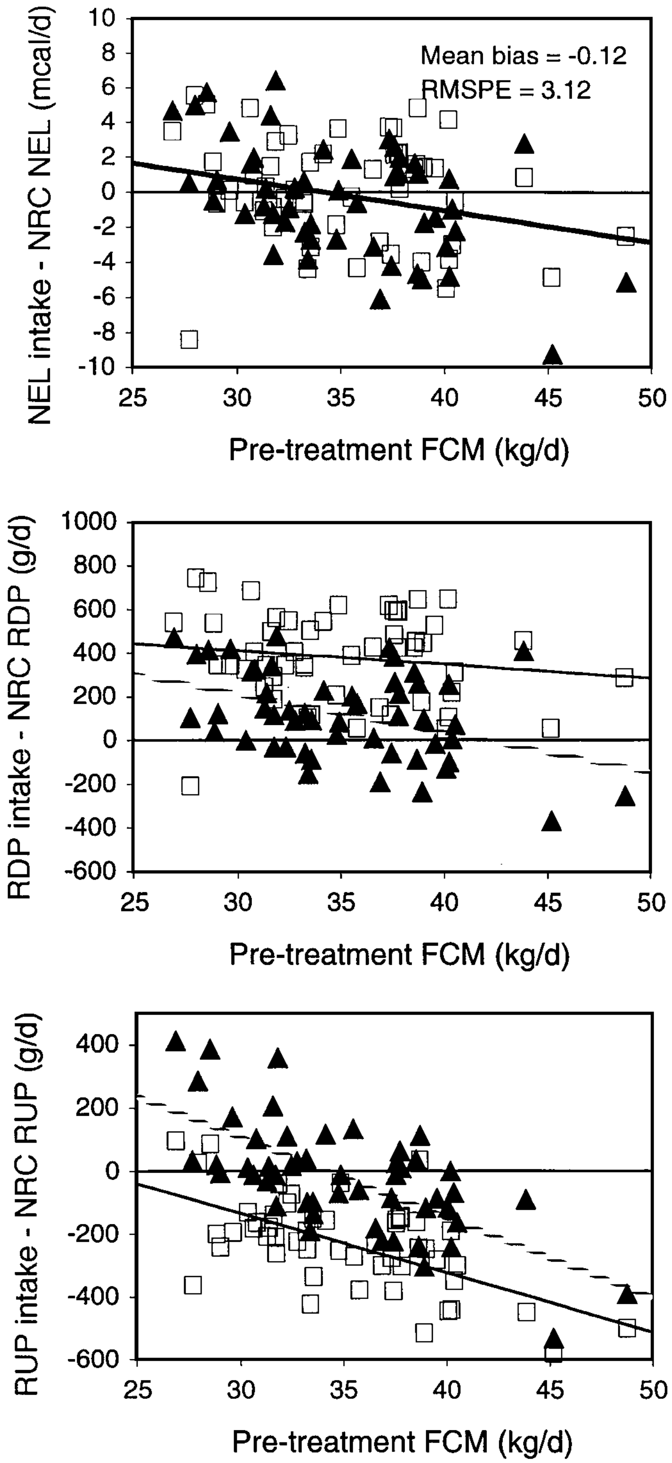

Figure 2. Observed NE_L intake minus NRC (10) predicted NE_L requirement, observed RDP intake minus NRC (10) predicted RDP Requirements, and observed RUP intake minus NRC (10) predicted RUP requirement for each treatment period versus pretreatment period 4% FCM production for cows consuming a ration high in RUP (HRUP, ▲), or low in RUP (LRUP, □). Outlying observations for one cow (pretreatment 4% FCM = 16 kg/d) were omitted to prevent crowding of remaining data. RMSPE = Root mean square prediction error.

