The effect of protein supplementation on nitrogen utilization in lactating dairy cows fed grass silage diets[1]

A. R. Castillo[2], E. Kebreab[3], D. E. Beever, J. H. Barbi[4], J. D. Sutton, H. C. Kirby, and J. France

Department of Agriculture, University of Reading, Earley Gate, P.O. Box 236, Reading RG6 6AT, U.K.

ABSTRACT: The study set out to examine the effects of supplementing grass silage with various levels of protein concentration and degradability on dietary nitrogen (N) excretion in lactating dairy cows consuming at least 60% forage. Six Holstein/Friesian cows in early to midlactation were offered six diets comprising two levels of crude protein (210 and 290 g/kg DM) and three levels of protein degradability in the concentrate achieved using different amounts of untreated or formaldehyde-treated soybean meal. Despite a difference of almost 100 g/d in N intake, apparent fecal and milk N outputs were not significantly affected. Protein degradability also had no effect on N outputs in feces and milk. However, there was a major effect of both level and degradability of CP on urinary N output. Moreover, an interaction between level and degradability of CP was detected, such that the rate at which urinary N increases with increasing CP degradability was higher on the high-CP than on the low-CP diet. A low level of protein (150 g/kg DM in the diet) and medium to low rumen-degradable protein supplements provided a significant reduction in N excretion without compromising lactational performance (mean 24.8 kg/d), in terms of both milk yield and composition. This study also demonstrated that a high efficiency of N utilization could be achieved on low-CP diets (supplying less than 400 g N/d), with feces being the main route of N excretion, whereas an exponential excretion of urinary N was observed as N intake exceeded 400 g N/d.

Key Words: Dairy Cows, Grass Silage, Nitrogen Metabolism, Pollution, Protein Supplements

 J. Anim. Sci. 2001. 79:247–253

Introduction

Grass silage is a major feed source for dairy cows. Although protein in grazed grass is used efficiently, the ensiling process can adversely affect protein quality. Therefore, there has been an increased use of protein supplements to improve milk production, particularly milk protein concentration. Merely increasing protein concentration in the diet, however, results in lower efficiency of protein utilization. Tomlinson et al. (1996), for example, observed a 77% increase in N excretion when CP concentration increased from 120 to 180 g/kg DM with no significant effect on milk N.

There has been much interest in supplementation of low degradable protein sources such as formaldehyde-treated soybean to improve AA availability by increasing protein supply as rumen-undegradable protein (**RUP**) (Beever, 1993). Lactational response to increased RUP supplementation, however, was inconclusive (Santos et al., 1998).

Nitrogen consumed in excess of animal requirement is excreted in feces and urine, contributing to environmental pollution (Kirchgessner et al., 1994). A 650-kg dairy cow is estimated to excrete 116 kg N/yr (Smith and Frost, 2000), 12% of which could be lost by ammonia volatilization (Lockyer and Whitehead, 1990), making dairy cows major N polluters in animal husbandry.

The objective of the present study was to examine the effects of various isoenergetic concentrates, containing two levels of protein concentration at three levels of degradability, on N utilization and milk production of dairy cows fed grass silage-based diets. At low CP, rumen-degradable CP would be more likely to be limiting, which might increase blood urea N to the rumen and reduce N in urine. At high rumen-degradable protein (**RDP**), more N would be absorbed as ammonia or more AA deaminated, which might increase N excretion in

[1]The authors would like to thank MAFF Environmental Protection Division for financial support and Dr. C. K. Reynolds for statistical advice.

[2]Present address: EEA Rafaela INTA, Casilla de correo 22, (2300) Rafaela, Santa Fe, Argentina.

[3]Correspondence: phone: +44 (0) 1189318498; fax: +44 (0) 1189 318297; E-mail: E.Kebreab@reading.ac.uk.

[4]Novus, Av. Carlos Gomes, 111 cj. 303/304 90480-003, Porto Alegre RS, Brazil.

Received April 11, 2000.

