Utilization and Partition of Dietary Nitrogen in Dairy Cows Fed Grass Silage-Based Diets

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ABSTRACT

Data from 207 production trials (998 treatment means) were used to study the effects of animal and dietary characteristics on the efficiency of N utilization for milk protein production, and on fecal N, urinary N, and total manure N output. The average efficiency of transferring dietary N to milk N (MNE; milk N/N intake) was 277 (SD = 36.0) g/kg. Nitrogen efficiency was poorly related to milk yield. Dietary concentrations of crude protein (CP) and protein balance in the rumen (PBV) were the best single predictors of MNE. Dietary CP concentration explained variation in MNE better than did N intake. Bivariate models with PBV or metabolizable protein (MP) explained the variation better than CP alone. The effects of protein feeding parameters on MNE were consistent among data subsets from studies investigating the effects of the amount and protein concentration of concentrate supplement, silage digestibility, silage fermentation quality, or substitution of grass silage with legume silage. The model with total dry matter and N intakes as independent variables explained fecal, urinary, and total manure N output more precisely than N intake alone. The model of fecal N output suggested that the true digestibility of dietary N was 0.91, and that metabolic and endogenous N was the major component in fecal N. The proportion of urine N in manure N was strongly related to dietary CP concentration. Including the concentration of dietary carbohydrates only slightly improved the models, indicating that the most effective strategy to improve MNE and to decrease N losses in manure, especially in urine, is to avoid feeding diets with excessively high CP concentration and especially excess ruminally degradable CP.

Key words: N utilization, dairy cow, grass silage, protein

INTRODUCTION

Dairy farming is known to contribute to both atmospheric and hydrospheric pollution (Tamminga, 1992). In spite of improved diet formulation systems and genetic potential of the cows, N efficiency for milk protein production (MNE) has remained relatively low. Small improvements in the efficiency of nutrient utilization by dairy cows have been counterbalanced by increased external inputs of concentrate and fertilizer N to dairy farms, and consequently the efficiency of N use in the farming systems has reduced. For example, in the Netherlands, the overall N efficiency of dairy farming systems decreased from 0.40 in the 1950s to less than 0.20 in the 1980s (Van Keulen et al., 1996). However, recent data (Groot et al., 2006) demonstrate that N efficiency in dairy farm increased from 0.20 to 0.30 in 5 yr by reducing external inputs (mainly fertilizers).

To keep the soil-plant-animal system ecologically sustainable, the 3 components should be balanced with each other so that losses of N and other nutrients are minimized (Tamminga, 1996). Balanced fertilizer and concentrate N imports to the farm and improved efficiency of N uptake from the soil are more effective strategies in reducing N losses from a dairy farm to the environment than is improving the N efficiency in the animal (Van Bruchem et al., 1999; Virtanen and Nousiainen, 2005). However, it is important to understand the effects of diet characteristics on MNE, manure N output, and especially distribution between fecal and urinary N, because urinary N is much more vulnerable to evaporative and leaching losses. This information is essential in the models predicting nutrient surplus from whole production systems.

Several analyses of factors influencing manure N output have been published, but most have been based on individual cow data (Castillo et al., 2000; Kebreab et al., 2001; Nennich et al., 2005; Yan et al., 2006). Animal and dietary factors can be confounded in models based on individual cow data, which can result in biased estimates of the effects of nutritional factors on MNE and manure N output. Our objectives were to conduct a meta-analysis of data from milk production

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trials conducted in dairy cows to 1) quantify the effects of animal and dietary factors on MNE, 2) produce equations to predict fecal and urinary N output, and 3) discuss possibilities to reduce N emissions at the farm level. The data are based mainly on grass silage-based diets typical for northern European countries.

MATERIALS AND METHODS

Experimental Data

The data analyzed in this study were collected from 207 lactation trials including 998 treatment means. The studies were conducted from 1979 to 2005. One treatment mean was based on 11.2 cows. Grass silage was the main forage source, but for 82 and 38 diets, grass silage was partly or completely replaced with legume (mainly red clover) and whole-crop silages (barley, wheat, corn), respectively. The forages were fed ad libitum in all trials and concentrates were fed on a flat-rate basis irrespective of milk yield. The main ingredients in concentrates were barley, oats, molassed sugarbeet pulp, and by-products from the milling and ethanol industry. Rapeseed meal, soybean meal, and fish meal were the main protein supplements. All variables showed a wide range of variation. The following parameters describing diet composition were used for statistical analyses: dietary concentration (g/kg of DM) of CP ($6.25 \times N$), MP estimated as amino acids absorbed from the small intestine, protein balance in the rumen (PBV), NDF, RDP, RUP, starch, NFC, lactic acid (LA), and VFA. Fermentation acids were analyzed from silages, ensiled cereal grains, and from some wet by-product feeds.

