Impacts of the Source and Amount of Crude Protein on the Intestinal Supply of Nitrogen Fractions and Performance of Dairy Cows*

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ABSTRACT

The objective of this article was to review and summarize the significance of the amount and source of dietary crude protein supplements on the supply of nitrogen fractions passing to the small intestine and the performance of lactating dairy cows. A meta-analysis was used to evaluate 2 data sets, one for nitrogen flow to the small intestine and one for performance of cows. The response of dairy cows to rumen-undegradable protein supplements is variable. A portion of this variable response from research trials is explained by the source of crude protein in the control diet, the proportion and source of rumen-undegradable protein in the experimental diet, the effect of rumen-undegradable protein on microbial protein outflow from the rumen, the degradability and amino acid content of the rumen-undegradable protein, and the crude protein percentage of the diet. Compared with soybean meal, the mean milk production responses to feeding rumen-undegradable protein supplements ranged from –2.5 to +2.75%. Because of the large variation and small magnitude of response when rumen-undegradable protein supplements are fed compared with soybean meal, efficiency of nitrogen utilization and the cost to benefit ratio for these crude protein supplements may determine the source and amount of crude protein to feed to dairy cows in the future.

(**Key words:** dairy cow performance, meta-analysis, nitrogen flow to small intestine, rumen-undegradable protein)

Abbreviation key: EAA = essential amino acids, NANMN = nonammonia, nonmicrobial N, RUP mix = a mixture of animal, marine, and/or plant protein, SBM = solvent-extracted soybean meal.

INTRODUCTION

At the onset of a new century, continued increases in cow productivity and growing concerns about the

environment and safety of food for human and animal consumption in combination with the shrinking profits of dairy producers have renewed a challenge faced by scientists for 100 yr. That challenge is that nutritionists must establish the minimal amount of protein required by high-producing dairy cows to achieve optimal, but not necessarily maximal, milk production. In addition, it is important to identify sources of supplemental protein that will accomplish this goal with maximal nutritional efficiency while minimizing environmental concerns and economical costs.

The objectives of this article were to review and summarize the effects of the amount and source of dietary CP supplements on the supply of N fractions passing to the small intestine and the performance of lactating dairy cows. Data from the literature were quantitatively summarized using meta-analyses. This summary will focus on providing estimates of the magnitude and significance of documented alterations in the supply of N fractions to the small intestine and performance of dairy cows in response to manipulations of the amount and source of dietary CP.

Statistical Analysis of Data from Literature

To summarize the available data from the literature, a meta-analytic technique (Curtis and Wang, 1998; Hedges et al., 1999) was used to evaluate 2 data sets: one for N flow from the rumen to the small intestine and one for performance of lactating dairy cows. Treatment comparisons from the compiled studies were expressed as the natural log of the response ratio, which was defined as the ratio between the responses to supplemental RUP sources in the experimental diets and supplemental CP sources in the control diets. For each data set, results from compiled studies were tested for homogeneity to determine if the data from different studies could be pooled. This was accomplished by grouping treatment comparisons into categories described by CP source in the control diet, supplemental RUP source in the experimental diet, percentage of CP supplied as RUP, and total N intake. Subsequently, groups of comparisons were tested for homogeneity between and within groups.

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Table 1. Descriptive statistics of the N flow data set.¹

