Ann Zootech (1996) 45, 327-341

Original article

Effect of protein content in the concentrate and level of nitrogen fertilization on the performance of dairy cows in pasture

L Delaby, JL Peyraud, R Vérité, B Marquis

Station de recherches sur la vache laitière, Inra, domaine de la Prise, 35590 Saint-Gilles, France

(Received 10 August 1995; accepted 11 December 1995)

Summary — The animal performance response to protein supplementation of grazing dairy cows was studied in relation to the level of nitrogen fertilization on swards. During two trials carried out in perennial ryegrass swards fertilized with a high (HN: 60 kg N/ha/cycle) or low (LN: 20 kg N/ha/cycle) level of nitrogen fertilization, comparisons were made between four isoenergetic doses of protected meal (PM) (trial 1: 0, 25, 50 and 100% PM; trial 2: 0, 15, 30 and 60% PM). The trials were carried out in the spring (8 weeks) with 32 dairy cows each year, according to a 4 x 4 intrafertilization latin square design. The swards were grazed at the same age of regrowth for the two levels of fertilization. Reducing nitrogen fertilization induced a sharper decrease in sward productivity in the first trial, as well as the foreseeable changes in the chemical composition of the grass, in particular a sharp decrease in crude protein (CP) content (100–130 g/kg dry matter [DM]). During trial 1, milk protein content (+1 g/kg) and yield were higher under LN treatment. Conversely, milk, fat and protein yields were sharply reduced on LN sward in year 2. Pasture management, characterized by herbage allowance, probably induced different levels of energy supply that could account for these discrepancies. On the HN swards, the response to PDIE increase was linear, but restricted (+180 g milk and +3.5 g protein/100 g PDIE). On the LN swards, the response was greater and curvilinear. It probably reflected a level of metabolizable protein supply lower than the cows' requirements when PDIE concentrate supplementation was kept below 300 g/day. A supply of 600 g/day PDIE on LN swards produced animal performance similar to that obtained under the HN treatment without protected meal supplementation. These responses were mostly due to the improved protein nutrition of the animals and also of the rumen bacteria. However, the hypothesis of an indirect beneficial effect on grass intake should not be ruled out. According to these results, protein supplementation is not justified on correctly fertilized swards in the spring. PM addition to the concentrate diet of grazing dairy cows should be worked out in consideration of the consequences of nitrogen fertilization on the grass PDI (protein truly digestible in the intestine) content.

pasture / dairy cow / nitrogen / supplementation / fertilization

Résumé — Effet de la teneur en protéines du concentré et du niveau de fertilisation azotée des prairies sur les performances des vaches laitières au pâturage. *La loi de réponse des performances zootechniques à l'apport de protéines dans le concentré chez des vaches laitières au pâtu-*

rage a été étudiée en interaction avec la fertilisation azotée des prairies. Au cours de deux essais, conduits sur prairies de ray-grass anglais fertilisées à un haut (HN : 60 kg N / ha /cycle) ou bas niveau d'azote (BN : 20 kg / ha /cycle), quatre doses isoénergétiques de tourteaux tannés (TT) (essai 1 : 0, 25, 50 et 100 % TT ; essai 2: 0, 15, 30 et 60 % TT) ont été comparées. L'expérience a été réalisée au printemps (8 semaines) avec 32 vaches laitières selon un schéma en carré latin 4 x 4 intrafertilisation. À même âge de repousses, la réduction de la fertilisation azotée a entraîné une baisse de la productivité des prairies plus marquée lors de l'essai 1 et les modifications attendues de la composition chimique de l'herbe offerte, avec notamment une baisse importante de la teneur en MAT (100 à 130 g/kg MS). Lors de l'essai 1, les animaux du traitement BN ont produit un lait plus riche en protéines (+ 1 point) et plus de matières protéiques. Les résultats inverses et concernant, cette fois, l'ensemble des performances ont été observés en année 2. La conduite du pâturage, caractérisée par la quantité d'herbe offerte aux animaux, a sans doute induit des niveaux d'apports énergétiques différents qui expliqueraient ces divergences. Sur les prairies HN, la réponse à l'accroissement des apports PDIE est linéaire mais de faible amplitude (+ 180 g de lait et + 3,5 g de MP / 100 g PDIE). Sur les prairies BN, la réponse est plus importante et curvilinéaire. Elle traduit sans doute un niveau de satisfaction des besoins azotés limitants avec des apports PDIE du concentré inférieurs à 300 g/jour. Un apport de 600 g/jour de PDIE aux animaux du traitement BN permet alors des performances similaires à celles du traitement HN sans apport de tourteaux. Ces réponses sont pour l'essentiel attribuées à l'amélioration de la nutrition azotée de l'animal et des microbes du rumen. Mais il ne faut écarter l'hypothèse d'un effet indirect favorable sur les quantités ingérées. Selon ces résultats, sur prairies bien fertilisées, au printemps, la complémentation protéique ne se justifie pas. L'introduction de TT dans le concentré des vaches laitières au pâturage est à raisonner en fonction des conséquences de la fertilisation azotée sur la valeur PDI de l'herbe offerte.

pâturage / vache laitière / azote / complémentation / fertilisation

INTRODUCTION

Dairy cow diets based on grazed or ensiled grass usually have a high crude protein (CP) content and therefore may not justify the use of protein-rich concentrates. However, with diets based on grass silage (Mayne, 1994) or alfalfa silage (Dhiman and Satter, 1993), dairy cow performance was increased by the introduction of vegetable or animal proteins in the concentrate. Likewise, recent studies on protein supplementation of grazing dairy cows have confirmed the interest of little degradable protein supplementation on animal performance, in most cases (Davison et al, 1990; Polan et al, 1991; Hamilton et al, 1992). Thus, in highly nitrogen-fertilized graminaceous swards (HN) (Delaby et al, 1995), total and isoenergetic replacement of cereal concentrate by protected soybean meal increased milk yield (+1.2 kg) and protein production (+26 g). In lower nitrogen- fertilized swards (LN), this response to protein-rich concentrate is higher for milk and protein yields (+45–60 g protein, Delaby et al, 1995). Thus, the decrease in grass CP content and/or the structural changes in the canopy induced by reducing nitrogen fertilization can prove to be a limiting factor of milk yield, at least under certain pedoclimatic conditions (Delagarde et al, submitted for publication).

