

# **CARBOHYDRATE-PROTEIN INTERACTIONS IN THE RUMEN AND POSSIBILITIES OF IMPROVING THE EFFICIENCY OF AMMONIA-N UTILIZATION IN DAIRY COWS**

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## **INTRODUCTION**

Efficiency of utilization of dietary N for milk protein synthesis by dairy cows has been calculated at 19-20% (Tamminga, 1992 and MacRae et al., 1995). These figures demonstrate the inefficient utilization of feed nitrogen in ruminants, which is due in large part to the wasteful process of N recycling occurring in the rumen. Tamminga (1992) estimated that up to 15% of the dietary N is lost to the dairy cow due to inefficient N metabolism in the rumen. Nitrogen that is not utilized by the cow is voided principally through urinary excretion of urea into the environment; some is utilized as fertilizer but the majority is wasted. In nature, urea is quickly converted to ammonia and then, when applied to cropland, to nitrate in the aerobic topsoil; substantial amounts of nitrate will leach into ground water supplies. Nitrogen, not digested and excreted with the feces, and ammonia volatilization during manure handling and application, further increase N losses from dairy operations.

## **RUMEN AMMONIA, MILK UREA N AND THE RUMEN SYNCRONY CONCEPT**

In the rumen, a significant portion of the dietary protein, amino acids and peptides are fermented to ammonia. Ammonia-N is the major source of N for ruminal bacteria, which in turn supply the cow with essential amino acids for synthesis of milk protein. We have estimated that 46% (alfalfa hay diet) to 82% (corn silage supplemented with urea) of rumen bacterial N is derived from ammonia N (Hristov and Broderick, 1996). Proportions of 70 to 80% were reported by others (Oldham et al., 1980). As a proportion of the total N pool in the rumen, free phase ammonia-N is relatively small -- less than 3% of the total non-ammonia N (NAN) pool in the rumen of alfalfa forage-fed cows (Hristov and Broderick, 1996). However, because the rate of irreversible loss of the ruminal ammonia-N is very high (calculated at 276, Oldham et al., 1980 or 480 g N/d,

Firkins et al., 1992) the irreversible N loss through this pool constituted 73% of the total N intake by the dairy cow (Oldham et al., 1980). Because microbial protein flowing out of the rumen is the major source of amino acids for the host -- accounting for up to 75 % of the total NAN flow from the rumen (Hristov and Broderick, 1996) -- ammonia produced in the rumen becomes the main source of the amino acids used for synthesis of milk proteins in the dairy cow. Therefore, the efficiency of ammonia utilization in the rumen is a critical factor determining the economic cost and environmental impact of milk protein production.

As ruminal ammonia levels correlate positively ( $r = 0.57$ ) with milk urea nitrogen (MUN, Broderick and Clayton, 1997) and increased ammonia levels in the intestine have resulted in increased NPN content of milk (Moorby and Theobald, 1999), improving the utilization of ammonia N in the rumen has the potential of reducing MUN content and consequently enhancing the processing quality of milk (Bachmann and Jans, 1995; Martin et al., 1997). Improvement in the efficiency of ammonia utilization for microbial protein synthesis in the rumen and reduction of MUN content of milk can be achieved through dietary factors such as regulating the ratio of fermentable carbohydrates (CHO) to nitrogen fractions and manipulating the composition of the CHO or nitrogen component of the diet (Rooke et al., 1987; Khalili and Huhtanen, 1991; Carruthers et al., 1997; Lyatuu and Estridge 1998; Migliorati et al., 1998). Rotz et al. (1999), using computer simulation showed that dietary manipulations could improve the efficiency of N usage on dairy farms. Some feeding systems for ruminants regulate dietary N utilization through availability of feed proteins in the rumen (the Dutch DVE and the Scandinavian AAT-PBV, for example). In the DVE system, reducing the degradable protein balance (OEB) in the rumen to 0.4 kg/d resulted in a steady decrease in N losses from dairy operations (Berentsen and Giesen, 1996) and concentration of MUN (Schepers and Meijer, 1998). Recent trials in the Netherlands have confirmed that a strong correlation between OEB and MUN exists. When OEB was corrected for the amount of absorbed protein that was not utilized for milk protein synthesis the coefficient of correlation was close to 1.0 (Ad Van Vuuren, personal communication). A strong correlation between dietary N to water-soluble CHO ratio and MUN in bulk milk was found in grazing conditions as well (Trevaskis and Fulkerson, 1999).