Dietary concentrations of MP and PBV were computed as described in the Finnish feed tables (MTT, 2006). Microbial MP (g/kg of DM) was calculated as $0.179 \times [\text{digestible crude carbohydrates (g/kg of DM)} + \text{effective protein degradability (EPD)} \times \text{CP (g/kg of DM)}] \times 0.70 \times 0.85$, where the coefficients are the efficiency of microbial protein synthesis (0.179), proportion of amino acid N in microbial N (0.70), and digestibility of microbial protein (0.85). Digestible carbohydrates were calculated as the sum of digestible crude fiber and nitrogen-free extracts determined at maintenance level of feeding. Tabulated values (MTT, 2006) for EPD were used to compute RDP and RUP. The EDP values were derived from in situ studies and from duodenal flow data. Passage rate values of 0.02/h and 0.03 to 0.04/h for forages and concentrates were used to compute EPD. Ruminant protein balance was the difference between the RDP supply and microbial requirements of RDP; that is, it is an estimate of rumen N losses. It was expressed in grams per day or grams per kilogram

of DMI. The supply of RDP (g/d) was calculated as $\text{EPD} \times \text{CP intake (g/d)}$ and microbial CP requirement (g/kg) as $0.179 \times \text{DMI (kg/d)} \times [\text{digestible carbohydrates (g/kg of DM)} + \text{EPD} \times \text{CP (g/kg of DM)}]$. Apparent diet digestibility was determined in 95 studies (509 diets) by total fecal collection ($n = 160$) or by using acid insoluble ash (Van Keulen and Young, 1977) as an internal marker ($n = 349$). The NFC concentration was calculated as $\text{OM} - \text{CP} - \text{ether extract} - \text{NDF}$. The concentration of ME was estimated using feed table values (MTT, 2006) for concentrates. Silage-digestible OM and subsequently ME concentration were estimated in vivo in sheep fed at maintenance level ($n = 467$), by in vitro methods using rumen fluid (Tilley and Terry, 1963; $n = 114$), or by using the pepsin-cellulase method (Nousiainen et al., 2003; $n = 224$), or by some other laboratory method ($n = 183$). The same method was used within a study.

The following animal measurements were included in the data: silage, concentrate and total DM intake, yield of milk, ECM, milk CP ($6.38 \times \text{total milk N}$), fat and lactose, BW, and average DIM during experiment. Milk urea N was analyzed in 91 trials (495 diets) from composite samples of a.m. and p.m. milkings as described by Nousiainen et al. (2004). Intake of nutrients was calculated as $\text{DMI} \times \text{respective nutrient concentration}$. The apparent MNE was estimated as milk N/N intake . Fecal N output was calculated as $(1 - \text{N digestibility}) \times \text{N intake}$. Urinary N output (unaccounted N) was estimated as $\text{N intake} - \text{milk N yield} - \text{fecal N output}$, assuming that N retention was zero.

The relationships between MNE and animal or dietary parameters were estimated from the whole data set (207 comparisons, 998 diets) or from 5 data subsets, in which data was divided into studies comparing effects of the level of concentrate supplementation (87 comparisons, 217 diets), CP concentration of the concentrate supplement (127 comparisons, 336 diets), silage digestibility influenced by the stage of maturity at harvest (24 comparisons, 81 diets), silage fermentation quality influenced by the type rate of additive applications (86 comparisons, 240 diets), and replacement of grass silage with legume silages (18 comparisons, 53 diets).

Statistical Analysis

Because part of the variation in N utilization in milk production can result from differences in, for example, the stage of lactation, genetic potential of the cows, and feeding strategies, it is important to exclude this variation when nutritional factors were investigated. Therefore, the relationships between N utilization and animal or dietary measurements within an experiment were investigated using the MIXED procedure of SAS

(Littell et al., 1996) using the model $Y = B_0 + B_1X_{1ij} + b_0 + b_1X_{1ij} + B_2X_{2ij} + B_3X_{3ij} + e_{ij}$, where Y is dependent variable, $B_0 + B_1X_{1ij} + B_2X_{2ij} + B_3X_{3ij}$ is the fixed part of the model; b_0 , b_1X_{1ij} , and e_{ij} are the random part of the model; $i = 1 \dots 207$ studies; and $j = 1 \dots n_i$ values.

In the models with 1 continuous independent variable an unstructured variance-covariance matrix for the intercepts and slopes were used. Using 2 or more continuous random independent variables can result in an overparameterized model and therefore only the random slope for the first independent parameter was used. In some cases, the models with 1 independent variable did not converge when the slope was random. In those cases, all the alternative models predicting the same dependent variable were estimated using only a random intercept statement. Further details of the mixed model methodology are reported in a review by St-Pierre (2001). The models were constructed by comparing first the single parameter models and then including other biologically relevant variables with the best single parameter. The goodness of fit of the models was compared according to Akaike's information criteria (AIC). The model with the smallest AIC value is likely to be the most correct. Residual mean square errors (RMSE) were calculated for the values adjusted for the random study effect.

RESULTS

Data Characterization

Parameters of feed and nutrient intake, diet composition, digestibility, and milk production are shown in Table 1. There was a large range in dietary CP concentration (from 101 to 252 g/kg of DM) and PBV (from -51 to 82 g/kg of DM). The cows were on average 103 DIM; that is, during the lactation phase when energy balance is generally close to zero. The average MNE, expressed as milk N output/N intake (g/kg), was 277 (SD: 36.0; range: 164 to 402).