Item	<i>n</i> ²	Minimum	Maximum	Median	Mean	SD
DIM, d	210	16	250	107	111	64.6
DMI, kg/d	224	10.8	26.8	19.6	19.5	3.4
Forage, % of diet DM	224	5.7	100	50.0	49.7	12.4
CP, % of diet DM	222	11.3	23.1	17.1	17.0	1.7
Protein source, % of diet CP						
Soybean meal	126	0.5	61.7	28.9	28.2	14.0
Heated soybean meal ³	20	11.3	60.3	40.8	35.2	12.7
Extruded soybean meal	3	18.1	24.1	18.1	20.1	3.4
Corn gluten meal	24	2.9	56.4	22.6	25.4	17.1
Fish meal	19	6.3	34.4	15.2	16.1	7.2
Animal meal	48	1.1	58.0	10.6	13.7	11.9
N intake, g/d	224	264	855	527	531	120.8
Flow to small intestine, g/d						
Total N	208	198	930	545	537	126.8
NAN	207	173	858	507	502	117.4
Microbial N	224	100	484	259	271	81.1
NANMN ⁴	221	61	576	234	237	87.1
Endogenous N ⁵	224	20.5	50.9	37.2	37.1	6.5
Total AA	106	917	4113	2778	2610	630
EAA ⁶	104	440	1970	1281	1218	302
Lys	108	58	264	167	168	48
Met	103	16	83	50	50	15
OMTD, ⁷ kg/d	177	5.1	15.4	9.2	9.3	2.0

¹All data were taken or calculated from experiments reported in 57 independent studies (Kung et al., 1983; Stern et al., 1983; Prange et al., 1984; Robinson and Sniffen, 1984; Santos et al., 1984; Robinson et al., 1985; Rode et al., 1985; Stern et al., 1985; Armentano et al., 1986; Lu et al., 1988; Price et al., 1988; McCarthy et al., 1989; Waltz et al., 1989; Herrera-Saldana et al., 1990; King et al., 1990; Klusmeyer et al., 1990, 1991a,b; Cameron et al., 1991; Lynch et al., 1991; Ohajuruka et al., 1991; Stokes et al., 1991; Erasmus et al., 1992, 1994; Aldrich et al., 1993; Christensen et al., 1993a, 1996; Cunningham et al., 1993, 1994, 1996; Feng et al., 1993; Poore et al., 1993; Tice et al., 1993; Benchaar et al., 1994; Mansfield and Stern, 1994; Pantoja et al., 1994, 1995; Calsamiglia et al., 1995; Oliveira et al., 1995; Overton et al., 1995; Yoon and Stern, 1996; Chan et al., 1997; Kalscheur et al., 1997a,b; Pires et al., 1997; Putnam et al., 1997; Yang et al., 1997; Zhu et al., 1997; Knowlton et al., 1998; O'Mara et al., 1998; Younker et al., 1998; Beauchemin et al., 1999; Ahvenjärvi et al., 2002; Ipharraguerre et al., 2002; Oba and Allen, 2003; Reynal et al., 2003; Voelker and Allen, 2003). Contributions of supplemental protein to dietary CP were estimated using the ingredient composition of experimental diets and the CP content of the protein sources either reported in the corresponding study or tabulated by NRC (2001).

²Number of treatment comparisons.

³Nonenzymatically browned soybean meal.

⁴Nonammonia nonmicrobial N.

⁵All data were calculated as DMI (kg/d) $\times$ 1.9 (NRC, 2001).

⁶Essential amino acids.

⁷Organic matter truly digested in the rumen.

Weighted means and 95% confidence intervals were calculated for each group of treatment comparisons using sample size and variation within and across (random) studies as weighting factors. Results are reported as the mean percentage change, which is equal to (response ratio $- 1$) $\times$ 100. If there were no differences between the control and RUP experimental diets, then the response for the control and RUP experimental diets would be the same and the response ratio would be equal to 1 and the mean percentage change would equal 0. If the response was greater for feeding RUP, then the response ratio would be >1 and the mean percentage change would be >0 or positive. If the response was less for RUP, then the response ratio would be <1 and the mean percentage change would be <0 or negative. In the figures, symbols represent the mean

percentage change, the bars represent the 95% confidence interval, and the number in parentheses is the number of treatment comparisons. If the 95% confidence interval bar does not overlap 0, then the mean percentage change is different from 0 ($\alpha = 0.05$). If the 95% confidence interval bars for the RUP supplements do not overlap, then they are significantly different ($\alpha = 0.05$) from each other.