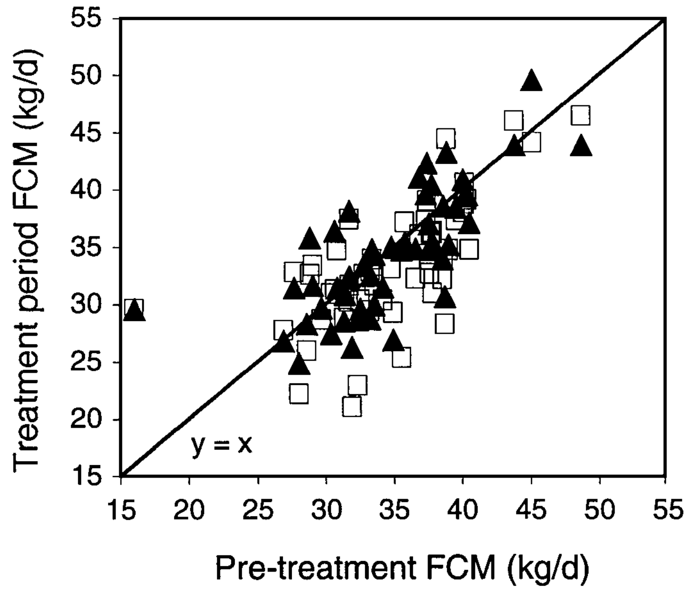

Figure 3. Treatment period 4% FCM production versus pretreatment period 4% FCM production for cows consuming a ration high in RUP (HRUP, ▲) or low in RUP (LRUP, □).

deficiencies become larger. In the middle to high ranges of production, RUP intake was predicted to be moderately deficient in the HRUP diet, and it was far below NRC (10) requirements for cows on the LRUP diet. For this reason, a loss in milk production due to the LRUP diet was most expected at high production levels when RUP deficiency was predicted to be the greatest.

Although expected RUP requirements varied widely among cows due to differences in milk production and milk composition, DIM, BW, and parity, and these differences resulted in some cows being considered far more deficient in RUP than others, the degree of predicted RUP deficiency (calculated from pretreatment parameters) had no effect on FCM in the treatment period. When testing the effect of the degree of deficiency, the effects of period and cow(sequence) were again significant ($P < 0.01$). Neither sequence ($P > 0.14$) nor predicted RUP deficiency ($P > 0.35$) affected FCM. Thus, feeding greatly below NRC recommendations in a style similar to that used in the field to feed a group of cows did not even affect milk production among the cows with the highest requirements. Figure 3 reinforces that there was no interaction between production and response to diet. Although RUP deficiency was predicted to increase with production for cows on the LRUP diet, this predicted deficiency did not depress milk production relative to cows on the HRUP diet. These results are somewhat different than those of Armentano et al. (1) in which a production response to RUP was found for the top 33% of milk producers but not for other cows. The lack of response to RUP in this experiment may have been due to unforeseen interactions between energy and protein content of the diets. Diets were equal in energy (Figure 2), and energy was fed according to the requirements of one of the higher producing cows in the group. However, this energy density appeared to cause a DMI reduction, such that diets were predicted to contain adequate energy for only 52% of the cows, primarily the lower producers. Energy balance was predicted to be most negative for the highest producing cows, and dietary energy may have limited production of these cows, preventing RUP effects on production. Similar effects would occur in the field when feeding a one-group TMR.

We conclude that there was no production effect of decreasing RUP to 200 g/d below average requirements for diets similar in energy to a one-group TMR and not limiting in RDP. National Research Council requirements for RUP may be too high for cows fed a one-group TMR. Other studies have shown that feeding RUP to individual mid- and late-lactation cows at 15 to 60% below RUP requirements did not depress milk production (11, 12). Rations might therefore be balanced at or below the 50th percentile for RUP, and this would result in lower ration costs. If NRC (10) RUP requirements are in fact too high, we may be able to reduce the total amount of protein fed to dairy cows and consequently decrease total N losses from dairy farms.

Alternatively, the RUP supplied by these diets may have been greater than predicted, potentially resulting in a lack of RUP deficiency in the LRUP diet. Diets were, however, formulated based on accepted values of undegradability for common feedstuffs. Similarly, there is only a slight difference in acid detergent insoluble CP (% DM) between the soybean meal (0.7%) and the Soy Best (2.0%), and this small difference does not reduce the predicted RUP intake of the cows on the HRUP diet substantially. Another possible explanation for the lack of difference between treatments is the difference in NSC between diets. Nonstructural carbohydrates were predicted to be 41.0% of diet DM on the HRUP diet and 43.8% on the LRUP diet. Perhaps this difference in available energy may have masked some of the treatment effects.

