Original article

Effect of nitrogen fertiliser rate and protein supplementation on the herbage intake and the nitrogen balance of grazing dairy cows

Laura ASTIGARRAGA*, Jean-Louis PEYRAUD, Luc DELABY

UMR INRA/ENSAR Production du Lait, INRA, 35590 Saint Gilles, France

Abstract — The objective of this study was to examine the effect of the level of reducing nitrogen (N) fertiliser rate on herbage intake and N balance of dairy cows grazing a pure perennial ryegrass pasture. The addition of a protein supplement to cows grazing the low N fertilised sward was also evaluated. Three treatments were compared over three periods of 2 weeks using 9 fistulated cows in a 3×3 Latin square design: HN (80 kg N·ha^{-1}·regrowth^{-1}), LN (0–20 kg N·ha^{-1}·regrowth^{-1}), LN+S (LN + 2 kg of soybean meal (SBM)). Daily herbage organic matter (OM) intake was estimated by chromic oxide dilution in the faeces. Nitrogen and ADF contents in faecal OM were used to estimate the herbage digestibility. Herbage mass, grazing behaviour and rumen fermentation pattern were measured. Nitrogen intake was estimated by the chemical composition of the defoliated herbage and urinary N was calculated by subtracting milk N and faecal N output from N intake. Digestibility (0.79), daily intake (16.4 kg OM), grazing time (512 min) and the proportion of volatile fatty acid were not affected by reducing the N fertiliser rate. These results may be explained by the moderate effect of N fertilisation on herbage mass which remained high in the LN swards (3.9 vs. 4.7 t OM·ha^{-1} for HN) and the rather large herbage allowance which allowed the cows to graze a herbage with a crude protein content that still remained higher than 160 g·kg^{-1} DM. On the LN sward, N intake was significantly lower (– 80 g·d^{-1}) ($P < 0.01$), faecal N and milk N output remained unchanged, whereas urine N output decreased (– 77 g·d^{-1}) ($P < 0.01$). Protein supplementation did not depress HOMI or grazing time, supplemented cows consumed 2.4 kg OM more ($P < 0.01$) and this increased milk yield by 1.3 kg·kg^{-1} SBM ($P < 0.01$). SBM supplementation largely increased N intake, and finally N excreted in the urine. It was concluded that N fertilisation, and N supplementation are efficient means to manipulate animal performances and N balance in grazing dairy cows.

dairy cow / grazing / nitrogen / supplementation / herbage intake

---

* Correspondence and reprints
Tel.: (5982) 3689914; fax: (5982) 3689913; e-mail: astigarr@fagro.edu.uy
Present address: Facultad de Agronomía, Universidad de la República, Montevideo, Uruguay.

Résumé — Influence du niveau de fertilisation azotée et de la complémentation azotée sur l'ingestion et le bilan azoté des vaches laitières pâturant du ray-grass anglais. L'effet du niveau de fertilisation azotée et de la complémentation avec du tourteau de soja (50 % normal et 50 % tanné) a été étudié chez des vaches laitières pâturant un ray-grass anglais. Trois traitements ont été comparés avec 9 vaches fistulées au niveau du rumen, selon un schéma en carré latin 3 × 3 sur des périodes de 2 semaines : HN (80 kg N·ha^{-1}·cycle^{-1}), LN (0–20 kg N·ha^{-1}·cycle^{-1}), LN+S (LN + 2 kg de tourteau de soja (SBM)). Les quantités d'herbe ingérée ont été calculées en mesurant la quantité de fèces par dilution de l'oxyde de chrome et en estimant la digestibilité de l'herbe ingérée à partir de la teneur en N et en ADF des fèces. L'azote ingéré a été estimé à partir de la composition chimique de l'herbe défoliée et l'azote urinaire a été calculé par différence entre l'azote ingéré et l'azote du lait et des fèces. La digestibilité (0,79), l'herbe ingérée chaque jour (16,4 kg MO), la durée d'ingestion (512 min) et les profils fermentaires du rumen n'ont pas été modifiés par le niveau de fertilisation azotée de l'herbe, sans doute en raison de l'effet modéré de la fertilisation sur la biomasse qui est restée élevée sur les prairies LN (3,9 vs. 4,7 t MO·ha^{-1} pour HN) et des quantités offertes élevées qui ont permis aux vaches d'ingérer une herbe avec une teneur en matières azotées supérieure à 160 g·kg^{-1} MS. L'azote ingéré a diminué (– 80 g·j^{-1}) ($P < 0,01$) dans le traitement LN, l'azote fécal et l'azote du lait n'ont pas été modifiés, tandis que l'azote urinaire a été réduit (– 77 g·j^{-1}) ($P < 0,01$). L'apport de concentré protéique n'a pas affecté l'ingestion d'herbe et la durée d'ingestion. Dans le traitement LN+S, les vaches ont ingéré 2,4 kg MO·j^{-1} ($P < 0,01$) en plus et la production de lait s'est accrue de 1,3 kg·kg^{-1} de concentré ($P < 0,01$). La complémentation a augmenté l'azote ingéré mais par contre l'azote du lait n'a été accru que de 14 g·j^{-1} ($P < 0,01$). En conséquence, l'azote urinaire a été fortement augmenté ($P < 0,01$) par rapport aux traitements sans complémentation. En conclusion, la fertilisation azotée et la complémentation azotée permettent de manipuler dans de larges proportions les performances zootechniques et les rejets azotés chez les vaches au pâturage.