Nitrogen Passage to the Small Intestine

Criteria required for a study to be included in the data set for N passage to the small intestine were 1) lactating cows had to be fed ad libitum; 2) the methodology had to be described and a minimum of 8 samples had to be collected within at least a 48-h period from

Figure 1. Effect of the proportion of dietary CP supplied from supplemental RUP sources in experimental diets compared with solvent-extracted soybean meal (SBM) control diets on nonammonia, nonmicrobial N (NANMN) flow from the rumen of lactating dairy cows. Means $\pm$ 95% confidence intervals (*n*; number of treatment comparisons) are shown.

the omasum or duodenum; 3) external markers had to be used to estimate digesta flow; and 4) means with standard errors or standard deviations had to be reported.

An N flow data set of 57 research trials (Table 1) provided a maximum of 224 treatment comparisons. Dry matter intake ranged from 10.8 to 26.8 kg/d, and dietary CP ranged from 11.3 to 23.1%. Only treatment comparisons that were isonitrogenous were included in the data set. The data set contained a wide range of values for passage of total N; NAN; microbial N; nonammonia, nonmicrobial N (NANMN); Lys; and Met. The mean and median for these parameters were very similar, indicating that approximately the same number of observations was either less than or greater than the treatment mean.

Regression analysis revealed a linear relationship between N intake and passage of NAN to the small intestine of lactating dairy cows fed unrestricted amounts of feed [NAN flow, g/d = 147 (SE 35.4, $P < 0.01$) + 0.676 $\times$ N intake (SE 0.06, $P < 0.01$); $R^2 = 0.74$]. These findings indicate that the amount of N consumed by cows dictates a part, but not all, of the variation in passage of NAN to the small intestine, which agrees with the conclusions of Clark et al. (1992).

Data from the meta-analysis indicated that feeding the supplemental RUP sources increased ($P < 0.05$) passage of NAN to the small intestine if soybean meal

(SBM), other plant proteins, or casein were used as the protein supplements in the control diets. However, if a source of supplemental RUP was in the control diet, then supplemental RUP in the experimental diet had no effect on NAN flow from the rumen (data not shown). These data also indicate that supplemental RUP in the experimental diet provided larger increases ($P < 0.05$) in NAN outflow when control diets contained casein rather than SBM (+25 vs. +7%). Therefore, the source of CP in the control diet to which the supplemental RUP in the experimental diet was compared had a significant effect ($P < 0.05$) on the magnitude of the response obtained from the RUP source. Because SBM was the most frequently used CP supplement in the control diets, and because SBM is widely used for feeding dairy cows, other N flow data in this paper will focus on the comparison between RUP experimental treatments and control diets that contain SBM.

Figure 1 shows the effects of including different proportions of CP in the experimental diets from supplemental RUP sources on ruminal outflow of NANMN compared with SBM control diets. All ratios of supplemental RUP inclusion increased the escape of NANMN from the rumen. The overall effect was a 26% increase ($P < 0.05$) in ruminal outflow of NANMN. The supplemental RUP increased the escape of NANMN from the rumen at all percentages of inclusion in the experimental diet. The smallest mean magnitude of response was

Figure 2. Effect of source of RUP supplement in experimental diets compared with solvent-extracted soybean meal (SBM) control diets on nonammonia, nonmicrobial N (NANMN) flow from the rumen of lactating dairy cows. Means $\pm$ 95% confidence intervals (*n*) are shown.

at the <25% concentration of RUP in the experimental diet, which is the concentration that most closely represents practical feeding recommendations of RUP supplements for lactating dairy cows. Although confidence intervals were too large to draw statistical inferences among proportions of supplemental RUP in the diet, it appears that the magnitude of the response to increasing proportions of supplemental RUP is not linear.