Accepted August 3, 2000.

urine. In addition, the study aimed to address environmental concerns regarding the form and amount of N excreted.

Material and Methods

Animals and Diets

The experiment was carried out at the Centre for Dairy Research in the University of Reading. Six multiparous Holstein-Friesian dairy cows were used from the main herd during early to midlactation. The initial average BW of the cows was 620 (SD ± 56) kg.

The diets were composed of early first-cut, partially wilted perennial ryegrass silage (*Lolium perenne*) prepared without additive and fed with six concentrate feeds. The concentrates were pelleted and offered at 7 kg DM/(cow·d) on top of grass silage (10 kg DM/[cow·d]) in two equal meals daily. The total amounts of silage and concentrates offered were isoenergetic with respect to ME and based on a predicted DMI of 17 kg/(cow·d) (AFRC, 1993), with 600 g/kg total DM intake derived from grass silage. An additional 10% of silage and concentrates were offered to the cows in case they consumed more than the predicted DMI.

The concentrates (as treatments) were formulated to supply 210 and 290 g CP/kg DM each, with three protein degradabilities (Table 1). To achieve these specifications, the supplements were formulated with different proportions of solvent soybean meal containing High degradable CP replaced by a Low degradable source of protein (Sopralin), a commercial soybean meal treated with formaldehyde (BP Nutrition SRL, U.K.), to provide three protein degradabilities (namely High, Medium, and Low) within each protein level.

Experimental Procedure

The experimental design was an incomplete block of four periods and six cows. Within each experimental period, wk 1 to 3 were used for dietary adaptation and determination of daily DMI and milk production. Week four was used to assess treatment differences in terms of DMI, milk production, and milk composition, and wk 5 was used for measurement of total N balance. The principal aim of wk 4 was to determine the amount of grass silage to be offered to the cows in wk 5 and to avoid possible differences in DMI and milk production among treatments when the animals were used to measure N balance. Methods of collection and sampling (milk, feces, and urine) are described by Sutton et al. (1997). Samples of feeds, feces, and urine were stored frozen and dried at 60°C as appropriate for subsequent analyses as described by Castillo et al. (2001).

Statistical Analysis

Data were analyzed statistically using the General Linear Models procedure of SAS (SAS Inst. Inc., Cary, NC) for a 3-way incomplete block design. The treatments were arranged as a 2 × 3 factorial, with two levels of protein, and three protein degradabilities. Treatment least squares means were assessed because of the incomplete block. When an interaction of CP level and degradability was detected ($P < 0.10$), linear × interaction and quadratic × interaction were assessed. When no interaction was detected ($P < 0.10$), main effects of CP level were assessed by ANOVA, and the linear and quadratic effects of protein degradability averaged over CP level were assessed using orthogonal contrasts with single degrees of freedom.

Results

Feed Composition

The concentrate formulations and chemical compositions, including the determinations of DM and CP degradabilities of the feeds, are presented in Tables 1 and

Table 1. Concentrate formulations

	Treatment[a]					
	Low (210 g CP/kg DM)			High (290 g CP/kg DM)		
Ingredient	H	M	L	H	M	L
	g/kg DM					
Soybean meal, 48% CP	234	117	—	430	215	—
Sopralin[b]	—	117	234	—	215	430
Ground barley grain	172	172	172	124	124	124
Ground corn grain	167	167	167	117	117	117
Ground wheat grain	172	172	172	124	124	124
Molassed sugar beet pulp	167	167	167	117	117	117
Molasses	50	50	50	50	50	50
Minerals and vitamins[c]	25	25	25	25	25	25
Sodium bicarbonate	13	13	13	13	13	13

[a]Within protein concentration (210 and 290 g/kg DM), high (H), medium (M), and low (L) rumen-degradable protein was formulated.

[b]A commercial soybean meal treated with formaldehyde (BP Nutrition SRL, UK).