These responses were obtained with very high doses of protected meal. In terms of nitrogen effectiveness, the responses were quite moderate, especially in HN sward (5%), and urine nitrogen excretion was hugely increased in proportion. It is important therefore to more accurately define the zootechnical response to nitrogen supplementation under various grazing conditions, and in particular according to the CP content of the grass offered.

With that as an objective, two trials were carried out in the spring of 1993 and 1994 in grazing dairy cows. In each trial, the effect of four levels of PDI (protein truly digestible in the intestine) supplementation in concentrate was described, in two contrasting situations of nitrogen fertilization. Some summarized results from the first trial have already been published (Delaby et al, 1994).

MATERIALS AND METHODS

During the two trials, the same experimental protocol was followed. Only the PDI supplementation levels under comparison were changed. In the absence of any particular specification, the processes and measurements described later apply to both trials.

Experimental design and treatments

Nitrogen fertilization was 20 and 60 kg nitrogen per hectare and per cycle in the LN and HN swards, respectively, ie, approximately 80 and 240–300 kg N/year. These levels of fertilization were applied to the same plots over the 2 years.

The different levels of PDI supplementation were obtained by partially replacing a cereal-rich concentrate by protected soybean–rapeseed meal (trial 1) and protected soybean meal (trial 2) (table I). The proportions of protected meal (PM) in the concentrate were 0, 25, 50 and 100% in trial 1, and 0, 15, 30 and 60% in trial 2. The amounts of concentrate given were the same in both fertilization treatments inside the year, and provided isoenergetic supplementation.

During each trial, each level of fertilization was permanently grazed by the same group of cows. The animals successively received the four concentrate treatments according to a 4 x 4 latin square design, over 14-day periods.

Sward and grazing management

The trials were carried out in the Rennes basin (France) on perennial ryegrass (RG, cv Belfort

Table I. Concentrate chemical composition and nutritional value.

Concentrate (g/kg MS)	*Trial 1*		*Trial 2*	
	Prot meal [a]	*Cereal C* [b]	*Prot meal* [c]	*Cereal C* [b]
Organic matter	927	946	928	946
Crude protein	458	114	469	112
Crude fiber	81	32	67	32
Fat	26	38	33	34
Neutral detergent fibre	274	132	251	138
Acid detergent fibre	111	40	91	39
Acid detergent lignin	27	9	11	7
Theoretical degradability of nitrogen [d]	0.412	0.665	0.452	–
UFL (/kg DM)	1.15	1.13	1.19	1.13
PDIE [e]	313	107	307	107
PDIN [f]	356	81	362	79

Concentrate composition (gross %): [a] soybean meal 80, rapeseed meal 20; [b] wheat 34, maize 35, barley 23, fat 1, molasses 3, bicalcium phosphate 1, calcium carbonate 1, sodium chloride 0.5, sodium bicarbonate 1.5; [c] soybean meal 96.5, fat 1, molasses 2, sodium chloride 0.5; [d] degradability measured in sacco (Michalet-Doreau et al, 1987); [e] protein truly digestible in the intestine from the energy available in the rumen; [f] protein truly digestible in the intestine allowed from the degraded dietary nitrogen.

and Fanal) sward. The LN plots were sowed in 1989 and received low level nitrogen fertilization from 1991 (Delaby et al, 1995). The HN swards were sowed between 1990 and 1993. About 30% of the HN area was sowed each year (autumn seeding). Since the first settlement, all plots received an annual base mineral fertilization composed of 120, 220 and 100 kg/ha of P, K and Mg, respectively.

The cows were turned out to pasture during the first week of April (6 April 1993 and 1 April 1994) and dietary transition was performed over 3 weeks, by keeping the animals in stables overnight. After the first cycle in pasture or on cut grass destined for silage, the experiment unfolded over May and June, during the second and third regrowths.

The swards were grazed at the same age of regrowth (in days), whatever the level of nitrogen fertilization. For a given fertilization level, the cows grazed on the same plot together, and were offered water ad libitum. The herds were managed in strip grazing without any rear wire to prevent them from moving back. After the morning milking, a new area corresponding to a day's grazing was provided to the animals. The area allocated was so determined as to offer the same herbage allowance to animals from both fertilization levels.

Supplementation

The total daily amounts of concentrate given were computed for each cow from its mean daily milk yield, as observed over the 2 weeks preceding the beginning of the trial. The supplementation principle was to provide 1 kg concentrate for 3 kg milk above 20 kg milk yield, with a maximum of 5 kg concentrate for the cows that produced more than 35 kg milk. These quantities were kept constant over the 8 experimental weeks. The animals received 500 g of a vitamin-rich mineral concentrate each day, composed of 22% minerals (18/22/12 P/Ca/Mg).

Concentrates were given after each milking in individual feeding troughs, in two equal meals per day. The experimental treatments were applied by mixing adequate proportions of the two concentrates. Between periods, the quantities of each concentrate were changed in a single day, except for extreme doses of protected meal (3 days).

Animals

In each trial, 32 multiparous Holstein dairy cows were divided into two identical groups of 16. The cows were assigned to the groups according to their mean performance characteristics, as observed during the 2 weeks preceding the beginning of the experiment: lactation stage, milk yield, fat and protein production, fat and protein content, live weight and body condition (table II).