vache laitière / pâturage / azote / complémentation / ingestion

1. INTRODUCTION

Since 1950, nitrogen (N) fertilisation of grassland has resulted in large increases in the stock carrying capacity and livestock production per unit surface area. Recommendations for grassland in most countries of northwest Europe have ranged from 100 to 300 kg N·ha^{-1}·year^{-1}, depending on soil management [41]. However, in recent years, changing technical and economic conditions of dairy production within the EU, and an increased concern about the effects of intensive production systems on the natural environment, have resulted in attempts to develop low input-low output systems, particularly with regards to reducing the use of N fertiliser on grass pastures [12, 14, 39].

Reducing N fertilisation rate decreases N content in grass and increases sugar content [3, 11, 32] but only marginally affects the nutritive value of grass. The voluntary dry matter intake, the digestibility and the quantity of N entering in the intestines seems to be little affected by the level of N fertilisation [11, 31, 42].

However, one of the main effects of reducing the level of N fertilisation is to decrease herbage mass and to modify sward structure. Therefore, to maintain the same allowance of herbage, it is necessary to increase the area allocated per cow. But, such an approach is not likely to always be effective, since the amount of herbage present as green leaf mass per unit area and sward canopy structure have been acknowledged to be important extrinsic factors limiting herbage

intake through their effects on the ease of prehension of herbage [29, 30]. When low N inputs lead to low sward mass, the generally associated low crude protein content in grass might worsen the detrimental effect of sward mass for high producing animals. Under these conditions, a slight increase in herbage intake was reported when cows were supplemented with 2 kg of a concentrate rich in proteins [9]. Similarly, supplements rich in protein increase the herbage intake of beef steers on tropical pastures [23].

The main objective of this experiment was to study the effects of a reduction in N fertiliser rate and the influence of a protein supplement on herbage intake, ruminal digestion and N balance in lactating dairy cows grazing a pure perennial ryegrass pasture.

2. MATERIALS AND METHODS

2.1. Experimental treatments and design

The grazing experiment took place at the Experimental Station of Méjusseaume near Rennes (Bretagne, France).

The effect of applying two levels of N fertiliser (Low N (LN): 0–20 and High N (HN): 60–80 kg N·ha^{-1} per cut) to a pure perennial ryegrass pasture and supplementing cows grazing the LN sward (LN+S) with 2 kg soybean meal (1 kg as protected cake) were examined in a 3×3 Latin square design. Nine ruminally cannulated lactating Holstein cows were used over 3 periods of 14 days (5 days-dietary adaptation, 9 days-excreta and digestion collection). The animals were allotted to the three treatments on the basis of the pre-experimental milk production (27.2 ± 5.8 kg·d^{-1} fat corrected milk (FCM)), live weight (646 ± 58 kg) and lactation stage (184 ± 30 days).

2.2. Pasture management

Two pastures of perennial ryegrass were used in the spring 1992: one of *Lolium perenne* L. cv. Fanal (Periods I and II) sown in September 1991 after a maize silage crop, and one of *Lolium perenne* L. cv. Belfort (Period III) sown in August 1989.

Three paddocks (one per period) of approximately 1 ha each, were longitudinally divided and each sub-paddock received one of the two experimental fertiliser treatments. The paddocks were cut in mid March and then a second time, so that the final cut allowed a herbage regrowth of 30 days at the start of each period. Nitrogen fertiliser (80 kg N·ha^{-1} per cut) was applied immediately after each cut. There was no fertilisation on the LN sward, except 20 kg N·ha^{-1} after the second cut for the plot used during the third period.