[c]Mineral and vitamin mixture contained 120 P, 170 Ca, 74 Na, 50 Mg, 187 salt, 2 Cu, 8 Mn, 0.2 Co, 6 Zn, 0.5 I, and 0.02 g Se/kg DM and 400,000 IU vitamin A, 80,000 IU vitamin D_3, and 1,000 IU vitamin E.

Table 2. Chemical composition and in situ degradability of feeds

	Concentrate[a]						
	Low (210 g/kg DM)			High (290 g/kg DM)			
Item	H	M	L	H	M	L	Grass silage
DM, g/kg as is	867	863	865	862	870	870	313
OM, g/kg DM	917	917	914	917	910	909	906
CP, g/kg DM	211	210	208	296	293	290	123
NDF, g/kg DM	162	165	184	136	163	184	553
Fat, g/kg DM	21	19	21	20	18	23	ND[b]
Starch, g/kg DM	374	382	367	252	273	297	ND
Water-soluble carbohydrates, g/kg DM	135	166	120	166	131	153	25
In situ degradability[c]							
DM, g/kg							
a	586	561	563	584	558	557	316
b	364	339	332	406	416	401	541
c, /h	0.062	0.054	0.029	0.064	0.046	0.035	0.031
ED	740	690	652	757	721	686	462
CP, g/kg							
a	338	340	339	371	324	324	493
b	662	660	661	629	676	676	204
c, /h	0.071	0.033	0.005	0.064	0.034	0.009	0.054
ED	644	518	377	639	513	389	571

[a]Within protein concentration (210 and 290 g/kg DM), high (H), medium (M), and low (L) rumen-degradable protein was formulated.
[b]ND = not determined.
[c]In situ degradability, a = water-soluble fraction; b = potentially degradable fraction; c = fractional rate of degradation of b fraction per hour; ED = Effective degradability calculated as a + (b·c)/(c + r), where r is the fractional rate of passage of the rumen digesta per hour.

2. The chemical analyses of the grass silage indicated a relatively mature forage with low CP concentration (123 g/kg DM).

The CP concentrations of the concentrates were close to expected values, at approximately 210 and 290 g CP/kg DM for the two protein levels. However, there was an increasing concentration of NDF in the supplements as CP degradability declined. This can be explained, in part, by the increased amount of insoluble protein in these feeds, which has been shown to affect NDF determinations (Van Soest et al., 1991). The fat content of the concentrates ranged from 18 to 23 g/kg DM, whereas all the concentrates had relatively high contents of starch and water-soluble carbohydrate (**WSC**). Starch and WSC together represented, on average, 515 and 424 g/kg DM, respectively, at each level of protein.

There was a trend for rate of DM degradation and effective DM degradability to decline with high to low degradability treatments within each CP level. Also, rate of degradation and effective degradability of CP in each supplement decreased with added increments of formaldehyde-treated soybean meal. Overall CP degradability values in the concentrates were 642, 516, and 383 g/kg CP for High, Medium, and Low CP degradability concentrates, respectively. Similar average values for the soluble fraction and for the potentially degradable fraction were observed for the two CP levels and the three CP degradabilities when compared for DM and CP, indicating that effective degradability was affected largely by rate of degradation.

Feed Intake

Feed intake and DM digestibility are presented in Table 3. Concentrate intake was 7.3 kg DM/d for all treatments because refusals consisted almost totally of grass silage. Increasing CP level or changing CP degradability in the supplements had no effect ($P > 0.05$) on silage DMI (mean 10.6 kg/d) and consequently on total DMI (mean 17.9 kg/d). Total diet DM digestibility was high (mean 762 g/kg DM) and unaffected ($P > 0.05$) by treatment (Table 3). On this basis, it was estimated that the mean CP concentration was 159 and 190 g CP/kg DM for low- and high-protein diets, respectively.

Milk Yield and Composition

Feeding high levels of protein did not affect ($P = 0.44$) milk yields despite a difference of 1.4 kg/d (Table 4). Furthermore, extra CP intake did not affect milk fat, protein, and lactose yield or composition. Similarly, protein degradability had no significant effect on milk yield and composition. There were no significant interactions between protein level and degradability (Table 4).