The pretrial milk yield was on average 3.4 kg higher in trial 1 than in trial 2, although the average lactation stage was the same (159 days). Therefore, the amounts of concentrate given were higher on average, ie, 3.9 and 2.8 kg per cow per day, respectively, for trials 1 and 2.

Measurements

Animals

Individual milk yield was measured daily in the milking parlor, using flowmeters (Westfalia), during the two milkings, starting at 0630 and 1630 hours. During eight consecutive milkings each week, fat and protein contents were determined individually with an infrared analyzer (Milkoscan, Foss Electric, DK-3400 Hillerod, Denmark). The animals were weighed each week, at a fixed time after the morning milking and before accessing to the feeding trough for concentrate intake.

The individual amounts of concentrate given and refused were weighed daily, regardless of the type of concentrate refused. Two concentrate samples were collected weekly: one for dry matter (DM) content determination (80 °C for 48 h), the other for proximal analysis. The latter sample was kept as it was.

Sward

The exact areas used weekly by the animals for each fertilization level were measured on Mondays. Each week, on the same day, the herbage mass was assessed by cutting six 0.5 x 10 m strips per hectare. To determine the DM content, a sample composed of two to three handfuls per strip was taken and immediately dried out in an oven (80 °C for 48 h). A second sample was taken

Table II. Animals' characteristics at beginning of trials.

Fertilization	*Trial 1*		*Trial 2*	
	Low	*High*	*Low*	*High*
No of cows	16	16	16	16
Lactation stage (days)	162	160	160	151
Milk (kg)	32.4	32.4	29.1	28.9
Fat-corrected milk (kg)	31.9	31.7	29.3	29.4
Fat (g)	1 263	1 250	1 179	1 188
Protein (g)	944	945	836	824
Fat content (g/kg)	38.9	38.9	40.8	41.3
Protein content (g/kg)	29.2	29.3	28.9	28.7
Live weight (kg)	626	622	605	619
Concentrate offered (kg)	3.90	3.81	2.86	2.76

under the same conditions and kept frozen before being lyophilized.

The mean height of the grass was measured weekly with an automatic plastic rising plate meter (Urban and Caudal, 1990), by performing 150 measurements per hectare. The volume of grass offered (VGO) was then defined as the available grass area x grass height product. Outside temperature and rainfall were recorded daily at the meteorological station, about 1 km away from the experimental plots.

Chemical analysis and nutritional value of the feeds

At the end of the trials, the undried average sample of each concentrate and the dried weekly grass samples were ground through a 0.8 mm grid for chemical composition analyses. Each year, organic matter (OM), total nitrogen (N x 6.25), crude fiber (CF), fat (only in concentrates) and cell wall constituents (neutral detergent fibre (NDF)-acid detergent fibre (ADF); acid detergent lignin [ADL] only in concentrates) were determined.

The OM digestibility of concentrate was estimated from the cell wall constituents (Giger-Reverdin et al, 1990). The OM digestibility of grass was computed from the measurements of pepsin-cellulase digestibility in oven-dried grass and from the predictive equation published by Aufrère and Demarquilly (1989). The in sacco N degradability of concentrate and of lyophilized grass was determined according to Michalet-Doreau et al (1987). Energy values (UFL: 1 700 kcal net energy) and PDI of the various feeds were computed from the equations described by Sauvant et al (1987) and Vérité et al (1987), respectively.

Statistical analyses

In both trials, the mean individual values of the second week in each period were taken for data analysis. Analysis of variance (GLM procedure, SAS, 1987) involved the amounts of concentrate ingested and, with regard to production parameters, the difference between the value before the experiment and the value of each experimental period.

The model chosen was that of the ranked latin square (Durier, personal communication) since the cows stayed on the same fertilization level. Mean effects were estimated according to the equation: $Y = Fi + Cj(Fi) + Pk + Dl + Fi * Pk + Fi * Dl + e$, where Fi = fertilization effect; Cj = cow effect (ranked in fertilization); Pk = period effect; Dl = dose effect of protected meal; and e = residual value. The fitted deviation means were then added to the preexperimental intraannual mean shown in table II. The response curve (linear or quadratical) to the increasing protected meal supply was analyzed by the orthogonal polynomial method (Gill, 1987).

For each trial, the effect of the fertilization level on the characteristics of the grass (biomass, height, chemical composition) was studied by analysis of variance (SAS, 1987) according to the ranked model: $Y = Fi + Rj + Fi * Rj + Wk(Rj) + e$, where Fi = fertilization effect; Rj = pasture regrowth effect; Wk = week effect (ranked in regrowth); and e = residual value.

RESULTS

Both trials were carried out in 8 weeks, starting on 2 or 3 May of each year. Mean monthly temperatures were identical in both years (ie, 13.4 and 16.6 °C in May and June, respectively). These temperatures were above the 30-year average (+0.6 and +0.8 °C). Cumulative monthly rainfall was very high in June of 1993 (127 mm, including 77 mm in 2 days) and in May of 1994 (108 mm), whereas it was below the 30-year average during the other experimental month. Although abundant, rainfalls had no significant consequences on the sward or on the unfolding of the trials.

During trial 1, one cow was dried-out in the third period, following colibacillary mastitis, and only the results obtained during the first two periods were considered in the analysis. Data from another cow were excluded from the first period, following digestive pathology. In total for trial 1, the analysis involved 125 individual data. In the absence of severe health problems, no data were eliminated from trial 2 and the analysis involved 128 individual data.

Grass production and chemical composition

The herbage mass per hectare differed little between the two trials. Trial 2 was characterized by a lower CP content of forage (180 and 138 g/kg DM, in trials 1 and 2, respectively). At the same age of regrowth (26 and 30 days for trials 1 and 2, respectively), reducing nitrogen fertilization decreased the biomass per hectare and the height of grass (table III). This decrease was more significant in year 1 (–700 kg DM/ha; –4.2 cm) than in year 2 (–260 kg DM/ha; –2.8 cm).