Ammonia concentration in the rumen can vary greatly depending on diet, feeding frequency, animal and other, unknown factors. In 8 feeding trials (11 diets varying from 0 to 95% grain content) ammonia concentrations in rumen fluid varied from undetectable to as high as 19.9 mmol/L (Fig. 1, data from Hristov and Broderick, 1996; Hristov et al., 1998a,b; Hristov et al., 1999a,b; Hristov et al.,

unpublished). Similar variation was observed in a recent trial where diets varying in ruminal CHO degradability were fed to dairy cows (Fig. 2).

Fig. 1 Ammonia concentration in ruminal fluid

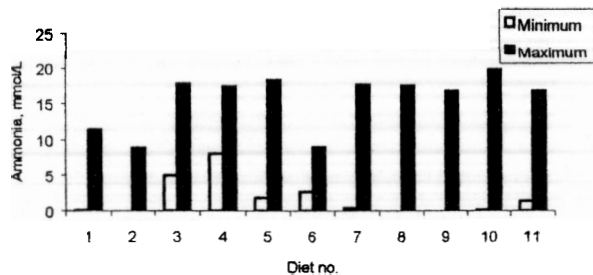
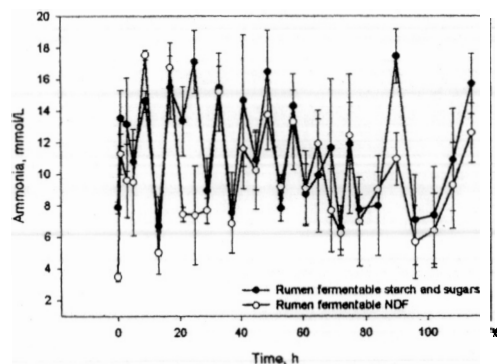


Fig. 2 Concentration of ruminal ammonia in dairy cows fed diets with varying ruminal degradability of the carbohydrate fraction (means  $\pm$  SE)



The sources of this variation have not been understood yet; some animals fed the same diets show extreme variation in ruminal ammonia concentrations not always following the pattern of feeding. The result of this phenomenon could be a lowered efficiency of microbial protein synthesis in the rumen and eventually, in N wastage. The extent to which ammonia is utilized in the rumen depends primarily on the rate of release and the balance of carbohydrates and N availability. A simplified calculation reveals that soluble sugars (expressed here as reducing sugars, RS), at the concentration they are usually found in the rumen, are responsible for utilization of a comparatively small proportion of ruminal ammonia N (rumen fluid volume and fermentation parameters from Hristov and Broderick, 1996 and Hristov et al., 1998a):

Rumen fluid volume - 41 L; concentration of RS - 630 mg/L; Total amount of RS - 31 g (increased by 20% to account for solid phase-associated RS not recovered in the fluid phase); ruminal ammonia N - 98 mg/L; Total amount of

ruminal ammonia N – 4.8 g (increased by 20% to account for solid phase-associated ammonia not recovered in the fluid phase); Theoretical maximum growth yield of ruminal bacteria – 0.4 g cells/g CHO (Russell et al., 1992); at 10% N content equals 0.04 g bacterial N/g CHO (RS in this example); Thus the RS in the rumen would support a bacterial mass growth of 1.24 g N; If an average of 70% of the bacterial N is derived from ammonia, then the soluble sugars in the rumen could account for incorporation into bacterial protein of only 18% of the ammonia N present at any time in the rumen. If the remaining 82% of ruminal ammonia N are to be utilized, the energy for this process has to come from starch and fibrous polysaccharides, CHO that, according to some workers, are inferior to soluble sugars as energy source for fixation of microbial N in the rumen (Chamberlain et al., 1993).

Excretion of urinary N in dairy cows shows a large variation, mainly related to differences in diet (including water consumption), production level, breed and management. Data summarized by Whitehead (1995) showed that variation in urinary N excretions ranged from 80 to 320 g/day. It has been suggested that the efficiency of ammonia utilization for microbial protein synthesis can be improved by dietary factors such as regulating the ratio of fermentable CHO to nitrogen fractions and manipulating the composition of the CHO or the nitrogen component of the diet. The rumen synchrony concept (Johnson, 1976) implies that microbial protein synthesis (and presumably ammonia uptake) in the rumen will be maximized if availabilities of fermentable energy and degradable protein are synchronized. Synchrony can be achieved either by changing the composition of the dietary CHO and N fractions, by altering the relative times of feeding of the dietary ingredients, or by a combination of both approaches. However, increasing the proportion of one fraction always results in a decrease in the proportion of another fraction, thus confounding the attempts to determine the relative importance of the different CHO or N fractions of the diet (Armentano and Pereira, 1997). In some animal experiments the provision of available CHO significantly reduced ammonia concentration in the rumen and increased the level and efficiency of microbial protein synthesis. Rooke et al. (1987) showed that supplementing the rumen with a source of available CHO resulted in a 45% decrease in ammonia-N concentration. The efficiency of ruminal fermentation was significantly improved as the CHO treatment increased the amount of microbial protein flowing out of the rumen by 29%, compared to control. In another study, continuous supply of CHO reduced rumen ammonia levels to 41% of the control (Khalili and Huhtanen, 1991). In this latter experiment, microbial N flow to the duodenum was increased by 31 and 46%, respectively. In an attempt to synchronize rumen availability of energy and protein, Shabi et al. (1998) concluded that the rate of