The sward was strip-grazed at a daily herbage allowance of 27 kg OM·cow^{-1}·d^{-1} (above 5 cm). This allocation was considered not to be limiting for herbage intake, according to data from previous experiments at the Station [30].

2.3. Herbage measurement

The pregrazing herbage mass (kg·ha^{-1}) was measured on days 1, 6, 10 and 13 in each plot by harvesting two diagonal strips (5 m $\times$ 0.5 m) with a motor scythe. The cut height was 8 cm of extended tiller height (i.e. 5 cm using a grass meter plate). Herbage samples were weighed fresh, sampled and approximately 500 g were dried at 80 °C for 48 h for DM determination. A separate subsample of fresh material from each cut was taken, and stored at –20 °C prior to freeze-drying for subsequent chemical analysis of grass on offer.

The mean sward height was measured before and after grazing on the area grazed on days 8, 10 and 12. At each time, 50 tillers were taken at random and the extended height to ground level of the longest leaf

and the longest sheath were measured. The differences between the two values were used to calculate the mean depth of defoliation for each treatment.

The morphological composition of the herbage offered and the composition of the herbage consumed was measured on days 6, 10 and 13. Two handfuls of herbage per treatment were cut to the ground level by hand. The samples were bulked and arranged correctly in a bag to keep the sward structure undamaged and then immediately frozen. For morphological analysis, 100 tillers were arranged so to preserve their original structure and placing their cut bases together. They were cut into sections of 50 mm, starting from the cut bases. Material of each section was separated into green leaf lamina, green stem and sheath (hereafter referred to sheath), flower heads (where present) and dead material. The morphological units were dried. These data were used to calculate the proportion of the total herbage mass of each morphological unit per 50 mm stratum, and the proportion of green lamina, sheath and dead material in the whole sward [44]. The chemical composition of the defoliated horizons was estimated from a second subsample of 100 tillers which was cut at the mean height of the post grazing sward height.

2.4. Animal measurements

Individual herbage OM intake was determined using chromic oxide (Cr_2O_3) to estimate faecal OM output, and N (Nf) and ADF (ADFf) contents in the faeces (g·kg^{-1} OM) to estimate digestibility of herbage, according to the following equation:

$$d = 0.791 + 0.0334\,Nf - 0.0038\,ADFf,$$
$$(R = 0.89,\ RSD = 0.013)$$

where d is herbage organic matter digestibility.

The equation used was established previously using herbage-based diets without supplements at the same experimental site [7]. Concentrate pellets containing chromic oxide (ca. 50 g Cr_2O_3·kg^{-1} DM) were given via ruminal cannula in two equal portions of 200 g each, at milking. All dung pats were identified from dosing each cow with coloured polystyrene particles. The dung pats were sampled each morning from day 9 to day 14 of each period. Faecal samples were oven-dried at 80 °C for 48 h, ground and bulked over the collection period for each cow for chemical analysis.

For supplemented cows, faecal OM output from herbage was calculated by subtracting the indigestible OM content attributable to the supplement (83 g·kg^{-1} DM) [22] from the total faecal OM output. The faecal N content for LN+S treatment was adjusted for the contribution of indigestible N from the supplement [22].

Time spent grazing and ruminating were recorded on day 7 to day 112 for each cow by the portable electronic device initially described by Brun et al. [4]. Stored data were transferred daily at morning milking to a microcomputer. An interpretative program was used to classify data into one of the three categories: grazing, ruminating or idling, in order to build up a behaviour profile over a 24 h-period. Biting rate was measured by visual observations one day at the end of each period. Cows were individually observed for 2 min every 15 min in the morning from 08:30 to 12:00 h and in the evening from 17:30 to 22:00 h (i.e. main periods of grazing activities). Total bites per day were calculated as the product of grazing time (automatically recorded) and mean biting rate.

Ammonia (NH_3), pH and volatile fatty acids (VFA) in the rumen were measured on day 9 by sampling rumen juice at 7:00, 9:30, 11:30, 13:30, 15:30, 16:30, and 22:00 h. At each time point, a 50 mL sample was taken and the pH was immediately measured. After straining, two subsamples

were frozen for NH_3 and VFA analysis according to the procedure described by Peyraud et al. [31]. A further sample (9 mL) was centrifuged to 3000 g for 20 min and filtered through a 0.2 μm pore membrane to measure osmolarity with a cryoscopic osmometer (Hemann Roebling, Berlin, Germany). Ruminal cellulolytic activity was estimated on d9 from the in sacco DM disappearance of the soybean hull (62 g NDF·kg^{-1} DM) after 24 h incubation in the rumen in duplicated nylon bags as described by Michalet Doreau et al. [25].