On LN sward, CP content was lowered ($P < 0.01$), in particular in trial 2 (105 g CP/kg DM), and DM content of grass was increased (+6 points, $P < 0.001$). It is noteworthy to mention that OM not accounted for by CP and NDF was higher (+ 115 g/kg DM, table III) on LN sward. These difference could be the expression of a higher content of sugar. OM digestibility and in sacco N degradability were slightly reduced in the grass of LN swards. Finally, reducing N fertilization hardly affected the energy value of the grass, but systematically decreased the PDIE value, by approximately 10 g/kg DM, and sharply decreased the PDIN value (–53 and –40 g for trials 1 and 2, respectively).

To make up for the loss in productivity of LN swards, the daily area offered to each cow was increased by approximately 75 and 29% during trials 1 and 2, respectively. The amounts and volumes of grass offered to the cows were similar in both fertilization levels in trial 2 ($P > 0.10$) but they were sensitively higher in the LN treatment (+5 kg DM and +3.2 m^3/cow/day, $P < 0.01$) during trial 1.

Animal performance

Trial 1

Mean concentrate intake amounts were 3.2 kg DM. PDIE supply varied by 350, 495, 645 and 995 g/day with the meal doses of 0, 25, 50 and 100%, respectively. Reducing nitrogen fertilization increased protein pro-

Table III. Effect of the level of nitrogen fertilization on sward productivity and chemical composition of the grass during the two trials.

Fertilization	*Trial 1*				*Trial 2*			
	Low	*High*	*RSD* [a]	*Fert effect*	*Low*	*High*	*RSD* [a]	*Fert effect*
Biomass (kg DM/ha)	1 937	2 648	426	0.016	2 147	2 409	577	NS
Height (cm)	11.8	16.0	1.72	0.003	13.3	16.1	1.89	0.025
Area (m^2/vl/j)	106	60	10.6	0.001	88	68	16	0.049
Volume offered (m^3)	12.6	9.4	1.1	0.002	11.5	10.8	1.57	NS
DM offered (kg DM/cow/day)	20.6	15.3	2.32	0.004	18.5	16.0	3.13	NS
DM (% gross)	20.5	14.3	1.98	0.001	22.1	15.6	1.60	0.001
OM (g/kg DM)	908	891	13.7	0.041	900	883	16.4	0.090
CP (g/kg DM)	135	225	33.5	0.002	105	172	26.4	0.003
CF (g/kg DM)	236	240	17.9	NS	219	231	11.1	0.074
NDF (g/kg DM)	505	498	22	NS	463	510	20.6	0.004
ADF (g/kg DM)	244	244	14.2	NS	223	238	7.3	0.006
OM digestibility [b]	0.75	0.78	0.03	0.106	0.77	0.78	0.021	NS
Theor degradability of nitrogen [c]	0.70	0.77			0.71	0.75		
UFL (/kg DM)	0.92	0.95			0.94	0.93		
PDIE (g/kg DM)	91	100			85	93		
PDIN (g/kg DM)	86	139			67	107		

[a] Residual standard deviation; [b] according to Aufrère and Demarquilly's equation (1989); [c] degradability measured in sacco (Michalet-Doreau et al, 1987). NS: not significant.

duction (+48 g/day, $P < 0.05$) and milk protein content (+1.0 g/kg, $P < 0.05$), without affecting milk yield ($P > 0.20$) which was 28.2 kg on average (table IV). Butter fat content, low on average (33.9 g/kg), and fat synthesis did not vary significantly with the level of nitrogen fertilization ($P > 0.20$).

Adding protected meal to the concentrate increased milk yield (+2.0 and +1.1 kg between extreme doses on LN and HN treatments, $P < 0.001$), fat ($P < 0.001$) and protein ($P < 0.001$). This effect was more sensitive in LN than in HN swards, but the interaction with the level of fertilization was only significant for protein ($P = 0.05$): +57 g ($P < 0.001$) in LN swards and only +21 g in HN swards between 0 and 100% of PM. Supplementation did not significantly affect butter fat ($P > 0.20$) and protein ($P > 0.05$) contents of milk. The effect of supplementation on live weight differed according to the level of fertilization ($P < 0.05$), but differences remained low (8 kg) and had no clear zootechnical meaning.

The increase in milk yield with the dose of PM was linear but low in the animals receiving the HN treatment (+0.16 kg milk/100 g PDIE intake, $P = 0.01$). In LN swards, the response evolution was quadratical ($P < 0.05$) and most of the milk yield response was achieved with 50% PM in the concentrate.

Trial 2

The mean concentrate intake amounts were 2.25 kg DM. Protected meal supplementa-

Table IV. Effect of the level of nitrogen fertilization and of the type of supplementation on zootechnical performance (trial 1).

Fertilization	*Low*				*High*				*RSD* [a]	*Fertilization effect prob <*	*RSD* [a]	*Concent effect prob <*	*Fert x conc effect prob <*
Protected meal (%)	*0*	*25*	*50*	*100*	*0*	*25*	*50*	*100*					
Intake (kg DM)													
Total concentrate	3.29	3.20	3.09	3.19	3.26	3.11	3.11	3.17					
including P meal	0.00	0.79	1.52	3.19	0.00	0.72	1.53	3.17					
Minerals	0.39	0.39	0.38	0.37	0.38	0.38	0.39	0.37					
Milk (kg)	27.4	28.9	28.8	29.4	27.2	27.7	28.0	28.3	4.09	NS	1.09	0.001	NS
Fat-corrected milk (kg)	24.8	26.0	25.9	26.8	24.9	25.1	25.4	25.7	2.99	NS	0.97	0.001	0.141
Fat (g)	923	960	956	1 000	933	935	948	959	148.5	NS	44.7	0.001	0.149
Protein (g)	808	861	857	865	786	800	804	807	111.0	0.021	30.9	0.001	0.053
Fat content (g/kg)	34.0	33.6	33.6	34.2	34.3	33.8	34.0	34.0	5.99	NS	1.20	NS	NS
Protein content (g/kg)	29.7	30.1	30.0	29.8	29.0	29.0	28.9	28.7	2.44	0.032	0.51	0.086	NS
Live weight (kg)	622	627	629	623	625	624	621	626	32.9	NS	8.4	NS	0.036

[a] Residual standard deviation. NS: not significant.

tion, which varied from 0 to 1.24 kg DM according to the fertilization level, supplied 245, 320, 375 and 480 g PDIE, respectively, for doses of 0, 15, 30 and 60% PM in concentrate.