Nitrogen Balance

Nitrogen balance data are presented in Table 5. There was a significant effect of CP level on N intake but not of CP degradability. The mean difference in N intake between protein levels was almost 100 g/d ($P <$

Table 3. Intake (overall mean, 10.5, 7.3, and 17.9 for silage, concentrates, and total DMI) and digestibility of DM for cows given concentrates of various CP concentrations and degradabilities

	Protein level[a]			Protein degradability[a]			
Item	H	L	SEM	H	M	L	SEM
DMI, kg/d							
Silage	10.8	10.3	0.16	10.4	10.6	10.6	0.25
Concentrates	7.3	7.3	0.014	7.3	7.3	7.3	0.021
Total DMI	18.1	17.6	0.17	17.7	17.9	17.9	0.27
DM digestibility, g/kg	763	759	15.5	759	754	770	24.8

[a]Within protein concentration (L, 210 and H, 290 g/kg DM), high (H), medium (M), and low (L) rumen-degradable protein was supplied. No interaction between CP concentration and degradability was observed ($P > 0.1$).

Table 4. Effect of protein level and degradability of the concentrate on milk production and composition

	CP level[a]			CP degradability[a]				CP level × degradability	Contrast[b]		
Item	L	H	SEM	H	M	L	SEM	*P*	1	2	3
									P		
Milk yield, kg/d	23.1	24.5	1.27	23.8	23.4	24.1	1.47	0.42	0.45	0.83	0.79
Milk composition, g/kg											
Fat	44.9	45.2	0.97	44.8	44.6	45.6	1.13	0.75	0.82	0.88	0.53
Protein	32.2	32.6	0.81	32.0	33.3	31.8	0.99	0.88	0.73	0.37	0.49
Lactose	47.7	47.6	0.74	48.4	46.9	47.8	0.91	0.91	0.93	0.29	0.89
Component yield, kg/d											
Fat	1.02	1.09	0.053	1.07	1.04	1.07	0.065	0.34	0.37	0.80	0.89
Protein	0.737	0.775	0.024	0.757	0.773	0.738	0.029	0.12	0.28	0.71	0.46
Lactose	1.09	1.17	0.074	1.16	1.10	1.13	0.087	0.52	0.42	0.68	0.96

[a]Within protein concentration (L, 210 and H, 290 g/kg DM), high (H), medium (M), and low (L) rumen-degradable protein was supplied.
[b]Contrasts: 1 = main effect of CP; 2 = linear effects of degradability and 3 = quadratic effects of degradability.

Table 5. Nitrogen balance for cows fed two levels of CP concentration and three levels of CP degradability

	CP level[a]			CP degradability[a]				CP level × degradability	Contrast[b]				
Item	L	H	SEM	H	M	L	SEM	*P*	1	2	3	4	5
											P		
N Intake, g/d	422	516	9.8	472	469	465	11.4	0.45	0.0001	0.88	0.71		
N output, g/d													
Feces	134	148	5.9	131	143	150	7.4	0.88	0.11	0.23	0.16		
Urine	151	225	10.0	219	184	162	10.5	0.24	0.0001	0.04	0.01		
Milk	110	122	4.4	116	118	114	5.5	0.06				0.29	0.20
Balance[c]	26	21	7.4	5.2	26	40	8.0					0.13	0.04
N output, g/kg of N intake													
Feces	318	287	9.0	277	307	323	10.5	0.52	0.03	0.07	0.03		
Urine	361	433	14.1	459	385	346	18.1	0.56	0.004	0.01	0.004		
Milk	261	237	9.3	246	255	245	11.7	0.09				0.12	0.64

[a]Within protein concentration (L, 210 and H, 290 g/kg DM), high (H), medium (M), and low (L) rumen-degradable protein was supplied.
[b]Contrasts: 1 = main effect of CP; 2 = linear effects of degradability; 3 = quadratic effects of degradability and 4 and 5 = linear and quadratic effects of interaction respectively.
[c]Probability estimate of CP × degradability interaction is inappropriate because simple means are not given.