The mean response for each source of supplemental RUP showed that treated soy products, corn by-products, a mixture of animal, marine, and/or plant proteins (**RUP mix**), and animal meal increased ($P < 0.05$) the amount of NANMN delivered to the small intestine compared with SBM control diets (Figure 2). Other protected plant proteins and fish meal also increased passage of NANMN to the small intestine, but the response was not significant. These findings agree with other reports (Clark et al., 1992; Santos et al., 1998b) that some RUP supplements increase passage of NANMN to the small intestine of lactating dairy cows. The magnitude of the mean increase of NANMN to the small intestine caused by the different sources of supplemental RUP ranged from about 12 to 52%, and the average for all sources of RUP was more than 20%. Even though this is a wide range in response, insufficient data resulted in large confidence intervals that did not allow for detection of differences among sources of supplemental RUP.

In contrast to the positive overall effect that the RUP supplements had on passage of NANMN to the small

intestine, there was a 7% depression ($P < 0.05$) in the overall response to RUP supplements on passage of microbial N to the small intestine (Figure 3). These data support the conclusions of Hoover and Stokes (1991), Clark et al. (1992), and Firkins (1996) that a shortage of energy, AA, peptides, or ammonia in the rumen can depress the growth of ruminal bacteria when RDP sources are replaced with RUP supplements. Animal meal, fish meal, and RUP mix depressed ($P < 0.05$) ruminal outflow of microbial N by 10 to 14%. There was also a mean decrease of 6 to 8% in ruminal outflow of microbial N caused by corn by-products and other protected plant sources, and a mean increase of 2% for treated soy products, but these differences were not significant compared with the SBM control diets. Therefore, feeding the supplemental sources of RUP increased ($P < 0.05$) the intestinal supply of NANMN (i.e. feed plus endogenous protein) but decreased ($P < 0.05$) the intestinal supply of microbial N compared with SBM.

The overall mean for ruminal outflow of NAN was increased ($P < 0.05$) about 6% when experimental diets that contained RUP were compared with SBM control diets (Figure 4). Compared with SBM control diets, experimental diets that contained treated soy products, corn by-products, and RUP mix increased ($P < 0.05$) ruminal outflow of NAN. Other protected plant proteins and fish meal did not increase ruminal outflow of NAN. Animal meal increased ruminal outflow of NAN by

Figure 3. Effect of source of RUP supplement in experimental diets compared with solvent-extracted soybean meal (SBM) control diets on microbial N flow from the rumen of lactating dairy cows. Means $\pm$ 95% confidence intervals (*n*) are shown.

about 5% compared with SBM, but this increase was not significant. Overlapping of the 95% confidence interval bars indicates that the ruminal outflow of NAN was not different among sources of supplemental RUP.

Across all comparisons, the ruminal outflow of essential amino acids (EAA) also was quantitatively improved by 9% when RUP supplements were compared

with SBM (Figure 5). All RUP supplements caused a positive response, but only the response to blood meal, RUP mix, and corn by-products was significant, ranging from 12 to 16%. Therefore, these data suggest that the benefit of the greater ruminal outflow of NANMN elicited by most RUP supplements was greater than their detrimental effects on ruminal outflow of microbial N.

Figure 4. Effect of source of RUP supplement in experimental diets compared with solvent-extracted soybean meal (SBM) control diets on NAN flow from the rumen of lactating dairy cows. Means $\pm$ 95% confidence intervals (*n*) are shown.

Figure 5. Effect of source of RUP supplement in experimental diets compared with solvent-extracted soybean meal (SBM) control diets on essential amino acid flow from the rumen of lactating dairy cows. Means $\pm$ 95% confidence intervals (*n*) are shown.

Corn by-products and fish meal were the most effective sources of RUP for enhancing the delivery of Met to the small intestine (Figure 6). Although not significant, the mean response was greater for corn by-prod-

ucts than for fish meal, probably because corn by-products supplied a larger percentage of the dietary protein. In contrast, the least effective sources of RUP for improving the intestinal supply of Met were animal meal

Figure 6. Effect of source of RUP supplement in experimental diets compared with solvent-extracted soybean meal (SBM) control diets on methionine flow from the rumen of lactating dairy cows. Means $\pm$ 95% confidence intervals (*n*) are shown.