Contrary to the previous trial, the animals grazing on the LN swards produced less milk on average (–2.0 kg; $P < 0.001$); the milk contained less fat (–1.4 g/kg; $P < 0.01$) and less protein (–1.0 g/kg; $P < 0.01$) relative to the HN treatment. As a consequence, fat and protein yields were significantly lower by 104 and 80 g, respectively, on LN swards ($P < 0.01$).

Increasing PM supplementation increased milk yield and fat production, without altering the butter fat and protein contents (37.3 and 29.3 g/kg on average, respectively) (table V). Milk yield and protein production responses, as well as the changes in live weight, differed in amplitude according to the level of nitrogen fertilization. On LN swards, the response was curvilinear ($P < 0.10$) and reached +2.0 kg milk and +49 g protein between 0 and 60% meal addition. Most of the response to the increase in concentrate PDI content was obtained by using 30% of protected meal (ie, 380 g PDIE/day). In HN swards, the increases in milk and protein yield, respectively +0.7 kg and +20 g, were clearly lower and nonsignificant.

DISCUSSION

Over the 2 years, the reduction of nitrogen fertilization had contrasting effects on the performance of dairy cows in pasture. During trial 1, the animals grazing from LN swards produced more protein, essentially because of improved protein content (+1 g/kg), relative to the cows managed on HN swards. Opposite results, this time involving overall production performances, were observed during trial 2. Nevertheless, the milk yield and protein production responses to an extra protected meal were the same in both trials at the same fertilization level.

On HN swards, increasing the PDI content of the concentrate induced a moderate zootechnical response that proved significant only in year 1, with the maximal dose of protected meal. In LN swards, the increase in production was more marked and exhibited a quadratical profile. The highest response in milk and protein yield was obtained when protected meal amounted to 40–50% of the concentrate (ie, 200 g PDIE/kg DM).

Effect of the level of nitrogen fertilization and of the amount of grass offered

The consequences of reducing nitrogen fertilization on sward productivity (Reid, 1970; Hopkins et al, 1990) and on the quality of grass, in particular low CP content (Demarquilly, 1977; Minson, 1990; Peyraud et al, 1993) are well established, when the swards are used at the same age of regrowth. The canopy structure is also modified, with reduced grass height in LN swards.

The difference in animal response between fertilization levels according to trials (milk yield, protein content) probably reflects differences in energy supply (Coulon and Rémond, 1991). Such a variable effect of reducing nitrogen fertilization could be due to the amounts of grass intake being modified as a result of variations in the availability of grass.

In trial 2, herbage allowances were similar at both fertilization levels. Delagarde et al (1996), under similar conditions, evidenced a marked decrease in the amounts of grass intake (–2.0 kg OM) and in milk yield (–2.0 kg) in little fertilized swards. These authors were able to link those decreases in herbage intake to changes in

Table V. Effect of the level of nitrogen fertilization and of the type of supplementation on zootechnical performance (trial 2).

Fertilization	*Low*				*High*				*RSD* [a]	*Fertilization effect prob <*	*RSD* [a]	*Concent effect prob <*	*Fert x conc effect prob <*
Protected meal (%)	*0*	*15*	*30*	*60*	*0*	*15*	*30*	*60*					
Intake (kg DM)													
Total concentrate	2.35	2.37	2.29	2.17	2.24	2.24	2.22	2.15					
including P meal	0.00	0.37	0.68	1.24	0.00	0.37	0.66	1.23					
Minerals	0.37	0.38	0.37	0.38	0.38	0.38	0.38	0.37					
Milk (kg)	23.1	23.8	24.7	25.1	25.7	26.3	26.0	26.4	4.04	0.010	1.15	0.001	0.024
Fat-corrected milk (kg)	22.0	22.6	23.5	23.5	24.7	25.4	25.1	25.6	4.20	0.004	1.12	0.001	0.109
Fat (g)	848	873	906	895	962	993	978	1 004	189.4	0.005	52.0	0.006	NS
Protein (g)	664	682	713	713	761	785	769	781	91.9	0.001	40.9	0.008	0.067
Fat content (g/kg)	37.1	36.8	37.0	35.7	37.8	38.3	37.9	38.2	4.04	0.060	1.58	NS	0.094
Protein content (g/kg)	28.9	28.8	29.0	28.6	29.7	30.0	29.7	29.7	1.79	0.005	0.62	NS	NS
Live weight (kg)	587	592	591	596	599	602	601	597	33.8	NS	8.7	NS	0.070

[a] Residual standard deviation. NS: not significant.

the canopy structure, and in particular to the green leaf biomass. Indeed, grass availability in pasture appears to be mainly dependent on the green leaf biomass (Penning et al, 1994; Peyraud et al, 1996). Furthermore, in this trial, the low CP content (105 g/kg DM) of LN grass and its high deficiency in degradable N, probably enhanced the deleterious effect on intake of the underfertilized grass structure.