Large variation in the proportion of concentrate, dietary concentrations of starch, and NDF also indicated that the data included a wide range of diets. The variation in production parameters reflected differences in diet composition, genetic production potential of the cows, stage of lactation, and different age distribution of the cows (from only primiparous to only multiparous) used in different studies.

Effect of Milk Yield on N Efficiency

The effects of milk yield on the apparent N efficiency are shown in Figure 1. When analyzed with simple regression model, milk yield was positively associated with MNE, but it explained only 14% of the variation.

With the mixed model analysis, milk yield did not have a significant effect on MNE; that is, within a study the feeding strategies increasing milk yield did not affect MNE. The regression coefficient of milk yield was rather similar between simple and mixed models (3.4 vs. 2.9 g/kg per kg) when dietary CP concentration was included in the bivariate model with milk yield (models not shown). The stage of lactation had a strong influence on the apparent MNE, but in the mixed model analysis, DIM had no effect, probably because the cows were in the same stage of lactation within a study.

Relationships Between N Efficiency and Dietary Protein

Linear and multiple prediction equations of MNE are presented in Table 2. The model with CP intake as an independent variable did not converge when both the intercept and slope were random, and therefore in all models only the intercept was kept random. Increased protein intake, irrespective of which parameter (CP, RDP, RUP, and MP) was used, was negatively related to MNE, with PBV intake being the best single predictor and MP the worst according to AIC. Addition of DMI to the model with CP intake improved the model markedly compared with CP intake alone (Table 2). The coefficient of DMI was positive indicating that the effect of increased CP intake on MNE was strongly dependent on how CP intake was increased (increased dietary CP concentration vs. DM intake). The best model based on RMSE and AIC was a bivariate model with PBV and MP intake as independent variables.

Milk N efficiency decreased as dietary CP concentration increased (Table 3). Including the quadratic effect improved the model according to the AIC value. Expressing CP as a ratio to ME rather than on a DM basis improved the model. Rumen PBV was a better predictor of MNE than CP, and the model was further improved when the MP concentration was used with PBV. The negative regression coefficient of PBV on MNE was much greater than that of MP (-1.49 vs. -0.57 g/kg per g/kg of DM).

Effects of Other Dietary Factors on N Efficiency

The effects of some other parameters in addition to PBV and MP intake are shown in Table 4. The model with PBV and MP intake as independent variables was improved when ME concentration was added in the model. However, it should be noted that the effect of ME was small in relation to the standard deviation of dietary ME concentration (0.48 MJ/kg of DM). Concentrations of the carbohydrate fractions had only minor effects on MNE. The quadratic models suggested that optimum NDF and NFC concentrations were 383

Table 1. Feed and nutrient intake, diet composition, and production parameters of the dairy cow data used for the assessment of N utilization

Item	n	Mean	SD	Minimum	Maximum
DMI (kg/d)					
Forage	998	10.5	1.94	4.6	17.4
Concentrate	998	7.4	2.33	0.0	18.4
Total	998	17.9	2.82	9.9	25.2
Concentrate (kg/kg of DMI)	998	0.41	0.100	0.00	0.80
Diet composition (g/kg of DM)					
CP	998	165	21.5	101	252
MP	998	95	4.9	80	118
PBV ¹	998	8	17.3	-51	82
RDP	998	132	17.1	82	205
RUP	998	33	6.1	17	59
NDF	998	407	50.0	195	571
Starch	998	147	60.0	0	364
Nutrient intake (g/d)					
CP	998	2,955	588	1,329	5,046
MP	998	1,696	301	904	2,744
PBV	998	136	298	-805	1,616
RDP	998	2,358	441	1,109	3,986
RUP	998	597	163	199	1,275
Starch	998	2,643	1,096	0	5,971
NDF	998	7,282	1,353	3,621	11,129
ME (MJ/d)	998	206	33.8	114	298
Digestibility					
OM	509	0.736	0.038	0.621	0.830
Nitrogen	483	0.691	0.041	0.542	0.819
NDF	400	0.629	0.072	0.408	0.830
Production data					
DIM	998	103	30	38	226
Milk yield (kg/d)	998	25.4	5.05	13.0	45.8
ECM yield (kg/d)	998	26.1	5.14	12.8	42.1
Milk composition (g/kg)					
Fat	998	42.8	3.96	31.9	55.0
Protein	998	32.1	1.70	25.9	37.8
Milk component yield (g/d)					
Fat	998	1,081	217	479	1,671
Protein	998	814	171	359	1,449
Milk N/N intake (g/kg)	998	277	36.0	164	402
MUN (mg/100 mL)	495	13.3	3.5	3.7	26.5

¹PBV = rumen protein balance.

and 313 g/kg of DM and optimum NFC/NDF was 0.99 (models not shown). However, the variation around the optimum was minimal.