Figure 7. Effect of source of RUP supplement in experimental diets compared with solvent-extracted soybean meal (SBM) control diets on lysine flow from the rumen of lactating dairy cows. Means $\pm$ 95% confidence intervals (*n*) are shown.

and treated soy products. One of the most distinctive differences among these RUP supplements is Met content, which is greater for corn and fish proteins than for animal and soy proteins (NRC, 2001). Therefore, the amount and source of RUP in the diet and the Met content of the RUP affects the ruminal outflow of Met.

The largest positive mean increase for the ruminal outflow of Lys was obtained with animal meal (Figure 7), which has one of the highest concentrations of Lys among these RUP supplements (NRC, 2001). In contrast, the largest mean decrease was for corn by-products that contain a low concentration of Lys (NRC, 2001). Responses to fish meal, RUP mix, and treated soy proteins were intermediate between animal meal and corn by-products. As was observed for Met, the amount and source of RUP in the diet and the Lys content of the RUP supplement affects the ruminal outflow of Lys.

These data indicate that there are at least 5 factors that affect the magnitude of the effect on ruminal outflow of AA in response to feeding RUP sources to dairy cows in research trials. These factors are the source of CP in control diet, proportion and source of RUP in experimental diet, effect of RUP on ruminal outflow of microbial protein, degradability of the RUP source, and the AA content of RUP outflow from rumen.

Performance of Lactating Dairy Cows

Underfeeding or overfeeding CP to dairy cows can have detrimental effects on milk production, efficiency

of nutrient utilization, reproduction, the environment, and the overall profit of the dairy operation (NRC, 2001; CAST, 2002). Therefore, the factorial effects of the amount and source of CP fed to dairy cows must be understood if we are to optimize productivity and maximize nutrient utilization.

The effect that the availability and source of protein have on productive performance of dairy cows was examined by creating a data set from published studies designed to investigate the response of lactating cows to changes in the amount and/or source of dietary CP. Studies were included in the data set only when cows were fed ad libitum, the CP percentage of the diet was reported or could be estimated, the experimental design was adequately described, DMI and DIM were reported, and means plus standard error or standard deviation for response variables were provided.

The data set (Table 2) contained results from 112 research trials in which cows were fed TMR (88 studies) or forage and concentrate separately (24 studies). In most cases, experiments started when cows were in early lactation and ended when cows were either in early or mid-lactation. Dry matter intake ranged from 10.2 to 29.4 kg/d, and CP in DM ranged from 12.1 to 25.8%. Milk yield varied from 15 to 46 kg/d. Maximum milk CP and fat yields were about 3 times greater than minimum yields. Mean and median values were similar for all variables in this data set.

A significant curvilinear relationship ($R^2 = 0.19$) was found between milk yield and the concentration of CP

Table 2. Descriptive statistics of the performance data set¹.

Item	<i>n</i> ²	Minimum	Maximum	Median	Mean	SD
Number of cows/treatment	4732 ³	4	75	12	13	10
CP, % of dietary DM	560	12.1	25.8	17.2	17.2	2.3
DMI, kg/d	560	10.2	29.4	21.1	21.0	3.2
Milk yield, kg/d	558	15.1	46.2	31.7	31.5	5.9
Milk CP content, %	547	2.54	3.70	3.07	3.08	0.20
Milk CP yield, kg/d	402	0.53	1.50	1.00	0.98	0.17
Milk fat content, %	543	2.08	5.12	3.45	3.43	0.44
Milk fat yield, kg/d	403	0.62	1.76	1.12	1.11	0.20