Figure 4 shows a plot of MUN observed minus PMUN (6) by pretreatment FCM production. There was a significant mean bias of 1.59 mg/dl indicating that, on the average, measured MUN was 1.59 mg/dl below PMUN. This bias may have resulted from differences in the lab that was used for this study compared to the labs that were used to derive the model (6). The root mean square prediction error for these observations was 3.48 mg/dl, and this was within the range of accuracy predicted for individual MUN predictions (7). There was no linear bias indicating that this MUN model (6) did adequately account for the effects of milk production and ruminal protein degradation on MUN. Baker et al. (2) suggested that MUN reflects the percentage of RDP in the diet. The data from the present study supports the hypothesis by Jonker et al. (6) that the relationship of MUN to RDP percentage results from decreases in milk protein yield that result from inadequate RUP.

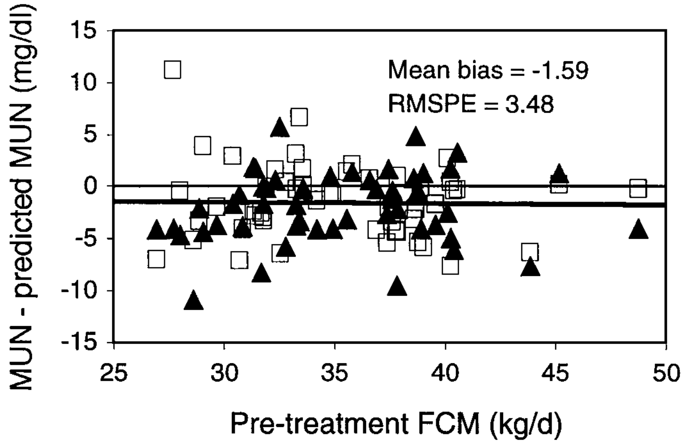

Figure 4. Observed milk urea nitrogen (MUN) minus predicted MUN (6) versus pretreatment 4% FCM production for cows consuming a ration high in RUP (HRUP, ▲) or low in RUP (LRUP, □). Outlying observations for one cow (pretreatment 4% FCM = 16 kg/d) were omitted to prevent crowding of remaining data. RMSPE = Root mean square prediction error.

There were significant effects of week and BW on MUN that were not accounted for by the model. Week effects seemed to be primarily the result of week to week biases in lab MUN analysis, and they did not show any recognizable pattern. The lab has since reduced this variation and revised the way standard curves are derived. Body weights of cows in this experiment ranged from 495 to 933 kg. There was a linear effect of BW on PMUN, and predictions were greater than observed at high BW and were lower than observed at low BW. The effect of BW (kg) upon PMUN (mg/dl) was:

$$\text{MUN observed} - \text{PMUN} = 15.55 - 0.02392 * \text{BW} \ (P < 0.001).$$

The model of Jonker et al. (6) estimates a renal clearance rate for all cows of 1254 L/d. The authors (6) state that larger cows will tend to have more blood than smaller cows, and at the same protein intake larger cows can be expected to have a lower concentration of blood urea than smaller cows. Moreover, larger cows are likely to have greater renal clearance rates and smaller cows are likely to have lower renal clearance rates than the 1254 L/d used in the model (6). Both of these effects have the consequence of reducing PMUN for heavy cows and increasing PMUN for light cows. Although these effects were noted by Jonker et al. (6), BW was not included in the PMUN model because the data used to develop the model were not robust enough to fit a parameter relating the effect of BW to MUN.

In conclusion, this study found that reducing dietary RUP by over 200 g/d below a group's average NRC (10) requirement did not depress milk production in cows fed a high energy and high RDP ration. This suggests that nutritionists might be able to reduce the level of RUP included in a 1-group TMR, potentially reducing ration costs and increasing N utilization efficiency.

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