During trial 1, the herbage allowance and the volume of grass offered were sensitively lower in HN swards, which may have reversed the zootechnical consequences of reducing nitrogen fertilization. Indeed, studies conducted by Le Du et al (1979) in Great Britain, Stakelum (1986) in Ireland and Peyraud et al (1996) in France have demonstrated the important role of the amount of herbage allowance on intake and on the performance of dairy cows in pasture. According to the data published by Peyraud et al (1996), obtained on the same experimental site with well fertilized RG swards, reducing the amounts of herbage allowance from 20 to 15 kg would induce a 1.5 to 2 kg decrease in herbage OM intake.

Therefore, during the two trials, the amounts of herbage allowance varied according to the consequences of the reduction of nitrogen fertilization on the sward structure and grass quality on the one hand, and according to the grazing management on the other. To maintain animal performance on underfertilized swards, it may therefore be necessary to make up for the reduced biomass per hectare by a more than proportional reduction of the stocking rate.

Response to increasing PDI supply according to the fertilization level

The results obtained during trial 1 between extreme doses of protected meal (0–100%) agree with previous data obtained under identical conditions of nitrogen fertilization (Delaby et al, 1995). In well-fertilized swards, the increase in production reached 1 kg milk and 21 g protein, for a 645 g PDIE supplementation. This response was doubled for milk yield and tripled for milk proteins in low fertilization swards. These results are globally concordant with those of Polan et al (1991), Davison et al (1990) and Penning et al (1988), who also found improved animal performance when using proteins that are little degradable in the rumen.

To establish the response curve to an increasing supply of PDI in the concentrate, it appears necessary to also take into consideration the supply of UFL and PDI from grass, according to its level of fertilization and grazing management (see earlier). Estimating the amounts of grass intake would thus make it possible to quantify the nutritional supply of forage, and to analyze the animal response in terms of total supplies, or even better in terms of PDI balance, as was achieved for winter feeding (Vérité et al, 1983). However, the models currently available (Peyraud et al, 1996) do not make it possible to integrate all the consequences of reducing nitrogen fertilization (changes in canopy structure, in DM content, in CP content of grass, etc) on intake in the presence of concentrate (Peyraud, personal communication).

Under these conditions, the average animal performance of a group of four cows undergoing the same sequence of PM supplementation during the trial was recorded on common bases, reflecting differences in nutrient supply. The analysis was common on both trials. The energy supply provided by grazed grass was assessed through parameters that are susceptible to acting on digestible OM intake, such as the herbage allowance, the volume of grass offered (VGO) and the grass OM digestibility (OMd). The level of PDI supplementation was characterized by the amount of PDIE provided by the concentrate (PDIEc) and by a fixed effect of the nitrogen fertilization level (LN vs HN). Lastly, the year, period effects and the year x fertilization interaction were also introduced in the model.

For each fertilization level, the best relation was obtained by simultaneously associating the volume of grass offered, grass OM digestibility and the PDIE supply in the concentrate. These descriptive variables of grazing conditions then accounted for most of the year effect and of the year x fertilization interaction, which were no longer significant. The herbage allowance proved to be less interesting than the volume of grass in describing the interannual interaction. This is probably due to the lesser accuracy of the measurements of herbage mass, and perhaps to the fact that the volume measured with the rising plate meter provides a better reflection of the grass prehensility and availability.

The equations obtained, which differed according to the level of nitrogen fertilization, were for milk and protein yield, respectively (fig 1):

LN: Milk (kg) = 5.9 + 0.34 x VGO + 0.15 x OMd + 1.51 x PDIEc – 0.09 x $PDIEc^2$

(R^2 = 0.96 ETR = 0.60)

HN: Milk (kg) = –4.6 + 0.98 x VGO + 0.27 x OMd + 0.18 x PDIEc

(R^2 = 0.95 ETR = 0.72)

LN: Protein (g) = 188 + 18.4 x VGO + 2.8 x OMd + 46.9 x PDIEc – 2.9 x $PDIEc^2$

(R^2 = 0.95 ETR = 21.1)

HN: Protein (g) = –306 + 37.7 x VGO + 8.9 x OMd + 3.5 x PDIEc

(R^2 = 0.92 ETR = 24.6)

where VGO is expressed as m^3/cow/day, dOM as % and PDIEc as 100 g of PDIE, which varied from 2.4 to 10.0 over the duration of the trials.

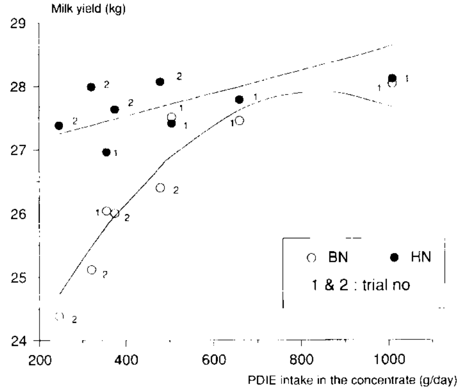

Fig 1. Effect of increasing protein supplementation in concentrate of grazing dairy cows. Influence of sward nitrogen fertilization level. The response curve was drawn by using the mean volume of grass offered and the mean OM digestibility of grass over the two trials, ie, 11.1 m^3 and 77%, respectively.

According to these equations, with the same level of PDIEc (500 g/day) and identical OMd (77%), milk yield reached approximately 27.0 kg milk with 10 and 12.2 m^3 of VGO in HN and LN swards, respectively. This result is consistent with the hypotheses given here of lower availability of grass on low fertilization swards.