¹All data were taken or calculated from experiments reported in 112 independent studies (Barney et al., 1981; Mielke and Schingoethe, 1981; van Dijk et al., 1983; Henderson et al., 1985; Polan et al., 1985, 1997; Van Horn et al., 1985; Broderick, 1986, 1992, 2003; Lundquist et al., 1986; Annestad et al., 1987; Garnsworthy and Jones, 1987; Howard et al., 1987; Jaquette et al., 1987; Kaim et al., 1987; Bowman et al., 1988; Hadsell and Sommerfeldt, 1988; Jones and Garnsworthy, 1988; Léonard and Block, 1988; Manson and Leaver, 1988; Robinson and Kennelly, 1988; Schingoethe et al., 1988; Sloan and Rowlinson, 1988; Voss et al., 1988; Bruckental et al., 1989, 2000; De Gracia et al., 1989; Garnsworthy, 1989; Herrera-Saldana and Huber, 1989; Higginbotham et al., 1989; Mäntysaari et al., 1989; Blauwiekel et al., 1990; Broderick et al., 1990, 1993; Canfield et al., 1990; Casper et al., 1990, 1999; Cody et al., 1990; Lees et al., 1990; McGuffey et al., 1990; Small and Gordon, 1990; Hoffman et al., 1991; Kim et al., 1991; Knapp et al., 1991; Owen and Larson, 1991; Petit and Veira, 1991; Robinson et al., 1991; Windschitl, 1991; Winsryg et al., 1991; Wohlt et al., 1991; Zimmerman et al., 1991, 1992; Atwal and Erfle, 1992; Calsamiglia et al., 1992; Grings et al., 1992; Harris et al., 1992; Nakamura et al., 1992; Aharoni et al., 1993; Armentano et al., 1993; Chen et al., 1993; Christensen et al., 1993b, 1994; Dhiman and Satter, 1993; Keery and Amos, 1993; Roseler et al., 1993; Grummer et al., 1994, 1996; Mansfield et al., 1994; Palmquist and Weiss, 1994; Robinson and McQueen, 1994; Tomlinson et al., 1994; Wattiaux et al., 1994; Atwal et al., 1995; Baker et al., 1995, 1996; Colin-Schoellen et al., 1995; Powers et al., 1995; Spain et al., 1995; Wheeler et al., 1995; Cunningham et al., 1996; Khorasani et al., 1996; Lines and Weiss, 1996; Pires et al., 1996; Son et al., 1996; Bernard, 1997; Dhiman et al., 1997; Henson et al., 1997; Komaragiri and Erdman, 1997; Maiga and Schingoethe, 1997; Rodriguez et al., 1997; Titgemeyer and Shirley, 1997; Weigel et al., 1997; Garcia-Bojalil et al., 1998; Grant and Haddad, 1998; Hongerholt and Muller, 1998; Nichols et al., 1998; Overton et al., 1998; Santos et al., 1998a; Kalscheur et al., 1999; McCormick et al., 1999; Mabjeesh et al., 2000; Wu and Satter, 2000; Abu-Ghazaleh et al., 2001; Bargo et al., 2001; McCormick et al., 2001; Allison and Garnsworthy, 2002; Davidson et al., 2003; Leonardi et al., 2003; Nofstger and St-Pierre, 2003; Reynal and Broderick, 2003). Dietary CP was taken as reported or estimated using the ingredient composition of experimental diets and the CP content of the ingredients either reported in the corresponding study or tabulated by NRC (2001).

²Number of comparisons.

³Total number of cows used in the data set.

in the diet (Figure 8). Diminishing returns in milk production to increases in dietary CP is a widely documented response. The multivariate regression analysis conducted by the 2001 NRC Dairy Committee resulted in an equation that predicts responses in milk production of 0.75 kg/d when CP increased from 15 to 16% and 0.35 kg/d when CP increased from 19 to 20%. Maximum milk yield is achieved at 23% CP in the diet. Using a larger data set and different methodology, estimations derived from the equation in this plot predict an increase of 0.94 and 0.42 kg/d when dietary CP is increased from 15 to 16 and 19 to 20%, respectively. Similarly, maximum milk production is achieved at 22.8% CP. These results are similar to results reported by NRC (2001).