OM degradability in the rumen was the factor mostly responsible for ruminal N utilization. The majority of the trials attempting to synchronize ruminal delivery of energy and nitrogen have, however, failed to demonstrate the practicality of this concept (published studies summarized by Dewhurst et al., 2000). Casper et al. (1999) found no effect of matching ruminal degradabilities of nonstructural CHO and protein on rumen fermentation or production parameters, although the authors questioned the validity of the feeds used in achieving the goals of their experiment. Witt et al. (2000) failed to demonstrate a response in milk production or composition and rumen fermentation in lactating ewes fed diets providing three levels of the so called 'synchrony index' (N to OM release in the rumen) and to confirm their previous findings with growing lambs (Witt et al., 1999). It is worth mentioning though that, in this latter study, plasma urea N levels were lower in ewes fed the synchronous as compared to the asynchronous diets. As noted by Dewhurst et al. (2000), it is possible that effects attributed to synchrony may be simple effects specific to the individual CHO and protein fractions of the diet.

#### **EFFECT OF DIETARY CHO COMPOSITION ON UTILIZATION OF RUMINAL AMMONIA N FOR MILK PROTEIN SYNTHESIS**

The only route of physiological significance through which rumen ammonia N can be incorporated into milk protein is via *de novo* synthesis of proteins in the mammary gland from microbial amino acids absorbed postruminally. The synthesis of non-essential amino acids in the liver is of little practical importance in the dairy cow. To be utilized for milk protein synthesis ammonia must first be captured by the ruminal microorganisms and utilized for cell protein synthesis. The complexity of this process makes the quantitative measurement of rumen ammonia-N utilization difficult and often unreliable. By using isotope dilution techniques it is possible to determine quantitatively the amount of milk protein (the secondary N pool) that originates from rumen ammonia N (via bacterial N, the primary N pool). The approach, with emphasis on bacterial N transfer, was used in studies with goats (Petri and Pfeffer, 1987 and Petri et al., 1988).

To validate the technique and investigate the effect of dietary CHO composition on ruminal ammonia utilization and transfer into milk protein a trial with four ruminally and duodenally cannulated, late lactation dairy cows (715±33.1 kg BW; 323±19.5 d DIM) was conducted. The cows were cared for according to the guidelines of the ACUC at the University of Idaho. Two diets were fed in a cross-over design. The diets (Rumen Fermentable Sugars and Starch, RFSS and Rumen Fermentable NDF, RFNDF; Table 1) were formulated

to provide high ammonia levels in the rumen and adequate metabolizable protein and energy and excess of absorbable N (CPMDairy).

Table 1. Estimated composition of the experimental diets based on three models.

Diet composition	RFSS	RFNDF
1. CPMDairy <sup>1</sup>		
Crude protein (% DM)	19.8	20.0
Soluble protein (% of CP)	33.8	31.3
NEL (Mcal/kg DM)	1.74	1.70
Fermentable CHO (% DM)	48.6	42.8
CHO fractions (% of CHO)		
A	16	7
B1	55	54
B2	18	28
C	11	12
Fermentable B2+C (% B2+C)	25	32
2. Tamminga et al (1990)		
Sugars and starch from energy concentrate fermentable in the rumen (% DM)	36.0	14.0
NDF from energy concentrate fermentable in the rumen (% DM)	6.1	13.2
3. Pancho <sup>2</sup>		
Ruminally degradable CHO, g		
Fractions A + B1	8357	5758
Fraction B2	2151	3799

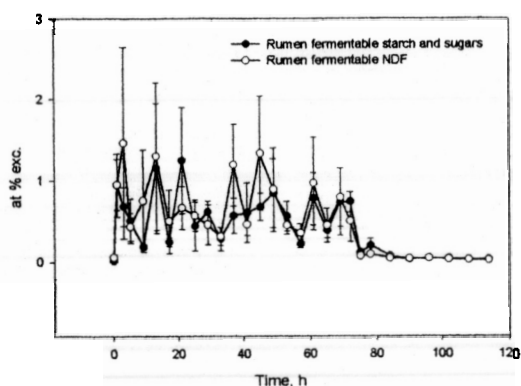
<sup>1</sup>Based on the Cornell Net Carbohydrate and Protein System and <sup>2</sup>Hubbard Feeds Inc., Mankato, MN.