Including silage LA, VFA, or total acid concentration ($n = 838$) did not improve the models. Only VFA showed a trend ($P = 0.07$) toward reduced MNE with increased concentration, but the slope was small (-0.14 g/kg per 1 g/kg of DM increase in silage VFA concentration). Increased soluble N concentration in silage N also tended ($P = 0.08$) to decrease MNE ($n = 537$), but the effect can almost entirely be attributed to silage ammonia N ($P = 0.01$), and the effect of soluble nonammonia N was nonsignificant ($P = 0.31$).

Addition of MUN ($n = 495$) with CP concentration or CP intake improved the model according to AIC, and the negative effects of MUN on MNE were highly significant ($P < 0.001$). However, the goodness of model was not improved when MUN was used together with PBV and MP concentrations or intakes.

Analysis of Data Subsets

Regression equations for MNE for the data subsets are shown in Table 5. Regression equations were rather similar irrespective of the method used to manipulate nutrient supply. Including the concentration of PBV always resulted in a better model compared with CP concentration according to AIC and, except for the concentrate supplementation studies, MP/CP ratio resulted in better models than PBV. Prediction error (RSME) was greatest in the concentrate level studies and the lowest in protein supplementation studies.

Partition of N

Fecal N output was more closely associated with DM than with N intake (Table 6). The prediction was further improved when they both were used in the model. Regression coefficients indicated that fecal N output in-

Table 2. Effects of nutrient intake on the efficiency of N utilization (milk N/N intake, g/kg) estimated by mixed model regression analysis ($Y = A + BX_1 + CX_2$)¹

X ₁	X ₂	A	SE	B	SE	P-value	C	SE	P-value	RMSE ²	AIC ³
CPI		397	4.6	-0.042	0.001	<0.001				10.29	8,512.0
RDPI		407	4.8	-0.057	0.002	<0.001				10.35	8,509.6
RUPI		343	3.9	-0.116	0.005	<0.001				11.91	8,741.9
PBVI		289	1.6	-0.089	0.002	<0.001				8.63	8,029.9
MPI		483	26.3	-0.048	0.004	<0.001				14.28	9,038.8
CPI	EPD	354	7.3	-0.044	0.001	<0.001	-99.1	29.7	<0.001	10.26	8,492.3
CPI	DMI	290	6.0	-0.065	0.002	<0.001	10.0	0.45	<0.001	8.87	8,129.6
RDPI	RUPI	404	5.0	-0.050	0.003	<0.001	-0.020	0.007	0.01	10.27	8,510.1
PBVI	MPI	312	4.5	-0.086	0.002	<0.001	-0.014	0.003	<0.001	8.29	8,013.5

¹CPI = CP intake (g/d); RDPI = RDP intake (g/d); RUPI = RUP intake (g/d); PBVI = protein balance in the rumen (g/d); MPI = MP intake (g/d); EPD = effective protein degradability (g/g); DMI = total DM intake (kg/d).

²RMSE = residual mean square error.

³AIC = Akaike's information criterion.

creased by 6.7 g per 1-kg increase in DMI and that the true CP digestibility of the incremental CP was 0.91. Fecal N output increased as the proportion of legume silage, mainly red clover, in forage increased. Urinary (unaccounted) N output increased with increased N intake, but at a constant N intake, DMI had a negative effect on urinary N output. The models were further improved by adding dietary ME concentration or milk yield as a third parameter in the model. Both fecal and urinary N output increased with milk yield, but the regression coefficient was much greater for urinary N compared with fecal N (11.4 vs. 5.5 g of N/kg of milk). The proportion of urinary N of the total manure N was closely related to dietary CP concentration. In the data with MUN analysis (n = 348), MUN improved the urinary N output model compared with dietary CP alone (Table 6).

DISCUSSION

Compared with previous studies (Wilkerson et al., 1997; Castillo et al., 2000; Nennich et al., 2005; Yan et al., 2006), this study comprised several different aspects. First, it is based on a large treatment means data set rather than on individual cow data. Second, the present study used a more comprehensive analysis of nutrient supply including a detailed description of dietary protein and carbohydrate composition. Third, the relationships were examined when diet composition was manipulated using different nutritional strategies; for example, increased concentrate feeding, improved forage digestibility, and so on. In previous meta-analyses, only Nennich et al. (2005) used a mixed model procedure excluding the random study effects from the variation. As noted by St-Pierre (2001), using simple regression analysis can result in misinterpretation of the biological relationships.

Effect of Milk Production

The relationship between milk yield and N utilization was poor ($R^2 = 0.14$; Figure 1), although significant with the simple regression model. The R^2 value increased

Figure 1. Relationship between milk yield and N efficiency analyzed by a simple (top) or mixed model regression (bottom). The values for mixed model data are adjusted for the random study effect.