0.001). As a consequence, significant differences in N excretion were obtained. The major effect was on urinary N output. The increase in dietary CP level increased urinary N excretion by 74 g N/d ($P < 0.001$). Urinary N excretion increased with increasing degradability ($P = 0.001$). Apparent fecal output was not affected by CP level ($P = 0.11$) or degradability ($P = 0.23$), but there was interaction of CP and degradability for milk N ($P = 0.06$). Although N retention was not affected by level of CP concentration, cows fed low degradability diets retained more N than cows fed the high-degradable diet (interaction × quadratic effect $P = 0.04$).

With respect to efficiency of conversion of dietary N, there was a marked effect of CP level on apparent fecal N excretion ($P = 0.02$) at the expense of urinary N output ($P = 0.001$), with no consistent effect on milk N output ($P = 0.12$).

The relationships between total N intake and output as milk, urine, and feces obtained from this study are illustrated in Figure 1. Fecal N and milk N were linearly related to N intake, with slopes of 20% and 12%, respectively. Below approximately 400 g N intake/d, feces were the main route of N output, with urinary N increasing exponentially above this level.

Discussion

The objective of this study was to evaluate the effect of different CP levels and protein degradability in the supplement on dietary N utilization by dairy cows with consideration given to reducing N pollution. The energy in the concentrates was a mixture of carbohydrates providing different rates of degradation and availability in the rumen, with the protein based on different proportions of untreated and formaldehyde-treated soybean meal. Grass silage quality was quite typical according to Steen et al. (1998) and MAFF (1992). The effect of the treatments on silage DMI was similar to the results of Sutton et al. (1996) and Santos et al. (1998). On a grass silage diet, Sutton et al. (1996) increased the CP concentration (200, 300, and 400 g CP/kg DM) in the concentrate using soybean meal and fish meal, and at equal concentrate intakes no significant effects on silage DMI were detected. Similar results were observed by Santos et al. (1998), who compared 15 trials with dairy cows supplemented with soybean meal vs treated soybean meal and concluded that total DMI was unaffected by increasing supplies of RUP.

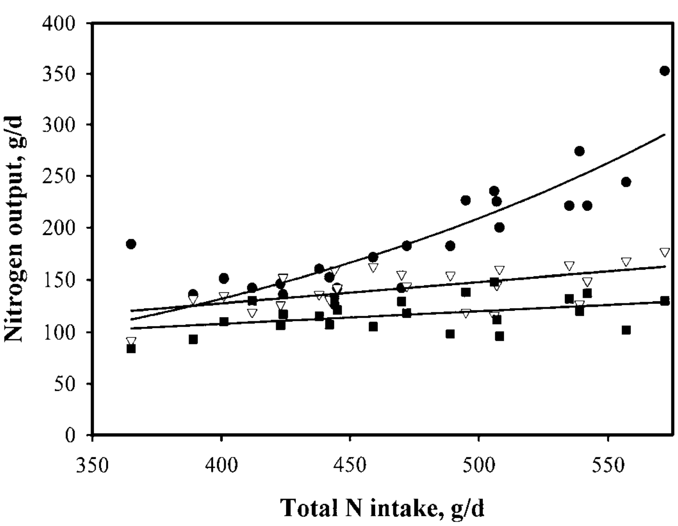

Figure 1. Relationship between total nitrogen intake (NI, g/d) and nitrogen output as urine (●), feces ▽), and milk (■). The fitted lines were as follows: urinary N (g/d) = $21.0^{0.0046\ (NI)}$ (SE = 6.21 and 0.0006 for the intercept and slope, respectively; $r^2 = 0.74$), fecal N (g/d) = 46.6 + 0.2 (NI) (SE = 30.2 and 0.064 for the intercept and slope, respectively; $r^2 = 0.32$) and milk N (g/d) = 59.1 + 0.12 (NI) (SE = 27.1 and 0.057 for the intercept and slope, respectively; $r^2 = 0.17$).