The forage component was the same for both diets: alfalfa haylage and hay (38 and 34% of DM, diets RFSS and RFNDF, respectively) hence, the difference in the ruminally available CHO was resulting from the concentrate portion of dietary DM: barley and molasses vs corn, beet pulp and brewers grains (Diets RFEE and RFNDF, respectively). The diets provided similar levels (estimated) of crude, undegradable, and soluble protein and ruminally fermentable CHO but differed in the composition of the CHO fractions. Diet RFSS contained a larger proportion of available CHO from concentrate in the sugars and starch fractions (terminology

differs between the three models used) and Diet RFNDF – a larger proportion of the available CHO from concentrates in the ruminally fermentable NDF fraction. Nitrogen-15 was used to label ruminal ammonia N and consequently the microbial and milk N pools. The isotope was infused into the rumen for 3 days. Samples from ruminal contents (analyzed for  $^{15}\text{N}$ -enrichment of the bacterial and ammonia-N pools) and milk (analyzed for  $^{15}\text{N}$ -enrichment of the protein and non-protein N pools) were taken for 114 (29 samples) and 155 (28 samples) hours, respectively. Rumen samples were also analyzed for fermentation parameters. The proportion of microbial N originating from ammonia N and the proportion of milk protein N originating from bacterial (and ruminal ammonia) N were calculated based on the areas under the respective  $^{15}\text{N}$ -enrichment curves during the infusion.

Ammonia concentration in the rumen varied extremely between cows and sampling time (Fig. 2). The average ammonia concentration was lower ( $P < 0.05$ ) when cows were fed the RFNDF diet (9.8) as compared to the RFSS diet (11.4 mmol/L). The large variation in the absolute concentration of ammonia in the rumen resulted in similar variation in the  $^{15}\text{N}$ -enrichment of the ammonia-N (Fig. 3). Relatively more stable  $^{15}\text{N}$ -enrichment was achieved in the bacterial (Fig. 4) and milk protein (Fig. 5) N pools.

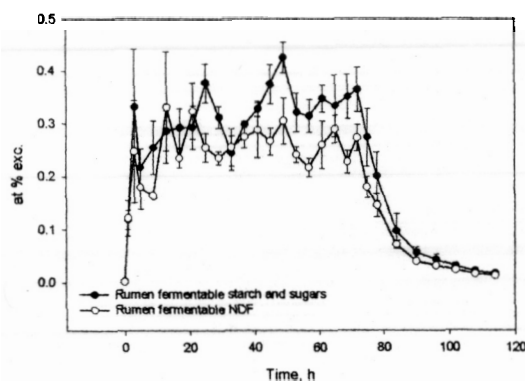
Fig. 3  $^{15}\text{N}$  enrichment of ruminal ammonia-N (means  $\pm$  SE)



Based on the areas under the  $^{15}\text{N}$  curves the proportion of ruminal bacterial N originating from ammonia N was calculated. Diet RFNDF resulted in lower ( $P < 0.1$ ) proportion of bacterial N derived from ruminal ammonia N (36.6%) as compared to diet RFSS (61.8%) (Fig. 6). These data suggest that rumen soluble sugars and starch promote more intensive ammonia uptake and incorporation into bacterial protein as compared to rumen fermentable fiber. It appears that the higher levels of rumen fermentable fiber stimulated the