Table 3. Effects of dietary protein parameters on the efficiency of N utilization (milk N/N intake, g/kg) estimated by mixed model regression analysis ($Y = A + BX_1 + CX_2$)¹

X ₁	X ₂	A	SE	B	SE	P-value	C	SE	P-value	RSME ²	AIC ³
CP		475	4.9	-1.20	0.028	<0.001				8.88	8,101.0
CP	EPD	549	21.8	-1.24	0.030	<0.001	-84.2	24.1	0.001	8.88	8,080.9
CP	CP × CP	636	17.6	-3.08	0.200	<0.001	0.0054	0.0006	<0.001	8.40	8,028.8
CP/ME		479	4.9	-14.2	0.319	<0.001				8.90	8,067.6
CP/ME	(CP/ME) × (CP/ME)	627	15.9	-33.9	2.05	<0.001	0.65	0.067	<0.001	8.40	7,981.1
PBV		289	1.6	-1.58	0.035	<0.001				8.86	8,044.5
PBV	PBV × PBV	288	1.6	-1.80	0.042	<0.001	0.0076	0.0008	<0.001	8.44	7,979.6
PBV	MP	342	12.6	-1.49	0.040	<0.001	-0.57	0.134	<0.001	8.68	8,029.7
MP/CP		2	6.1	473	10.2	<0.001				8.74	7,996.6

¹CP = crude protein (g/kg of DM); EPD = effective protein degradability (g/g); CP/ME = CP/metabolizable energy (g/MJ); PBV = protein balance in the rumen (g/kg of DM); MP = metabolizable protein (g/kg of DM).

²RMSE = residual mean square error.

³AIC = Akaike's information criterion.

Table 4. Effects of some dietary factors in addition to PBV and MP intake (g/d) on the efficiency of N utilization estimated by mixed model regression analysis ($Y = A + BX_1 + CX_2 + DX_3 + EX_4$)¹

X ₁	X ₂	X ₃	X ₄	A	B*	C*	D†	E	SE	P-value	RSME ²	AIC ³
PBVI	MPI			312	-0.086	-0.015					7.23	7,951.2
PBVI	MPI	ME		248	-0.085	-0.020	6.2				7.07	7,931.2
PBVI	MPI	ME	Starch ⁴	234	-0.085	-0.019	6.9	0.012	0.0144	0.40	7.07	7,937.1
PBVI	MPI	ME	NDF ⁴	224	-0.088	-0.015	8.2	-0.006	0.0135	0.68	7.05	7,939.7
PBVI	MPI	ME	NFC ⁴	263	-0.083	-0.021	4.9	0.025	0.0094	0.01	7.06	7,932.1

¹PBVI = protein balance in the rumen (g/d); MPI = MP intake (g/d); ME = ME concentration (MJ/kg of DM); Starch, NDF (g/kg of DM); NFC = nonfiber carbohydrates (g/kg of DM).

²RMSE = residual mean square error.

³AIC = Akaike's information criterion.

* $P < 0.001$; † $P < 0.01$.

Table 5. Effects of protein parameters on the efficiency of N utilization when nutrient supply was manipulated by different strategies ($Y = A + BX_1$)¹

Item/parameter ¹	n	A	SE	B	SE	P-value	RSME ²	AIC ³
Concentrate level								
CP	217	492	17.4	-1.29	0.105	<0.001	8.26	1,866.4
PBV	217	293	2.0	-2.07	0.130	<0.001	7.94	1,803.6
MP/CP	217	-58	24.9	581	41.9	<0.001	8.78	1,808.6
Protein supplementation								
CP	336	485	7.6	-1.25	0.041	<0.001	4.64	2,677.0
PBV	336	292	1.9	-1.73	0.055	<0.001	4.50	2,651.8
MP/CP	336	-23	7.1	522	13.4	<0.001	4.77	2,594.9
Silage digestibility								
CP	81	462	19.2	-1.16	0.103	<0.001	6.25	694.0
PBV	81	290	3.9	-1.78	0.131	<0.001	7.45	687.6
MP/CP	81	-12	18.4	496	31.7	<0.001	7.41	667.3
Silage fermentation quality								
CP	240	482	19.1	-1.31	0.112	<0.001	5.57	1,912.2
PBV	240	282	2.6	-1.69	0.128	<0.001	5.64	1,881.1
MP/CP	240	-30	17.6	520	32.1	<0.001	5.60	1,850.7
Inclusion of legume silages								
CP	53	466	28.2	-1.22	0.16	<0.001	6.82	448.2
PBV	53	281	5.0	-1.61	0.17	<0.001	5.62	426.8
MP/CP	53	-41	16.8	543	29.4	<0.001	6.05	395.7

¹CP = crude protein (g/kg of DM); PBV = protein balance in the rumen (g/kg of DM); MP = metabolizable protein (g/kg of DM); MP/CP = metabolizable protein (g/kg of DM)/CP.

²RMSE = residual mean square error.

³AIC = Akaike's information criterion.