Blood samples were obtained by venepuncture from the tail at the morning milking on d14 of each period. The samples were placed into heparinised tubes and centrifuged immediately to separate plasma, which was then stored at –20 ºC, until analysis for urea, non-esterified fatty acid (NEFA) and glucose contents.

The cows were milked twice, from 7:00 to 8:00 h in the morning and from 16:30 to 17:30 h in the afternoon. Individual milk production was measured each day. Milk fat and protein contents were determined on four consecutive days each week using a Milko Scan 605 (Foss Electric, Denmark). Only mean values obtained from day 6 to day 14 were used for the final analysis. Cows were weighed on the last day of each period.

Nitrogen secreted in the milk was calculated by the equation of Alais [1].

N (milk) = milk yield (kg·d^{-1}) × (milk protein (g·kg^{-1}) + 1.6) / 6.38.

Nitrogen intake was calculated from the N content of estimated herbage consumed. Urine N was calculated by subtracting milk N and faecal N output from intake, assuming there was no N retention.

2.5. Chemical analysis

Chemical analyses were determined on dry samples of feeds and faeces after grinding the samples and passing them through a 0.8-mm screen. Organic matter (OM), N, NDF and ADF in the feedstuffs and faeces, and NH_3 and VFA in ruminal fluid were analysed as described by Peyraud et al. [31]. Chromic oxide content in faecal samples was estimated following acid digestion using the method of Mathieson and Davidson [24] modified by Poncet and Rayssiguier [33] to an auto-analyser (Technicon). Plasma non-esterified fatty acids (NEFA) were determined colorimetrically (NEFA-C Kit, Wako Chemicals Gmbh, Neuss, Germany) using the method described by Chilliard et al. [5]. Glucose was analysed by the enzymatic method with glucose oxidase on an Isamat Autoanalyzer as described by Hurtaud et al. [21]. Plasma urea was analysed with a Technicon continuous flux analyser (Technicon Industrial Systemems, New York) by a colorimetric diacetyl monoxime procedure [27].

2.6. Statistical analysis

Data were analysed by ANOVA using the GLM procedure of SAS [38] for a 3 × 3 Latin square design. The model sums of squares were separated into the effects of treatments, periods and cows. Time sequence data (behavioural and ruminal data) were analysed as a split-plot design with sampling time, and the interactions of time × treatment, time × period, and time × cow added to the model.

3. RESULTS

One cow was removed from the trial during Period I for reasons unrelated to dietary treatments and another cow in Periods II and III substituted it. Data collected from that cow for Period I was not utilised in the statistical analysis, thus least square means are reported.

Total rainfall (113 mm) and mean temperature (16.5 ºC) over the two months of the experiment were close to seasonally normal climatic conditions.

3.1. Sward measurements

Herbage mass and extended tiller height were reduced on the LN swards ($P < 0.05$) (Tab. I). The height of sheath represented 59% of ETH in both HN and LN swards. There was no significant effect of the N fertiliser rate on the morphological composition of the swards. The green leaf mass was lower on the LN sward.

Reducing the N fertiliser rate significantly decreased the crude protein content in the grass by 44 g·kg^{-1} OM ($P < 0.01$), and increased the DM content ($+ 24$ g·kg^{-1}, $P < 0.05$). Water soluble carbohydrates (WSC) were numerically increased ($+ 51$ g·kg^{-1} OM) but the difference failed to be significant. NDF and ADF contents did not differ.

Cows on the LN sward were given an average 22% higher area than cows on the HN sward, in order to allocate the stipulated herbage allowance (Tab. II). The post grazing sward height was high (228 mm) and did not significantly differ between treatments. The depth of grazing was similar for LN and LN+S treatments but was 66 mm lower than for the HN sward ($P < 0.01$). The defoliated volume (calculated as the product of the area allocated per cow and the depth of defoliation) was similar for the three treatments.