incorporation of N other than ammonia (amino acid and peptide N) into the bacterial cells. The milk produced from the cows fed on the RFNDF diet had higher ( $P < 0.05$ )  $^{15}\text{N}$ -enrichment of the protein as compared to the milk from the cows consuming the RFSS diet (areas under the  $^{15}\text{N}$  curve of 9.36 and 7.99 at % exc.  $\times$  h, respectively) (Fig. 7). The proportion of milk protein N originating from ruminal microbial N was found to be higher ( $P < 0.001$ ) on the RFNDF diet as compared to the RFSS diet (44.0 and 29.4%, respectively), possibly indicating higher level of microbial protein production in the rumen or higher efficiency of microbial N transfer into milk protein on the former diet. It remains to be determined what was the total amount of ammonia N utilized for microbial growth in the rumen on the two diets. Our preliminary data indicate that the RFNDF diet resulted in a more intensive microbial growth in the rumen; consequently the total amount of ammonia-N utilized may be higher on the RFNDF diet as compared to the RFSS diet. Based on the areas under the  $^{15}\text{N}$ -enrichment curves data the proportion of ruminal ammonia N transferred into milk protein was calculated: under the conditions of the trial it was determined that 18.7 and 16.4% (Fig. 8) of the milk protein-N originated from ruminal ammonia-N (diets RFSS and RFNDF, respectively,  $P > 0.05$ ).

Fig. 4  $^{15}\text{N}$  enrichment of bacterial N (means  $\pm$  SE)



Milk production and composition did not differ ( $P > 0.05$ ) between the two diets: 26.5 and 28.4 kg, 3.80 and 3.76%, 3.35 and 3.27%, 25.3 and 26.6 kg, 1.01 and 1.06 kg, and 0.89 and 0.92 kg for milk yield, milk fat and true protein percent, 4% FCM, and milk fat and protein yields (diets RFSS and RFNDF, respectively).

The absolute recovery in milk protein of the  $^{15}\text{N}$  isotope infused into the rumen can also be used to estimate the proportion of milk protein N originating from ruminal ammonia N (and verify the isotope-based approach). Within the duration of milk sampling (155 h) 10.9 and 12.9% (SE = 0.75,  $P > 0.05$ , diets



RFSS and RFNDF respectively) of the  $^{15}\text{N}$  infused into the rumen was recovered in milk protein N. These values were lower ( $P < 0.1$ ) than the percentages estimated through the isotope dilution methods: the areas under the  $^{15}\text{N}$  curves (18.7 and 16.4%, RFSS and RFNDF respectively) or the plateau  $^{15}\text{N}$ -enrichments 16.4 and 15.5% (Fig. 8 and Table 2). Nevertheless, all methods gave recoveries within 11 to 19%. The curve fitting process gave comparatively low rates of  $^{15}\text{N}$  disappearance from the ruminal ammonia N pool (Table 2). The disappearance of the  $^{15}\text{N}$  isotope from the ruminal ammonia pool is very rapid within the first hour after infusion is ceased; Firkins et al. (1992) and Koenig et al. (2000) found slopes for this part of the curve of 0.942 and 1.673  $\text{h}^{-1}$ , respectively. The model used in our trial was successful in describing the entire curve of  $^{15}\text{N}$ -enrichment but was not designed to separate the rapid rate of decline in isotope concentration immediately after the infusion was ceased. The rates shown on Table 2 are comparable to those reported by Firkins et al. (1992) and Koenig et al. (2000) for sampling beyond 1 h: 0.047 and 0.013  $\text{h}^{-1}$ , respectively.

Table 2. Isotope kinetics data from the N pools studied and estimated isotope transfer based on plateau enrichments.

Nitrogen pool	RFSS	RFNDF	SE
<b>Rumen ammonia</b>			
Estimated plateau (at % exc.)	0.701	0.937	0.1279
Slope <sup>1</sup> , ( $\text{h}^{-1}$ )	0.049	0.051	0.0125
<b>Rumen bacteria</b>			
Estimated plateau (at % exc.)	0.333 <sup>a</sup>	0.264 <sup>b</sup>	0.0030
Slope, ( $\text{h}^{-1}$ )	0.132	0.111	0.0174
<b>Milk protein</b>			
Estimated plateau (at % exc.)	0.092 <sup>c</sup>	0.112 <sup>d</sup>	0.0051
Slope, ( $\text{h}^{-1}$ )	0.055	0.059	0.0026
Proportions (%) calculated based on plateau enrichment			
Bacterial N from ammonia N	58.1 <sup>c</sup>	34.0 <sup>d</sup>	5.65
Milk protein N from bacterial N	27.6 <sup>a</sup>	42.8 <sup>b</sup>	1.51
Milk protein N from ammonia N	16.4	15.5	2.12

<sup>1</sup>Estimated slope after isotope infusion was ceased (descending part of the  $^{15}\text{N}$ -enrichment curve).

<sup>ab</sup> Means within a row having different superscripts differ ( $P < 0.05$ ).

<sup>cd</sup> Means within a row having different superscripts differ ( $P < 0.1$ ).