Table 6. Effects of dietary factors on fecal and urinary N (unaccounted N) output ($Y = A + BX_1 + CX_2 + DX_3$)¹

Item	X ₁	X ₂	X ₃	n	A	B	C	D	RSME ²	AIC ³
Fecal N (g/d)	NI			515	54	0.197			10.08	4,214.9
	Milk			515	7	5.49			10.31	4,252.7
	DMI			515	-28	9.90			9.38	4,127.4
	DMI	NI		515	-21	6.73	0.101		8.92	4,064.1
	DMI	NI	Legume	515	-21	7.00	0.090	20.0	8.81	4,047.0
Urinary N (g/d)	NI			515	-126	0.676			12.3	4,484.1
	Milk			515	-91	11.4			24.6	5,259.2
	NI	DMI		515	27	0.844	-13.0		9.28	4,134.2
	NI	DMI	ME	515	104	0.855	-13.2	-6.8	9.19	4,118.6
	NI	DMI	Milk	515	40	0.879	-9.0	-3.9	8.00	4,052.0
Urinary N/manure N (g/kg)	CP			515	155	2.48			19.32	5,028.1
	CP	CP ²		515	-241	7.11	-0.013		19.54	5,018.2
	CP			348	120	2.70			17.08	3,304.2
	CP	MUN		348	203	1.61	7.0		16.21	3,272.3
Manure N (g/d)	NI			515	-65	0.859			4.33	3,593.8
	NI	DMI		515	-1	0.937	-5.7	-5.7	3.66	3,298.6
	NI	DMI	ME	515	81	0.947	-5.9	-5.9	3.35	3,229.6

¹NI = N intake (g/d); DMI = total DMI (kg/d); Legume = proportion of legumes in the forage (kg/kg); ME = Dietary ME concentration (MJ/kg of DM); Milk = milk production (kg/d); CP = dietary CP concentration (g/kg); MUN = milk urea N (mg/100 mL).

²RMSE = residual mean square error.

³AIC = Akaike's information criterion.

to 0.73 when dietary CP concentration was added into the model, which suggests that diet composition rather than production level is the main determinant of MNE in dairy cows. Based on regression coefficients of both simple and mixed bivariate models, 10 g/kg of DM in dietary CP concentration and 1,200 to 1,300 kg in annual milk yield have similar effects on MNE. A recent study by Rechtenwald (2007) demonstrated that high (>40 kg/d) milk yields can be obtained feeding a diet containing 140 g of CP/kg of DM with only marginal production losses compared with a higher CP diet.

The different effect of milk yield on MNE when a random study effect was included in the statistical model is an example of the simple regression model leading to biological misinterpretation of the data (St-Pierre, 2001). When the random study effect was ignored, the positive relationship between milk yield and N efficiency arose evidently from the improved genetic milk yield potential during the time course of the present data (from 1979 to 2005) and from variation in DIM between the studies. Generally, it is assumed that MNE is improved with increased milk yield due to reduced maintenance costs (Tamminga, 1992). However, when the annual milk yield exceeds 7,500 kg, only small progresses in MNE can be expected from increased milk yield (Tamminga, 1996). Van Bruchem et al. (1999) suggested that breeding more-productive dairy cattle is unlikely to be effective in reducing the N surpluses of milk production, because highly produc-

tive dairy cows need diets with greater CP and energy concentrations. In a recent study based on field data, Virtanen and Nousiainen (2005) did not observe any relationship between milk yield and farm N efficiency.

In the present data, the marginal increase in milk protein yield within a study was 153 g/kg increase in CP intake, which is markedly lower than the mean efficiency (277 g/kg). Furthermore, increased milk yield within a study was associated with a much greater increase in urinary than fecal N output per kilogram of extra milk (Table 6). Because urinary N is more susceptible to leaching and volatilization than fecal N, a shift toward greater urinary N excretion with increased milk yield is undesirable. However, the effects of increased milk yield on partitioning of manure N are equivocal. Broderick (2003) substituted a mixture of alfalfa and corn silage isonitrogenously with a mixture of high-moisture corn and soybean meal. In that study, increased milk yield with enhanced levels of high-moisture corn were associated with decreased urinary N output with no changes in fecal N output.

Effect of Diet Composition (All Data)

Reduced N efficiency with increased N intake (Table 2) is consistent with the observations of Castillo et al. (2000), who reported decreases in MNE especially with daily N intakes exceeding 400 g. Including EPD in the CP intake model or dividing CP intake into RDP and

RUP intake did not markedly improve the goodness of fit of the model according to AIC, which suggests that estimates of protein degradability may not be accurate. It is also possible that expected benefits from the reduced degradability were not fully realized because of reduced microbial protein synthesis (Santos et al., 1998; Ipharraguerre and Clark, 2005), lower RUP digestibility of treated protein supplements (Rinne et al., 1999), or nonideal AA composition of RUP.

The better predictions with PBV intake could be expected, because it is an estimate of excess rumen degradable N, which is absorbed as ammonia from the rumen (Madsen, 1985). In addition to degradability, PBV takes into account the energy supply for microbial protein synthesis. Prediction was further improved including MP intake in the model, but the negative coefficient for MP intake was proportionally only 0.16 of that for PBV. This suggests that if the MP supply could be increased with minimal increases in PBV, MNE could be improved without compromising milk yield. Børsting et al. (2003) showed that positive PBV had only minor effects on milk protein yield, and they concluded that maximal yield and high MNE could be reached at a rumen protein balance close to zero.

Previously, MNE (Castillo et al., 2000) and manure N output (Kebreab et al., 2001; Yan et al., 2006) have been predicted as a function of N intake. In the present study, using DMI with CP intake in the model developed a more precise prediction than CP intake alone. It should also be noted that these variables had a different coefficients (Table 2). When the increase in CP intake is due to greater DMI, the supply of fermentable OM also increases, allowing a more efficient capture of RDP by rumen microbes and utilization of absorbed AA for milk protein synthesis.