The N fertiliser rate and soybean meal (SBM) supplementation did not significantly modify the morphological composition of the herbage consumed (Tab. II). Due to the high post grazing sward height, the proportion of leaf (62 vs. 48%) and the crude protein content (180 vs. 146 g·kg^{-1}OM) were much higher in the consumed than in the offered herbage. In particular, cows grazing on the LN sward ate a grass which contained more than 160 g·kg^{-1} OM crude protein. For the same reason, the sheath proportion and the fibre content were lower in the consumed than in the offered grass. However, as already observed for the offered grass, reducing the N fertiliser rate significantly decreased the crude protein content and increased the

Table I. Effect of nitrogen fertiliser rate on herbage mass (t OM·ha^{-1}), extended tiller height (mm), morphological and chemical compositions of the perennial ryegrass sward.

Sward parameters	HN	LN	SEM
Herbage mass (t OM·ha^{-1})[1]	4.7[a]	3.9[b]	0.18
Herbage mass as green leaves (t OM·ha^{-1})[1]	2.6[a]	2.1[a]	0.17
Extended tiller height (mm)	564[a]	481[b]	8.6
Leaf sheath height (mm)	327[a]	288[b]	5.8
Morphological composition (% DM)[1]			
Leaf	48.6[a]	47.8[a]	2.52
Sheath	42.1[a]	41.6[a]	1.10
Dead material	4.6[a]	3.6[a]	0.34
Flower head	4.7[a]	7.0[a]	1.26
Chemical composition (g·kg^{-1} OM)[1]			
DM (g·kg^{-1})	162[b]	186[a]	5.3
OM (g·kg^{-1} DM)	896[b]	912[a]	2.9
Crude protein	168[a]	124[b]	4.8
Water soluble carbohydrates	180[a]	231[a]	16.7
NDF	558[a]	535[a]	8.5
ADF	331[a]	314[a]	8.4

[a, b] Means in the same row with different superscripts differ significantly ($P < 0.05$).
[1] Above the motor scythe cutting height (8 cm).

Table II. Effect of nitrogen fertiliser rate and protein supplementation on morphological and chemical characteristics of the herbage consumed by grazing dairy cows.

	HN	LN	LN+S	SEM
Offered area ($m^2 \cdot cow^{-1}$)	59[a]	72[b]	71[b]	3.0
Herbage allowance (kg OM·cow^{-1})[1]	27[a]	27[a]	28[a]	1.3
Depth of defoliation (mm)	328[a]	262[b]	261[b]	6.5
Depth of defoliation (ETH)	0.58[a]	0.54[a]	0.52[a]	0.019
Volume defoliated ($m^3 \cdot cow^{-1}$)	18.6[a]	17.9[a]	17.5[a]	1.22
Morphological composition (% DM)				
Leaf	64.5[a]	60.6[a]	61.8[a]	3.74
Sheath	25.4[a]	24.1[a]	23.4[a]	0.97
Flower head	8.2[a]	14.2[a]	13.9[a]	3.35
Chemical composition ($g \cdot kg^{-1}$ OM)				
OM ($g \cdot kg^{-1}$ DM)	902[b]	914[a]	911[a]	2.1
Crude protein	197[a]	163[b]	166[b]	2.6
Water soluble carbohydrates	186[b]	204[a]	203[a]	3.9
NDF	484[a]	494[a]	502[a]	7.7
ADF	262[a]	264[a]	270[a]	5.4

[a, b, c] Means in the same row with different superscripts differ significantly ($P < 0.05$).
[1] Above the motor scythe cutting height (8 cm).

water soluble carbohydrate and DM content ($+ 51$ and $+ 24$ g·kg^{-1} respectively) ($P < 0.05$) in the consumed grass.

3.2. Animal measurements

Faecal OM output was similar for LN and HN treatments, but was significantly higher for LN+S ($P < 0.05$). The OM digestibility was not affected by the level of N fertiliser or by supplementation, and remained high (0.79 on average). Daily herbage OM intake was similar among the treatments and averaged 16.4 kg OM·cow^{-1}, but total OM intake increased significantly as a result of SBM supplementation ($P < 0.01$). Finally, digestible OM intake was 2.0 kg·d^{-1} ($P < 0.01$) higher in the LN+S treatment.

The time spent grazing was unaffected by the N fertiliser rate or supplementation, and averaged 8.5 h (Tab. III). The time spent ruminating was not affected by the level of the N fertiliser when the cow did not receive a supplement but increased by 30 min ($P < 0.05$) when the cows received a supplement. The rate of biting increased on the LN sward ($P < 0.05$) but was unaffected by supplementation. Total bites per day did not differ significantly between the treatments and averaged 21 800 bites·d^{-1}.