Considerable variation in the relationship between the percentage of dietary CP and milk yield might be accounted for by the source of CP. To test this hypothesis, the data set was divided into 2 smaller data sets. The first data set contained 193 comparisons in which supplemental CP was provided entirely from SBM. The

second data set contained 149 comparisons in which supplemental CP was supplied partially from animal, marine, or blends of these 2 protein sources. A quadratic relationship provided the best fit for describing the relationship between milk yield and dietary CP percentage for both SBM ($R^2 = 0.35$) and the animal/marine proteins ($R^2 = 0.30$). According to the equation for the SBM data set, increasing CP from 14 to 18% with SBM increased milk 2.7 kg/d and milk production was maximized (32.8 kg/d) at 21.4% CP in the diet. The regression equation for the animal/marine protein data set indicated that increasing CP in the animal/marine protein diets from 14 to 18% increased milk yield by 2.9 kg/d and yield was maximized (33.8 kg/d) at 19.1% CP in the diet. These results indicate that the source of CP contributes variability to the response in milk production explained by changes in dietary CP percentage.

Ninety-four research trials that provided 244 treatment comparisons were selected from the overall data set, and meta-analyses were used to compare control diets with experimental diets supplemented with RUP

Figure 8. Prediction plot of the relationship between milk yield and dietary CP percentage for lactating dairy cows. A mixed model approach was used based on the assumption of random variation in the effect of study (St-Pierre, 2001). Treatment means were weighted by the reciprocal of the variance of the means (i.e., n/s^2 scaled using the average weight) to account for unequal replications and heterogeneous variances across studies (Oldick et al., 1999). The best-fit model (Milk yield, kg/d = 1.04 [SE 8.91, $P < 0.91$] + $2.92 \times \text{CP\%}$ in the diet [SE 1.00, $P < 0.004$] - $0.064 \times \text{CP\%}^2$ in the diet [SE 0.028, $P < 0.02$]; $R^2 = 0.19$) was estimated using the Mixed procedure of SAS (SAS Inst., Inc., Cary, NC).

sources. To prevent confounding the effect of the source of CP with the amount of CP in the diet, only treatment comparisons that were isonitrogenous were included in this data set. Cows in this data set consumed 14.0 to 29.4 kg of DM/d, which contained 12.1 to 23.3% CP. Cows produced 15.1 to 45.5 kg/d of milk. The effects of CP percentage in the diet and the source of CP in the control and experimental diets were used to evaluate variation in cow performance.

Figure 9 shows the effect of CP source in the control diet on the cows' response to RUP supplement source in experimental diets. Although not significant, the magnitude of the mean increase in milk yield when a variety of RUP experimental diets was fed was smallest when the control diet contained SBM. Data suggest that the source of CP in the control diet may influence the mean production response obtained from feeding supplemental RUP in experimental diets in research trials. Because supplemental RUP in experimental diets provided the smallest mean increase in milk yield when SBM control diets were fed, and because SBM also was the most frequently used CP supplement in control diets, data were selected from the overall data set to compare control diets that contained SBM with experimental diets that contained various sources of

supplemental RUP. Dry matter intake decreased ($P < 0.05$) when experimental diets that contained fish meal or RUP mix were compared with control diets that contained SBM (Figure 10). Some of the other RUP sources caused a slight increase and others a slight decrease in DMI compared with the SBM control, but these differences were not significant.

Examination of the mean response for RUP supplements that were compared with SBM revealed that fish meal, animal proteins, treated soy products, and RUP mix improved milk production, but the increase was significant only for treated soy products (Figure 11). In contrast, replacing SBM with corn by-products, RUP mix plus urea, and other plant proteins of reduced ruminal degradability tended to depress milk yield, but these differences were not significant. Fish meal and soy products have been suggested to have an AA profile that best resembles milk protein (Santos et al., 1998b; NRC, 2001) and that have high intestinal digestibilities (NRC, 2001). Thus, it appears that the quality of protein, the amount of protein, and the source of protein are major determinants of the milk production response from protein. Compared with the SBM control, treated soy products provided the only increase ($P < 0.05$) in milk yield, and this increase was less than 3%. The

Figure 9. Effect of source of CP in the control diet compared with RUP experimental diets on milk production of dairy cows. Means $\pm$ 95% confidence intervals (*n*) are shown.

response to other CP supplements ranged from -2.6% to $+1.4\%$. The question then becomes if or when is it economical to feed each of these protein supplements. Based on these data, it is apparent that these RUP supplements result in small differences in milk production compared with SBM.