Dietary CP concentration explained the variation in MNE better than did CP intake. The ratio CP/ME resulted in a more precise model than CP (Table 3), probably because the supply of fermentable substrate for rumen microbes increased with ME. However, PBV (which takes into account both ruminal degradability and energy supply for microbial protein synthesis) was better than CP or CP/ME. The ratio MP/CP was the best single predictor of N efficiency (Table 3). This parameter takes into account fermentable energy supply for rumen microbes and protein degradability in addition to CP concentration. In a bivariate model with PBV and MP as independent variables, the slope was greater for PBV than for MP (−1.57 vs. −0.49). The negative slope indicates that N efficiency is also decreased by overfeeding MP, even without an increase in rumen N losses.

The diets of the present study were based largely on grass silage as forage. Soluble sugars of grass are

converted to LA and VFA through the action of anaerobic bacteria in the silo, and grass CP is extensively degraded to NPN. Silage CP is rapidly and extensively fermented to ammonia in the rumen, and therefore, synchronizing energy and N release in the rumen should improve MNE (Sinclair et al., 1993). However, this study did not provide support for benefits from including more starch or NFC in the diet. It is possible that the opportunity to improve MNE with increased starch or NFC supplementation was not realized because of limits imposed by excess acid production and depressed pH in the rumen. In other studies, some positive responses to improved synchronization of energy and N in terms of rumen microbial N production or improved milk production have been reported (see Castillo et al., 2000). On the other hand, the review of Chamberlain and Choung (1995) suggests that no benefits of synchronization (timing the energy and N release) can be expected.

Fermentation of sugars to LA and VFA in the silo decreases microbial N synthesis in the rumen (Jaakkola et al., 2006), but the extent of silage fermentation in the silo did not influence MNE in the present meta-analysis. Increased blood glucose supply from the fermentation of silage LA to propionic acid could counterbalance the reduced microbial N synthesis (Harrison et al., 2003); and MNE is not reduced when extensively fermented silages are fed.

Analysis of Data Subsets

The effects of dietary CP and PBV concentrations or the ratio of MP/CP on the efficiency of N utilization were similar regardless of how the diet composition was changed (Table 5). This demonstrated that increased dietary CP concentration uniformly decreases MNE. The effects of the level of concentrate supplementation on MNE depend on the changes in dietary CP and especially in PBV concentration. When high protein forages are supplemented with increasing amounts of low CP concentrates, MNE improves, whereas the reverse is true when low CP silages are supplemented with increasing amounts of high CP concentrates. The slope of concentrate DMI on MNE was 3.8, 0.1, and −3.7 g/kg for concentrates containing <150, 150 to 220, or >220 g of CP/kg of DM, respectively.

Attempts to improve MNE by feeding more concentrates with grass silage-based diets have not been very successful, especially if high CP concentrates are fed. This is at least partly because the marginal production responses of 0.09 to 0.10 kg of ECM or milk per MJ of ME (Thomas, 1980; Huhtanen, 1998) to extra ME from increased concentrate feeding are relatively small compared with the expected response of 0.194 (MTT,

2006). As discussed previously, optimum dietary NDF and NFC concentrations suggest that N efficiency is, in most cases, maximized using moderate levels (0.35 to 0.50) of concentrates.

Reducing the ruminal degradability of protein supplements has been suggested as a strategy to improve MNE (Tamminga, 1992; Kirchgessner et al., 1994). However, in protein supplementation studies, MNE only tended ($P = 0.08$) to improve with reduced EPD when it was used with CP concentration in the model (data not shown). This is surprising, because different protein supplements ranging from urea to fish meal were used. Different explanations can be suggested for this. First, the current methods and protein evaluation models may overestimate the range in protein degradability. Indeed, using a constant degradability for all feeds resulted in better predictions of milk protein yield responses compared with using *in situ* determined values (Tuori et al., 1998) or tabulated values (Schwab et al., 2005). Second, a review of large number of studies by Santos et al. (1998) and Ipharraguerre and Clark (2005) suggested that increased supply of RUP often resulted in a reduced supply of rumen microbial protein. More specifically, with grass silage-based diets, heat-moisture-treated rapeseed cake did not elicit milk or milk protein yield responses compared with untreated rapeseed meal despite the fact that graded doses of each supplement produced substantial production responses (Rinne et al., 1999). The true digestibility of supplemental protein derived from heat-treated rapeseed expeller was significantly lower than that of solvent-extracted rapeseed meal (Rinne et al., 1999) or soybean meal (Shingfield et al., 2003). Kebreab et al. (2001) also reported greater fecal N output with decreased protein degradability, but urinary N output was decreased.

Partitioning of Dietary N

The relationship between N intake and fecal N output was in agreement with the equation presented by Castillo et al. (2000) based on individual cow data. Fecal N output was predicted more precisely using a model with DM and N intakes as independent variables rather than N intake alone. The bivariate model better takes into account the origin of fecal N losses, which are derived from excretion of undigested feed N, undigested microbial N, and endogenous N. Metabolic and endogenous N, which has a major contribution to fecal N, is related to DMI (Van Soest, 1994), whereas undigested feed N is related to N intake.