When the cows grazed on unfertilised swards, ruminal ammonia concentration was sharply reduced ($P < 0.01$, Tab. IV) and this effect was consistent over 24 h (Fig. 1) Osmolality, total VFA and the proportion of iso acids also decreased. Reducing N fertilisation did not influence ruminal pH and the proportions of acetate, propionate and butyrate and the cellulolytic activity. Ruminal pH declined progressively during the day (Fig. 1).

On unfertilised swards, the feeding supplement slightly increased ruminal ammonia concentration but the ammonia level still remained lower ($P < 0.01$) than on the HN sward, the difference being consistent during the day (Fig. 1). Supplementation also increased osmolality, total VFA and the proportion of iso acids but had no significant

Table III. Effect of nitrogen fertiliser rate and protein supplementation on faecal output (kg OM·d^{-1}), herbage organic matter digestibility, intake (kg·d^{-1}), grazing (min·d^{-1}) and ruminating (min·d^{-1}) behaviour of dairy cows.

	HN	LN	LN+S	SEM
Faecal output (kg OM·d^{-1})	3.33[b]	3.39[b]	3.64[a]	0.063
Herbage OM digestibility	0.795[a]	0.788[a]	0.793[a]	0.006
Herbage OM intake (kg·d^{-1})	16.2[a]	16.2[a]	16.9[a]	0.28
Total OM intake (kg·d^{-1})	16.2[b]	16.2[b]	18.6[a]	0.28
Total digestible OM intake (kg·d^{-1})	12.9[b]	12.8[b]	14.8[a]	0.23
Grazing time (min·d^{-1})	515[a]	509[a]	500[a]	15.5
Ruminating time (min·d^{-1})	489[b]	502[b]	526[a]	7.6
Biting rate (bites·min^{-1})	41[b]	45[a]	43[ab]	1.1
Total bites (× 1000·d^{-1})	21.0[a]	22.8[a]	21.5[a]	0.75

[a, b, c] Means in the same row with different superscripts differ significantly ($P < 0.05$).

Table IV. Effect of nitrogen fertiliser rate and protein supplementation on daily mean ruminal fermentation parameters, particulate passage rate and cellulolytic activity of the ruminal fluid in grazing dairy cows.

	HN	LN	LN+S	SEM
Rumen pH	6.2[a]	6.1[a]	6.1[a]	0.04
Osmolarity (mOsm·L^{-1})	332[a]	314[b]	321[b]	2.6
Ammonia (mmol·L^{-1})	11.1[a]	5.5[c]	7.0[b]	0.32
VFA (mmol·L^{-1})	116[a]	108[b]	112[ab]	1.9
Acetic (%)	61.7[a]	61.2[a]	60.5[a]	0.37
Propionic (%)	22.1[b]	22.9[a]	22.4[b]	0.16
Butyric (%)	11.9[b]	12.5[ab]	13.0[a]	0.26
Isoacids (%)	2.4[a]	1.7[c]	2.0[b]	0.06
Particulate passage rate (%·h^{-1})	4.6[b]	5.2[a]	4.8[b]	0.11
In situ degradability of soybean hulls	0.542[a]	0.533[a]	0.540[a]	0.0066
ADF total tract digestibility	0.767[a]	0.762[a]	0.769[a]	0.0036

[a, b, c] Means in the same row with different superscripts differ significantly ($P < 0.05$).

effects on the proportion of other VFA and the cellulolytic activity.

Neither milk yield nor milk composition were affected by the level of N fertilisation (Tab. V). Supplementation with SBM significantly increased milk yield ($P < 0.01$) by 2.4 kg FCM per day, but did not modify the milk composition. Live weight was un-affected by the N fertiliser rate, but when supplementing, live weight significantly increased ($P < 0.01$).