In lactating goats, Petri et al. (1988) found higher (compared to the present study) proportions of milk protein originating from ruminal bacterial N and bacterial N originating from ruminal ammonia N (on average 43% and 73% compared to 29.4 and 44.0% and 61.8 and 36.6% found in the present study for diets RFSS and RFNDF, respectively) and respectively higher proportion of milk protein N originating from ruminal ammonia N (average of 31% compared to 15.3 and 14.6% found in this study for diets RFSS and RFNDF, respectively). This difference is probably a reflection of the comparatively higher level of metabolism in the lactating dairy cow and higher requirements of feed by-pass protein as compared to the lactating goat.

Fig. 5 15N enrichment of milk protein-N (means±SE)

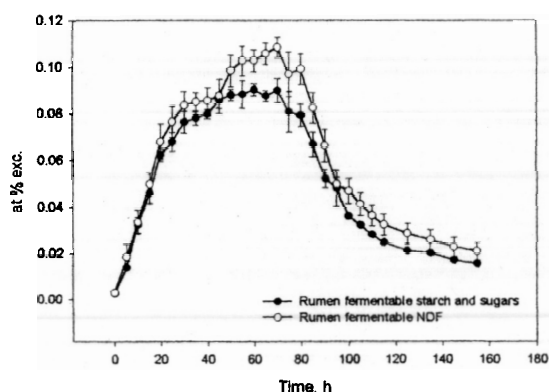


Fig. 6 Areas under the 15N curve (LS means and SE) for ruminal bacterial- and ammonia-N and proportion of bacterial protein-N originating from ammonia-N

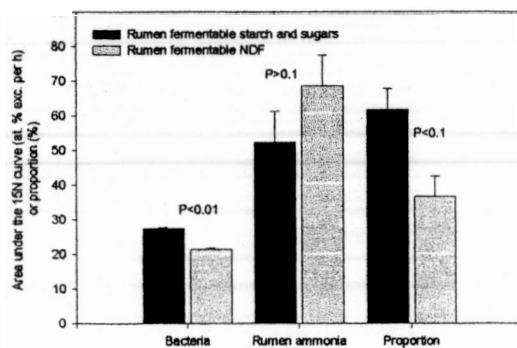


Fig. 7 Areas under the 15N curve (LS means and SE) for ruminal bacterial-N and milk protein-N and proportion of milk protein-N originating from ruminal bacterial-N

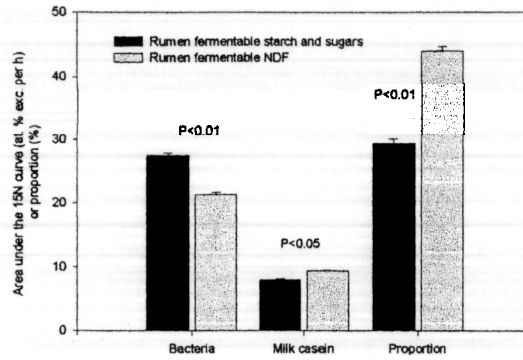


Fig. 8 Recovery of 15-N (ammonia) infused into the rumen (LS means and SE)

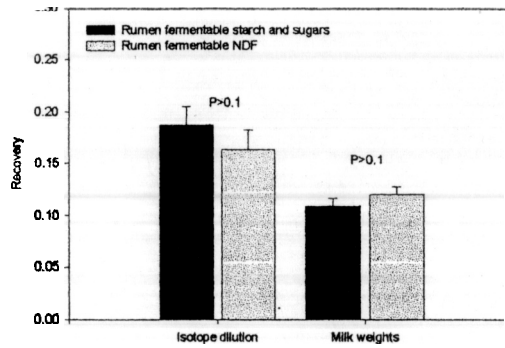
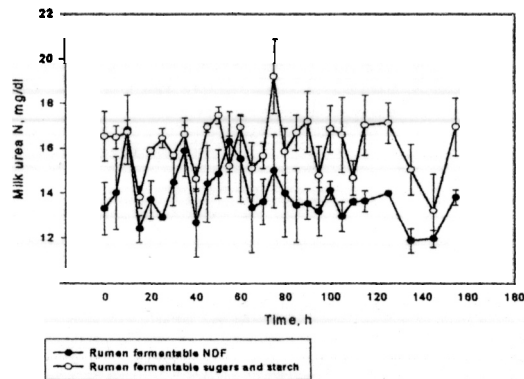


Fig. 9 Milk urea N content as affected by carbohydrate composition of the diet (means±SE)



The MUN content of the milk in this trial varied greatly with time of sampling and individual cows and was lower ( $P < 0.05$ ) on the RFNDF than on the RFSS diet: 13.9 and 16.1 mg/dl, respectively (Fig. 9) indicating more efficient utilization of dietary N with the former diet. The large variation in MUN observed in this trial questions the usefulness of spot- or individual cow-sampling for MUN analysis as an indicator of the N status of the cow (Trevaskis and Fulkerson, 1999).