Despite increased N intake, the level of CP concentration in the concentrate had no effect on milk yield or composition. Similarly, protein degradability did not have a significant effect. Thomas (1984) and Sutton (1989) mentioned possible beneficial effects on milk yield when supplementing diets with extra CP, irrespective of degradability, but considering the specific effect of RUP, the current experiment is in agreement with Santos et al. (1998), who concluded that increased RUP per se in dairy cows does not consistently improve milk yield and composition. However, in most of the studies reviewed by Santos et al. (1998), RUP was not limiting. The results of the present experiment suggest that the provision of additional RUP did not overcome AA limitation for the production of milk or milk protein.

Level or degradability of protein in concentrate feeds did not significantly affect total fecal N excretion although it increased in response to reduced protein degradability. Tamminga (1992) analyzed the composition of fecal N output in lactating dairy cows and concluded that a reduction in the fecal excretion of indigestible feed protein was not the most promising way to achieve substantial reductions in N loss by the animal. Little improvement in digestibility is possible because true digestibility of feed protein in dairy cow rations is generally high. The higher proportion of dietary N excreted as apparent fecal N for the Low treatment compared with the High is most likely due to metabolic fecal N representing a higher proportion of total fecal N on this low-protein diet.

In agreement with Sutton et al. (1998) and Wright et al. (1998), the most significant effect of protein concentration in the diet on N outputs was on urinary N excretion. In this study, not only was there a significant increase of urinary N with the higher degradable protein-supplemented diet, but the rates at which urinary N increased with increasing CP degradability were much greater on the high-CP compared with the low-CP diet. This is probably due to decreased efficiency of N capture by microbes in the rumen as the fractional rate of degradation of the potentially degradable fraction of CP in the diet is increased, leading to an increase

Table 6. Predicted, required, and estimated effective rumen-degradable protein (ERDP) and digestible rumen-undegradabale protein (DUP) in the diet

	Treatment[a]					
	Low CP (210 g/kg DM)			High CP (290 g/kg DM)		
Item	H	M	L	H	M	L
Required ERDP, g[b]	1,823	1,811	1,799	1,824	1,802	1,780
Predicted ERDP, g[c]	1,801	1,655	1,508	2,101	1,837	1,568
Estimated ERDP, g[d]	1,511	1,365	1,122	1,886	1,628	1,370
Required DUP, g	335	428	521	320	334	483
Predicted DUP, g	670	818	961	908	1,175	1,431
Estimated DUP, g	887	1,073	1,249	1,129	1,351	1,595

[a]Within protein concentration (210 and 290 g/kg DM), high (H), medium (M), and low (L) rumen-degradable protein was supplied.
[b]ERDP requirement of the cows was calculated basedon AFRC (1993).
[c]Predicted ERDP was the value that the experiment was aimed to achieve.
[d]Estimated calculation of the actual ERDP in the diet.

in urinary N output. In the high-CP and high degradable diet, effective RDP was estimated to be oversupplied (Table 6), which might have resulted in the excess N being excreted as urea in urine and reduced recycling of blood urea N to the rumen. It is also possible that any excess AA in the high-RDP diet may be deaminated and used as a source of energy, thus contributing to higher N excretion in urine. Studies by Metcalf et al. (1996) clearly showed that increasing the DUP content of the diet by the inclusion of Sopralin increased the arterial supply of essential AA to the udder but did not result in corresponding increases in milk protein yield, implying that factors other than the essential AA supply limit the milk protein synthesis in the udder.

From an environmental perspective, losses of N from the animal as urine should be kept to a minimum because urinary N, which is principally in the form of urea, is likely to be more rapidly degraded than fecal N, thus contributing additional N to the environment through the action of bacterial ureases present in the environment (Tomlinson et al., 1996), who also presented evidence, similar to the results of this study, that dairy cows have the ability to conserve N when dietary protein is low and to keep urinary N to a minimum.