In HN swards, the response to introducing protected meal into the concentrate was linear and reached 0.180 kg milk ($P < 0.01$) and only 3.5 g protein ($P > 0.13$) for each 100 g of additional PDIE. Davison et al (1990), using increasing doses of meat-and-bone meal (MBM), also obtained a positive and linear milk yield response in RG swards, but nonsignificant on RG-clover swards. Likewise, Davison et al in 1991 did not obtain any beneficial effects on the animal performance of dairy cows when introducing a small amount of MBM (15%) in a maize grain-based concentrate. It appears that any significant increase in milk and protein yield can only be achieved with a high supplementation of bypass proteins. These results are consistent with a high level of satisfaction of the nitrogen needs of dairy cows grazing on well-fertilized swards. In the spring, the fertilized swards (CP between 18 and 22%) provide enough metabolizable protein to allow a milk yield of 25–27 kg at least when ample grass is offered with a small amount of cereal concentrate.

In LN swards, the curvilinear profile of the response is perfectly consistent with the law of decreasing yields described by Vérité and Peyraud (1989). Under our experimental conditions, an additional supply of 250 g PDIE, ranging from 250 to 500 g/day, increased milk and protein yield by 2.1 kg and 63 g, respectively, equivalent to a marginal response of milk protein yield as high as 25%. This response was reduced to 1.0 kg milk and 27 g protein when the same amount of PDIE supplementation was provided in the concentrate beyond 500 g/day. On kikuyu swards (15.5% CP), Hamilton et al (1992) also obtained a positive response, although less significant (+1 kg milk without fat and protein content changes) by introducing 30% formaldehyde-treated sunflower meal in a barley-based concentrate.

These significant responses characterize a state of nitrogen undernutrition in animals which were producing around 30 kg milk at the beginning of the trials. Additional bypass protein supplementation in the form of protected meal, even at low doses, increased the flow of proteins in the intestine which, in a deficiency situation, were largely used by the mammary gland. In addition, this positive effect on the animals' nitrogen status may be associated to a stimulating effect of protected meal on microbial syntheses, through urea recycling into the rumen and to a lesser extent to degradable nitrogen supplementation. Indeed, during trial 2, the deficiency of degradable protein of the grass offered reached −19 g/UFL, which is far below the acceptable level of −4 to −8 g/UFL (Vérité and Peyraud, 1989).

This improvement of nitrogen nutrition, both in the rumen and in the animal as a whole, could also have stimulated intake. This favourable effect was described by Journet et al (1983) and Roffler et al (1986), from feeding trials based on maize silage diets. Similarly, in a comparison with unsupplemented animals, Delagarde et al (1996) showed a slight increase of herbage intake when cows on LN swards where supplemented with 2 kg of soybean meal. Thus, in limiting situations, such as those described in LN swards, the increase in PDI supply could have an indirect beneficial effect on energy supply. The production responses observed would then be the consequence of a global improvement of nutritional balances, energy as well as nitrogen.

These response curves show that, with the same mean volume of grass offered (11.1 m^3/cow/day) obtaining the same dairy performance in LN swards as that obtained in HN swards without protected meal, requires supplementation of about 600 g PDIE in the concentrate, or 60% protected meal in our trials. Admitting that beyond that level, zootechnical response hardly varies, and that 600 g PDIE supplementation by the concentrate meet the PDI requirements, these requirements then reach approximately 1 650 g/day, equivalent to an estimated grass intake of 13–14 kg DM. Considering the net energy content of the grass offered, this intake level would satisfy the animals' energy requirements (ie, maintenace, 23 kg fat-corrected milk and 1 UFL to cover extra energy linked to the grazing activity).

The essential advantage of using nitrogen fertilization in pasture is to increase sward productivity, and consequently to increase the number of grazing days (GD) per hectare. Simultaneous increase in grass CP sensitively increases nitrogen excretion by the cows, also enhanced by adding meal to the concentrate. This combined effect of fertilization and concentrate was quantitated from the nitrogen input–output balances on the plot scale (Peyraud et al, 1995). During these trials, performed over two grazing cycles in HN swards, nitrogen restitutions per hectare increased by 30 kg N when the proportion of protected meal in the diet was increased from 0 to 60%, mainly because of the high stocking rate permitted by fertilization (312 GD/ha over two cycles, equivalent to 842 kg concentrate intake per hectare). In LN swards, with 206 GD/ha (556 kg concentrate), adding 60% protected meal to the concentrate increased restitutions by only 8 kg N per hectare.

In practice, this reasoning, which integrates the law of decreasing milk protein production, cannot be separated from dairy herd management, in particular that of the grazing area available on the one hand, and of the agricultural consequences of reducing nitrogen fertilization on plot productivity, on the other.

CONCLUSION

The zootechnical interest of increasing the PDI content of concentrate in grazing dairy cows varies according to the level of nitrogen fertilization applied to the swards. When reducing N fertilization induces a marked decrease in sward productivity and CP content of the grass, using quality proteins in the concentrate permits correcting deficits that, with moderate meal doses, result in a high marginal response of protein synthesis. In high fertilized swards in the spring, the nitrogen value of the grass does not justify the use of meal, which would remain little used even by high producing dairy cows.

ACKNOWLEDGMENTS

The authors wish to thank C Durier (Inra Biometry Station, Versailles) for his learned and valued advice regarding the analysis of variance model. We also thank the Méjusseaume farm personnel (Inra SRVL, Saint-Gilles) for their efficient cooperation in performing the measurements. P Rousseau-Cunningham translated the text into English.