### **EFFECT OF RUMINAL FERMENTABILITY OF CHO AND PROTEIN FRACTIONS ON DMI AND MILK PROTEIN YIELD**

The newer feeding systems for ruminants partition the major nutrients into fractions having different availability in the rumen. It is of interest to find out if any specific CHO or protein fraction (or fractions) can impact milk protein yield (MPY) in dairy cows. In an attempt to investigate these possible relationships 794 individual diets from a data set involving 10 years of published research (Journal of Dairy Science, volumes 73 through 82, 229 references) were analyzed for CHO and protein fraction composition based on the Cornell Net Carbohydrate and Protein System (CPMDairy program). Only trials with Holstein cows conducted in the US and Canada were considered for this study. Diets containing ingredients with unknown composition were excluded from the analysis. Data were grouped based on DIM ( $< 100$  DIM = early lactation;  $> 100$  DIM = mid and late lactation). Thus, the early lactation group (EL) included 472 diets (DMI varied from 12.9 to 29.7, average 22.0 kg/d; milk yield – from 16 to 46.0, average 32.9 kg/d) and the mid- and late lactation group (MLL) included 298 diets (DMI varied from 12.6 to 28.4, average 21.6 kg/d; milk yield – from 15.1 to 41.6, average 28.3 kg/d); 24 experimental units did not specify lactation stage of the cows. In selecting parameters for the regression models PROC CORR, PROC PRINCOMP and PROC REG and the colinearity, and forward and backward selection options of PROC REG were used (SAS, 1996).

Energy and DM intakes accounted for most of the variation in MPY in the dataset studied (Table 3). Metabolizable energy or NEL intake (identical variables,  $r = 0.9999$ ) accounted for 46 and 42% of the variation in MPY in the all lactations (AL) and EL groups. DMI accounted for 45% of the variation in MPY in the MLL group. None of the studied CHO or protein fractions had a considerable impact on MPY. Intake of fermentable CHO accounted for 1.8, 2.6, and 2.4% of the variation in MPY in AL, EL, and MLL groups, respectively. In the MLL cows NDF intake accounted for 4.1% of the variation.

Table 3. Relationships between nutrients intake and milk protein yield in lactating dairy cows.

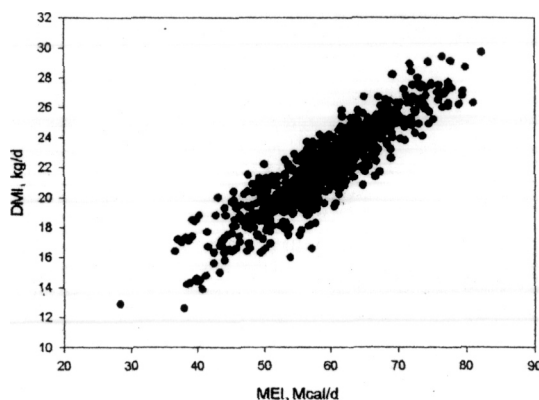
Dependent and model variables	Parameter estimate	SE	Partial $r^2$	Model $r^2$	P
All lactations (model $r^2=0.53$ , $n=794$ )					
Intercept	-0.1022	0.0644			0.1131
MEI <sup>1</sup>	0.0199	0.0016	0.461	0.461	<0.0001
BW	0.0006	0.0001	0.027	0.488	<0.0001
FermCHOI	-0.0462	0.0074	0.018	0.507	<0.0001
DMI	0.0288	0.0052	0.011	0.517	<0.0001
eNDF	-0.0431	0.0152	0.032	0.521	0.0048
FermB2CI	0.0913	0.0261	0.004	0.525	0.0005
UndegPI	-0.0440	0.0172	0.005	0.529	0.0108
CHOB2I	-0.0319	0.0129	0.005	0.534	0.0142
Early lactations (model $r^2=0.54$ , $n=472$ )					
Intercept	-0.0688	0.0754			0.3622
NELI	0.0150	0.0028	0.418	0.418	<0.0001
BW	0.0008	0.0001	0.079	0.496	<0.0001
FermCHOI	-0.0399	0.0078	0.026	0.522	<0.0001
DMI	0.0189	0.0052	0.007	0.529	0.0003
eNDF	-0.0359	0.0108	0.014	0.543	0.0010
Mid- and Late lactations (model $r^2=0.62$ , $n=298$ )					
Intercept	-0.0525	0.1054			0.6190
DMI	0.1168	0.0170	0.453	0.453	<0.0001
NDFI	-0.1130	0.0211	0.041	0.495	<0.0001
MPFeedI	0.1194	0.0477	0.026	0.521	0.0133
NSCI	-0.0914	0.0358	0.023	0.544	0.0115
BW	0.0004	0.0002	0.013	0.557	0.0180
CPI	-0.0835	0.0257	0.015	0.572	0.0014
Ferm B1I	0.0895	0.0362	0.008	0.580	0.0144
MPBactI	-0.7248	0.1658	0.009	0.589	<0.0001
FermCHOI	0.0975	0.0322	0.024	0.613	0.0029
CHOB1I	-0.0729	0.0353	0.009	0.622	0.0406