The overall effect of replacing SBM with RUP supplements at the same level of CP in the diet decreased (P

< 0.05) the percentage of CP in milk by approximately 2%. Only when fish meal replaced SBM in the diet did the percentage of CP in milk increase (0.8%), but this increase was not significant. Because fish meal decreased ($P < 0.05$) DMI (approximately 2.5%) and tended to increase milk yield when it replaced SBM in the diet, the trend for fish meal to increase milk CP percentage might be because of increases in the amount

Figure 10. Effect of source of RUP supplement in experimental diets compared with solvent-extracted soybean meal (SBM) control diets on dry matter intake of lactating dairy cows. Means $\pm$ 95% confidence intervals (*n*) are shown.

Figure 11. Effect of source of RUP supplement in experimental diets compared with solvent-extracted soybean meal (SBM) control diets on milk production of dairy cows. Means $\pm$ 95% confidence intervals (*n*) are shown.

and/or proportion of AA supplied to the cows. The negative response ($P < 0.05$) in milk CP percentage to replacing SBM with the RUP mix plus urea (-3.4%) and mixtures of plant protein (-1.3%) may be related to a numerical decrease in DMI. The poor quality of metabolizable protein supplied by corn by-products deficient in lysine may be the reason for the depressed (-3.1% ; $P < 0.05$) milk CP percentage when they were fed to the cows. Because treated soy products increased ($P < 0.05$) milk production, their negative effect ($P < 0.05$) on milk CP percentage may be a dilution effect.

The replacement of SBM with other plant proteins, RUP mix plus urea, corn by-products, and a mixture of animal proteins decreased the mean production of milk CP, but this effect was significant only for RUP mix plus urea and the other plant proteins (Figure 12). The magnitude of the response in milk CP output to replacement of SBM with treated soy products or the animal, marine, and plant protein mixtures was small. Although not significant, replacing SBM with fish meal increased the yield of milk CP by about 2.5%.

The largest depression in milk fat yield, although not significant, occurred when SBM was replaced with other plant proteins (-5.2%), fish meal (-3.5%), and RUP mix plus urea (-3.2%). The largest increases in milk fat yield (also not significant) occurred when treated soy products (3.0%) and mixtures of animal proteins (1.5%) replaced SBM in the diet. With the exception of fish meal, these responses suggest that the effect of RUP supplements on milk fat output was largely related to their effects on milk production. The

detrimental effect of fish meal on milk fat secretion has been attributed to impairments in ruminal fermentation and/or the postabsorptive metabolism of lipids elicited by polyunsaturated fatty acids in fish oil.

CONCLUSIONS

Large variation exists in the responses of dairy cows to RUP supplements in research trials. A portion of this variation is explained by the source of CP in the control diets, the source of RUP fed to the cows, and to a lesser extent the CP percentage of the diet. The quantity and quality of the protein in the control diet and the amount and source of RUP in the experimental diet, therefore, influence the magnitude, either positively or negatively, of the response obtained from RUP supplements in experimental diets in research trials. Compared with SBM, fish meal provided the largest improvement in milk CP yield (2.5%) but depressed milk fat yield (-3.5%). Compared with SBM, milk production responses to RUP supplements ranged from -2.5% to $+2.75\%$. Treated soy products provided the largest increase in milk production (2.75%) and that in part may be explained by increased DMI. Because of the large variation and small magnitude of response when RUP supplements are fed compared with SBM, efficiency of N utilization and the cost-to-benefit ratio for these CP supplements may determine the source and amount of CP to feed to dairy cows in the future.

Figure 12. Effect of source of RUP supplement in experimental diets compared with solvent-extracted soybean meal (SBM) control diets on milk CP yield of dairy cows. Means $\pm$ 95% confidence intervals (*n*) are shown.

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