Reducing the level of N fertilisation significantly reduced plasma urea concentration ($P < 0.01$), while supplementing with soybean meal increased plasma urea to the same level as that observed on the HN
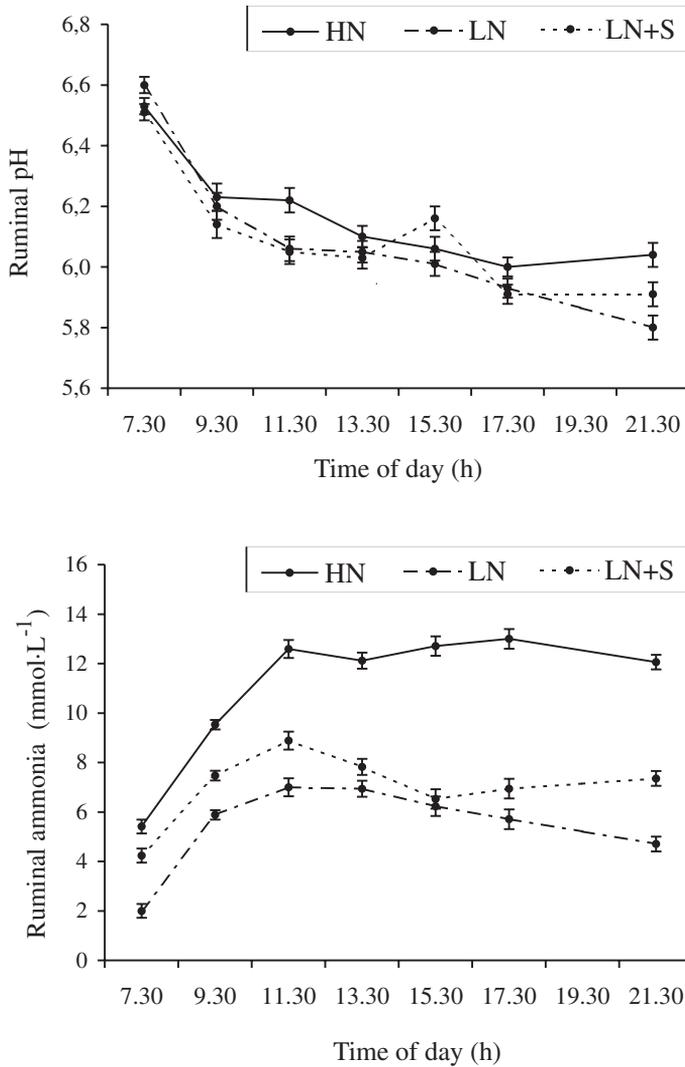
Figure 1. Effect of nitrogen fertiliser rate and protein supplementation on diurnal kinetics of ruminal pH and ammonia concentration (mmol·L^{-1}) in grazing dairy cows.

treatment (Tab. V) NEFA was significantly lower ($P < 0.05$) for the LN+S than for the HN treatment, and intermediate for the LN treatment. The supplemented treatment presented a higher ($P < 0.05$) glucose content in plasma than the unsupplemented treatments.

3.3. N intake and N output

Reducing the level of N fertilisation decreased N intake by 80 g·d^{-1} ($P < 0.01$). Milk N and faecal N output remained unchanged, whereas urine N output was significantly reduced ($P < 0.01$) (Tab. VI).

Table V. Effect of nitrogen fertiliser rate and protein supplementation on milk yield (kg·d^{-1}), milk composition (g·kg^{-1}), live weight (kg) and plasma metabolite concentrations (mmol·L^{-1}) of grazing dairy cows.

	HN	LN	LN+S	SEM
Milk yield (kg·d^{-1})	22.2^b	22.5^b	25.1^a	0.38
Fat content (g·kg^{-1})	39.6^a	38.8^a	38.3^a	0.42
Protein content (g·kg^{-1})	30.0^a	29.9^a	29.8^a	0.25
Fat corrected milk (kg·d^{-1})	22.1^b	22.1^b	24.5^a	0.41
Live weight (kg)	653^b	655^b	663^a	2.4
Plasma urea (mmol·L^{-1})	4.22^a	2.57^b	4.32^a	0.515
Non esterified fatty acids (mmol·L^{-1})	0.124^a	0.092^{ab}	0.073^b	0.0143
Plasma glucose (mmol·L^{-1})	3.59^b	3.61^b	3.72^a	0.035

$^{a, b, c}$ Means in the same row with different superscripts differ significantly ($P < 0.05$).

Table VI. Effect of nitrogen fertiliser rate and protein supplementation on nitrogen intake (g·d^{-1}) and nitrogen outputs (g·d^{-1}) of grazing dairy cows.