REFERENCES

Aufrère J, Demarquilly C (1989) Predicting organic matter digestibility of forage by two pepsin-cellulase methods. *XVI Congrès International des Herbages 4–11 octobre* (AFPD, ed), Nice, France, 877-878

Coulon JB, Rémond B (1991) Réponse de la production et de la composition du lait de vache aux variations d'apports nutritifs. *INRA Prod Anim* 4, 49-56

Davison TM, Jarrett WD, Clark R (1990) Effect of level of meat-and-bone meal and pasture type on milk yield and composition of cows grazing tropical pastures. *Aust J Exp Agric* 30, 451-455

Davison TM, Williams D, Orr WN, Lisle AT (1991) Responses in milk yield from feeding grain and meat-and-bone meal to cows grazing tropical pastures. *Aust J Exp Agric* 31, 159-163

Delaby L, Peyraud JL, Vérité R (1994) Influence du niveau de fertilisation azotée des prairies et de la complémentation protéique sur la production laitière au pâturage. *Ann Zootech* 43 (suppl 1), 62s-63s

Delaby L, Peyraud JL, Vérité R, Marquis B (1995) Effet de la complémentation protéique sur les performances des vaches laitières au pâturage conduit à deux niveaux de fertilisation. *Ann Zootech* 44, 173-188

Demarquilly C (1977) Fertilisation et qualité du fourrage. *Fourrages* 69, 61-84

Dhiman TR, Satter LD (1993) Protein as the first limiting nutrient for lactating dairy cows fed high proportions of good quality alfalfa silage. *J Dairy Sci* 76, 1960-1971

Giger-Reverdin S, Aufrère J, Sauvant D, Demarquilly C, Vermorel M, Pochet S (1990) Prévision de la valeur énergétique des aliments composés pour ruminants. *INRA Prod Anim* 3, 181-188

Gill JL (1987) *Design and Analysis of Experiments in the Animal and Medical Science,* vol 1. The Iowa State University Press, Ames, IA, USA

Hamilton BA, Ashes JR, Carmichael AW (1992) Effect of formaldehyde-treated sunflower meal on the milk production of grazing dairy cows. *Aust J Agric Res* 43, 379-387

Hopkins A, Gilbert J, Bowling PJ, Murray PJ (1990) Response of permanent and reseeded grassland to fertilizer nitrogen. 1. Herbage production and herbage quality. *Grass Forage Sci* 45, 43-55

Journet M, Faverdin P, Rémond B, Vérité R (1983) Niveau et qualité des apports azotés en début de lactation. *Bull Tech CRZV Theix, INRA* 51, 7-17

Le Du YLP, Combellas J, Hodgson J, Baker RD (1979) Herbage intake and milk production by grazing dairy cows. 2. The effects of level of winter feeding and daily herbage allowance. *Grass Forage Sci* 34, 249-260

Mayne CS (1994) Recent research on supplement feed level and protein content of the supplement for dairy cows – practical implications for dairy feeding. In: *66th Annual Report 1992–1993*. Agricultural Research Institute of Northern Ireland, Hillsborough, 29-39

Michalet-Doreau B, Vérité R, Chapoutot P (1987) Méthodologie de mesure de la dégradabilité in sacco de l'azote des aliments dans le rumen. *Bull Tech CRZV Theix, INRA* 69, 5-7

Minson DJ (1990) Digestible energy of forage. II. Variation of forage digestibility. In: *Forage in Ruminant Nutrition* (TJ Cunha, ed), Academic Press, London, UK, 85-149

Penning PD, Orr RJ, Treacher TT (1988) Responses of lactating ewes, offered fresh herbage indoors and when grazing, to supplements containing differing protein concentrations. *Anim Prod* 46, 403-415

Penning PD, Parsons AJ, Orr RJ, Hooper GE (1994) Intake and behaviour responses by sheep to changes in sward characteristics under rotational grazing. *Grass Forage Sci* 49, 476-486

Peyraud JL, Astigarraga L, Faverdin P (1993) The digestion of fresh perennial ryegrass fertilized at two levels of nitrogen in lactating dairy cows. *Ann Zootech* 4, 138

Peyraud JL, Vérité R, Delaby L (1995) Rejets azotés chez la vache laitière : effet de l'alimentation. *Fourrages* 142, 131-144

Peyraud JL, Comeron EA, Wade MH, Lemaire G (1996) The effect of daily herbage allowance, herbage mass and animal factors upon herbage intake by grazing dairy cows. *Ann Zootech* 45, 201-218

Polan CE, Garrett JL, Novaes LP, Stieve DE (1991) Milk production response to rumen degradable and undegradable protein fed to grazing cows (abstract). *J Dairy Sci* 74 (suppl 1), 306

Reid D (1970) The effects of a wide range of nitrogen application rates on the yields from a perennial ryegrass with and without white clover. *J Agric Sci (Camb)* 74, 227-240

Roffler RE, Wray E, Satter LD (1986) Production responses in early lactation to additions of soybean meal to diets containing predominantly corn silage. *J Dairy Sci* 69, 1055-1062

SAS Institute, Inc (1987) *SAS/STAT Guide for Personal Computer. Version 6.* Cary, NC, USA

Sauvant D, Aufrère J, Michalet-Doreau B, Giger S, Chapoutot P (1987) Valeur nutritive des aliments concentrés simples : tables et prévision. *Bull Tech CRZV Theix, INRA* 70, 75-89

Stakelum G (1986) Herbage intake of grazing dairy cows. 2. Effect of herbage allowance, herbage mass and concentrate feeding on the intake of cows grazing primary spring grass. *Ir J Agric Res* 25, 41-51

Urban B, Caudal JP (1990) Herbomètre automatisé. In: *Les journées de la mesure. Électronique, Informatique, Automatique* (Inra Dépt Informatique, ed), 3 and 4 October, Port-Leucate, II, 57-59

Vérité R, Peyraud JL (1989) Protein: the PDI system. In: *Ruminant Nutrition. Recommended Allowance and Feed Tables* (Jarrige R, ed), Inra, John Libbey, Paris, 33-47

Vérité R, Dulphy JP, Journet M (1983) La complémentation azotée des rations d'ensilage pour les vaches laitières. *Bull Tech CRZV Theix, INRA* 52, 43-51

Vérité R, Michalet-Doreau B, Chapoutot P, Peyraud JL, Poncet C (1987) Révision du système des protéines digestibles dans l'intestin (PDI). *Bull Tech CRZV Theix, INRA* 70, 19-34