Overall there was a significant repartitioning of excreted N between urine and feces due to changes in protein degradability. Wright et al. (1998), using diets at restricted DMI observed that both N outputs in feces and urine increased linearly when RUP intake was increased. In this experiment and in accordance with Lines and Weiss (1996), who examined different protein sources without any apparent restrictions in DMI, it was evident that apparent fecal N excretion increased progressively (when RUP supply increased), accompanied by a substantial decline in urinary N output. However, total milk N or milk N expressed as a proportion of N intake were not affected by changing protein source or degradability in the concentrates, in agreement with Lines and Weiss (1996).

Nitrogen intake ranged from 419 to 516 g/d and the main route for additional N output was as urinary N. Thus, of the extra 94 g N/d supplied between the two CP levels, 15 and 11% appeared in fecal and milk N, respectively, compared with 74% as extra urinary N, suggesting a low overall efficiency of utilization. According to Peyraud et al. (1995), all dietary N in excess of animal requirements is excreted in urine, which is confirmed by our results.

It is clear from the results of this study that lactating dairy cows with relatively modest yields are quite sensitive to changes in both the amount and form of protein included in the diet. When total ration protein levels were reduced, total excreted N levels fell from 15.2 to 12.3 g/kg milk produced and, if such results could be imposed in normal livestock systems, they would represent a net reduction in N excretion from 10.6 to 8.6 tonnes/yr for 100 dairy cows producing average milk yields of 7,000 kg/yr. However, these changes were accompanied by a small reduction (5.7%) in milk yield and would necessitate more cows being kept in order to meet individual farm quotas. St-Pierre and Thraen (1999) also observed a similar trend and argued that there will be considerable societal cost associated with enforcing a maximum N efficiency strategy. However, most developed countries are signatories to various treaties to reduce N excretion and the observed changes are not necessarily accompanied by financial penalties, and the opportunity to further develop more environmentally sensitive practices would be increased if milk producers were in the future to be charged for N excretions.

Implications

It is possible to improve N utilization in dairy cows by decreasing protein intake in balanced diets. A reduction of protein concentration from 190 to 150 g/kg dry matter substantially reduced N wasted in urine without compromising lactational performance. Diets with low-degradable protein sources also reduced N output in urine with little change in milk production. The form in which N is excreted is relevant to environmental N pollution. On low-protein concentration diets, a high efficiency of N utilization is achieved, with feces being the main route of N excretion. An exponential increase in urinary N occurs as intakes increase above 400 g/d. More than 70% of N in excess of animal requirements is excreted in urine, which contributes to ammonia emissions and pollution of the environment. These findings must be considered according to the environment impact of N excretion by feces or urine and the possibilities of manipulating these processes.

Literature Cited

AFRC. 1993. Energy and Protein Requirements for Ruminants. CAB International, Wallingford.

Beever, D. E. 1993. Rumen function. In: J. M. Forbes and J. France (ed.) Quantitative Aspects of Ruminant Digestion and Metabolism. pp 187–215. CAB International, Wallingford.

Castillo, A. R., E. Kebreab, D. E. Beever, J. H. Barbi, J. D. Sutton, H. C. Kirby, and J. France. 2001. The effect of energy supplementation on nitrogen utilization in lactating dairy cows fed grass silage diets. J. Anim. Sci. 79:240–246.

Kirchgessner, M., W. Windisch, and F. X. Roth. 1994. The efficiency of nitrogen conversion in animal nutrition. Nova Acta Leopold. 70:393–412.

Lines, L. W., and P. W. Weiss. 1996. Use of nitrogen from ammoniated alfalfa hay, urea, soybean meal and animal protein meal by lactating cows. J. Dairy Sci. 79:1992–1999.

Lockyer, D. R., and D. C. Whitehead. 1990. Volatilization of ammonia from cattle urine applied to grassland. Soil Biol. Biochem. 22:1137–1142.