The negative intercept of the bivariate model, which is biologically impossible, suggests a curvilinear relationship between DMI and metabolic and endogenous

fecal N. Indeed, a quadratic model solved the intercept problem, but the standard errors of parameters were high. Another alternative to predict fecal N output is to use the Lucas test and include DMI in the model to predict fecal N output per kilogram of DMI. For a cow consuming 20 kg of DM/d (160 g of CP/kg of DM), the bivariate model (N intake and DMI) predicted fecal N output of 165 g/d, of which 69% was of metabolic and endogenous origin and 31% undigested feed N.

As discussed by Tamminga (1992), possibilities to improve MNE by improving N digestibility seem to be limited because the true digestibility of dietary N is rather constant and high. Endogenous N losses seem mainly to be related to DM passing the small intestine and undigested DM or OM excreted in feces (Tamminga, 1992). However, addition of predicted diet digestibility in the model had no influence on fecal N output in the present study.

Accurate prediction of urinary N output is more difficult due to errors in N balance measurements (Spanghero and Kowalski, 1997; Reynolds and Kristensen, 2007). Typically, measured N balances in ruminants are greater than expected, based on the changes in body mass or measured protein accretion (Reynolds and Kristensen, 2007). In the present study, the urinary N output was estimated as N intake – (milk N + fecal N) and manure N as N intake – milk N; that is, assuming zero N balance. Although true N balance can be substantially positive or negative in short-term measurements, it cannot be markedly different from zero during the whole lactation. Live weight gain of 50 kg in a year corresponds to a daily average N retention of 3.5 g/d assuming 160 g of CP/kg of live weight gain. This value is markedly lower than the mean measured values of 38.8 (SD: 52.2) and 26.0 (SD: 35.0) g/d in the data sets of Spanghero and Kowalski (1997) and Yan et al. (2006), respectively. These values would correspond to 300 to 450 kg of live weight gain during a 305-d lactation.

The minor losses in hair and scurf are usually not accounted for in N balance studies in dairy cows. This, together with some gaseous losses, nitrate formation, and fecal ammonia N losses will result in slight overestimation of N balance. The proportion of ammonia N in fecal N increased from 4 to 7% with increased dietary CP concentration in the diets of growing heifers (Marini and Van Amburgh, 2005). This fraction is lost unless fecal samples are acidified or analyzed as fresh. In the present data the possible loss of fecal ammonia N was accounted for in urinary N. Abrupt changes in N intake can alter the size of labile N pools (Reynolds and Kristensen, 2007), which may lead to errors in estimating urinary and manure N output in short-term N balance measurements. In relation to urinary (unac-

counted) N output (206 g/d; SD: 57.8) the error related to realistic N retention during the whole lactation is <2%. Van Horn et al. (1994) stated that manure N can be estimated as a difference between N intake and N output in milk.

Urinary N originates from various sources such as rumen loss, metabolic losses in the gut, incorporation of dietary protein to microbial nucleic acids, maintenance requirement, and losses because of inefficient conversion of absorbed amino acids to milk protein (Tamminga, 1992). In particular, rumen losses and losses due to inefficient conversion of amino acids to milk protein can be reduced by manipulating diet composition. The bivariate model with N intake and DMI as independent variables suggested that proportionally 84.4% of the incremental N was excreted in urine (Table 6).

Similarly as with fecal N, including DMI in the model with N intake improved the precision of urinary N output prediction. The much greater coefficient for N intake in the bivariate model is related to the fact that increased N intake at a constant DMI (i.e., increased CP concentration) increases both rumen N losses from increased RDP supply and metabolic N losses from increased MP supply. In the present study, total manure N output was predicted more accurately compared with the studies of Kebreab et al. (2001) and Yan et al. (2006), in part because zero N retention rather than determined urinary output was used, and in part because treatment mean rather than individual cow data were used. Manure N output increased much less with increasing N intake in their studies (0.62 and 0.72) compared with the present study (0.86).

CONCLUSIONS

Dietary concentrations of CP and PBV were strongly and negatively related to MNE. The model parameters were consistent for different data subsets, indicating that the relationship between dietary CP or PBV concentration and MNE were consistent. Dietary CP concentration predicted N efficiency better than CP intake, because increased CP intake derived from increased concentration or DMI has contrary effects on ruminal and metabolic N losses. The models based on MP and PBV predicted MNE better than those based on CP alone. Fecal, urinary, and total manure N outputs were predicted more precisely using a bivariate regression model with DM and N intakes as variables compared with the N intake alone. Nitrogen efficiency can be improved and manure N (especially urinary N) output reduced by decreasing dietary CP and PBV concentrations. Decreasing dietary CP concentration will also decrease the proportion of less desirable urinary N in manure N. Feeding strategies that increase milk

yield do not unequivocally improve N efficiency, especially if milk yield responses are derived from increased dietary CP concentration,

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