<sup>1</sup>MEI=metabolizable energy intake, Mcal; BW=body weight, kg; FermCHOI=intake of fermentable CHO, kg; DMI=dry matter intake, kg; eNDF=effective NDF intake, kg; FermB2CI=fermentable CHO B2+C fractions intake, kg; UndegPI=undegradable protein intake, kg; CHOB2I=CHO B2 fraction intake, kg; NELI=net energy of lactation intake, Mcal; NDFI=NDF intake, kg;

MPFeedI=metabolizable protein from feed intake, kg; NSCI=nonstructural CHO intake, kg; CPI=crude protein intake; FermB1I=fermentable CHO fraction B1 intake, kg; MPBactI=metabolizable protein from bacteria intake, kg; CHOB1I=CHO B1 fraction intake, kg.

Dry matter intake (DMI) was found to correlate positively ( $r = 0.65$ ) to MPY and therefore analysis on the dietary factors responsible for the variation in DMI was performed. A comparatively larger set of variables was needed to describe the variation in DMI (data not shown, model  $r^2$  were 0.67, 0.72, and 0.73 for AL, EL, and MLL cows, respectively). In all groups the largest partial  $r^2$  was associated with the concentration of Fermentable B2 (available fiber)+C fraction (as % of dietary NDF): 19.3, 20.0, and 18.3%. Of the other variables left in the models the fermentability of the B1 fraction (starch and soluble fiber), metabolizable protein content (metabolizable protein from bacteria in the EL group) and effective NDF content of the diet (and protein B1 fraction, soluble proteins, in the MLL group) accounted for 7 to 9% of the variation in DMI. When DMI was related to intake of nutrients, most of the variation ( $\geq 80\%$  in all three lactation groups) in DMI was accounted for by variation in ME intake (Fig. 10). In conjunction with intakes of NDF and NSP (non-structural polysaccharides), and fermentable B1+C CHO fractions for the EL group, ME intake accounted for up to 95% of the variation in DMI (data not shown).

Fig. 11 Relationship between DMI and ME intake in lactating dairy cows (all lactations, n=794)



## CONCLUSIONS

Complex relationships between carbohydrates and protein exist in the rumen. Testing the 'synchrony' concept of timely release of available energy and protein for maximizing microbial protein synthesis in the rumen is confounded by interactions between dietary nutrients. The results from the study presented here suggested that at the same levels of total rumen fermentable carbohydrates, compared to diets with higher rumen fermentable starch and sugars (more rapidly degradable in the rumen energy), diets containing higher concentration of rumen fermentable NDF (more slowly degradable in the rumen energy) enhanced the transfer of ruminal microbial N into milk protein. This effect was probably a result of more intensive microbial growth in the rumen. Ammonia incorporation by the ruminal microorganisms was elevated when higher proportions of rumen fermentable starch and sugars were fed but the total amount of ruminal ammonia N transferred into milk protein was not different between the two dietary treatments. The different concentrations of milk urea nitrogen between the two diets demonstrate the possibility of improving milk quality by altering the carbohydrate composition of the diet. A review of a large dataset of published nutritional trials indicated that milk protein yield is mostly related to metabolizable energy (in early lactation cows) and DM intakes (in mid- and late lactation cows) and that carbohydrate and protein fraction have little effect on this production parameter in Holstein cows. Concentration of fermentable dietary fiber and, to a lesser extent, concentration of fermentable starch and soluble fiber and effective NDF had the largest impact on DMI independently of the lactation stage of the cows. In all cows, more than 80% of the variation in DMI was accounted for by variation in metabolizable energy intake.

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