MAFF. 1992. Feed Composition—UK Tables of Feed Composition and Nutritive Value for Ruminants. 2nd ed. HMSO, London.

Metcalf, J. A., D. Wray-Cahen, E. E. Chettle, J. D. Sutton, D. E. Beever, L. A. Crompton, J. C. MacRae, B. J. Bequette, and F. R. C. Backwell. 1996. The effect of dietary crude protein as protected soyabean meal on mammary metabolism in the lactating dairy cow. J. Dairy Sci. 79:603–611.

Peyraud, J. L., R. Vérité, and L. Delaby. 1995. Rejets azotés chez la vache laitiére: effets du type d'alimentation et du niveau de production des animaux. Fourrages (Frankfort on the Main) 142:131–144.

Santos, F. A. P., J. E. P. Santos, C. B. Theurer, and J. T. Huber. 1998. Effects of rumen undegradable protein on dairy cow's performance: a 12-year literature review. J. Dairy Sci. 81:3182–3213.

Smith, K. A., and J. P. Frost. 2000. Nitrogen excretion by farm livestock with respect to land spreading requirements and controlling nitrogen losses to ground and surface waters. Part 1: cattle and sheep. Bioresour. Technol. 71:173–181.

Steen, R. W. J., F. J. Gordon, L. E. R. Dawson, R. S. Park, C. S. Mayne, R. E. Agnew, D. J. Kilpatrick, and M. G. Porter. 1998. Factors affecting the intake of grass silage by cattle and prediction of silage intake. Anim. Sci. 66:115–128.

St-Pierre, N. R., and C. S. Thraen. 1999. Animal grouping strategies, sources of variation, and economic factors affecting nutrient balance on dairy farms. J. Dairy Sci. 82(Suppl. 2):72–83.

Sutton, J. D. 1989. Altering milk composition by feeding. J. Dairy Sci. 72:2801–2814.

Sutton, J. D., A. L. Abdalla, R. H. Phipps, S. B. Cammell, and D. J. Humphries. 1997. The effect of the replacement of grass silage by increasing proportions of urea-treated whole-crop wheat on food intake and digestibility and milk production by dairy cows. Anim. Sci. 65:343–351.

Sutton, J. D., K. Aston, D. E. Beever, and M. S. Dhanoa 1996. Milk production from grass silage diets: Effects of high-protein concentrates for lactating heifers and cows on intake, milk production and milk protein fractions. Anim. Sci. 62:207–215.

Sutton, J. D., S. B. Cammell, D. E. Beever, D. J. Humphries, and R. H. Phipps. 1998. Energy and nitrogen balance of lactating dairy cows given mixtures of urea-treated whole-crop wheat and grass silage. Anim. Sci. 67:203–212.

Tamminga, S. 1992. Nutrition management of dairy cows as a contribution to pollution control. J. Dairy Sci. 75:345–357.

Thomas, P. C. 1984. Feeding and milk protein production. In: M. E. Castle and R. G. Gunn (ed.) Milk Compositional Quality and its Importance in Future Markets. pp 53–68. Br. Soc. Anim. Prod. Occasional Publ. No. 9, Edinburgh.

Tomlinson, A. P., W. J. Powers, H. H. Van Horn, R. A. Nordstedt, and C. J. Wilcox. 1996. Dietary protein effects on nitrogen excretion and manure characteristics of lactating cows. Trans. Am. Soc. Agric. Eng. 39:1441–1448.

Van Soest, P. J., J. B. Robertson, and B. A. Lewis. 1991. Methods for dietary fiber, neutral detergent fiber, and nonstarch polysaccharides in relation to animal nutrition. J. Dairy Sci. 74:3583–3597.

Wright, T. C., S. Moscardini, P. H. Luimes, and B. W. McBride. 1998. Effects of rumen-undegradable protein and feed intake on nitrogen balance and milk protein production in dairy cows. J. Dairy Sci. 81:784–793.