	HN	LN	LN+S	SEM
Nitrogen intake (g·d^{-1})	503^b	423^c	594^a	6.5
Milk nitrogen (g·d^{-1})	109^b	109^b	123^a	2.0
Faecal nitrogen (g·d^{-1})	113^b	110^b	132^a	2.3
Urine nitrogen output (g·d^{-1})	281^b	204^c	340^a	5.0
(% N intake)	56^a	46^b	56^a	1.0
(g·kg^{-1} milk)	13.4^a	9.2^b	13.7^a	0.45
(g·m^{-2})	5.1^a	3.1^b	5.3^a	0.19

$^{a, b, c}$ Means in the same row with different superscripts differ significantly ($P < 0.05$).

When expressed as a percentage of total N intake, N in urine was 10 percentage units lower for LN than for HN treatment.

SBM supplementation substantially increased N intake ($P < 0.01$) but milk N output was only 14 g·d^{-1} higher ($P < 0.01$). Thus, N excretion was significantly increased when supplementing, namely urine N output, which increased by 59 or by 136 g·d^{-1} ($P < 0.01$) as compared to HN or LN treatments, respectively.

4. DISCUSSION

4.1. Grazing management and sward characteristics

Because herbage allowance is a major factor affecting intake [30], the two N fertiliser rates were compared at the same herbage allowance. This was achieved by increasing the offered area on unfertilised swards in proportion to the difference

between herbage mass measured on the two types of swards. Cows received 27 kg OM·d⁻¹ above the cutting height of the motor scythe which was higher than the values used by Delagarde et al. [9].

The variations between perennial ryegrass sward treatments according to the level of N fertiliser were as expected [35, 45]. For the LN sward, herbage mass per unit area ($- 0.8\,t\,OM\cdot ha^{-1}$) and sward height ($- 83$ mm) were lower than for the HN sward. However, these differences were smaller than intended. This was primarily due to high residual fertility remaining after manure fertilisation from the preceding maize silage culture.

Regardless of the herbage mass, the swards presented a similar morphological composition. In particular, the proportion of green leaf remained constant among both swards and the sheath height remained a fairly constant proportion of the ETH. This was in accordance with the effects of N fertilisation already reported [9, 44].

With reducing fertilisation, the increase of WSC matched the decrease in CP content of the grass, the DM content increased while the cell wall content was not greatly altered. Several authors [3, 11, 32] have reported similar changes due to the N fertilisation level in herbage chemical composition.

Due to the high herbage allowance and the relatively high levels of herbage height in both swards, the post grazing height of the tillers remained substantially higher (230 mm on average) than the cutting height of the motor scythe (80 mm on average). Therefore, the composition of herbage consumed by the cows was substantially different from that determined at the cutting height, and one essential prerequisite was its estimation. In the present study, a subsample composed of sections lying between the top of the tillers and the mean height of sward after grazing, was taken as a representative of the consumed herbage. From this analysis, it appeared that defoliated herbage was composed mainly of leaf (62% on average). This led to an increase from 124 to 164 g·kg⁻¹ OM of the crude protein content of the cows' diet on LN or LN+S treatments. This level was far higher than the protein content that is critical for ruminal digestion [26] and the level which may affect the appetite of the cow [43].

4.2. N fertilisation

The determined levels of OM intake were in accordance with the requirements, calculated from the standards given by INRA [22] and adjusted by the energy expenditure used for muscular activity while grazing [36], indicating that any bias in the estimation of OM intake was not significant.

Reducing N fertilisation had no effect upon the quantity of herbage consumed by cows and it averaged 16.4 kg OM·d⁻¹. Similarly, the time spent grazing did not differ among treatments and was 508 min on the average, which is in accordance with grazing periods observed by Arriaga-Jordan and Holmes [2] and Delagarde et al. [9] with strip grazing cows. Finally, total harvesting bites amounted to 21 800 per day and the defoliated volume of both LN and HN swards were similar (18 m·cow⁻¹·d⁻¹), which confirmed that neither the sward mass/structure nor the herbage composition influenced the intake or grazing behaviour components.

The effect of fertilisation on herbage intake at grazing would appear to be highly variable. Some studies have pointed out a major increase of intake as a function of fertilisation [9, 17], while others showed no such effect [6]. In fact, fertilisation may have an indirect effect by modulating the green leaf mass per unit area, which is a determinant of herbage availability [19, 29, 30]. The relationship between herbage intake and herbage as green leaf mass per hectare is most probably a curvilinear one.

Since the amount of green leaf mass increases, the response is likely to become progressively smaller and a point will be reached beyond which further increments have no effects on herbage intake. Delagarde et al. [9] reported a decrease of herbage intake of 2.6 kg OM·d^{-1} by grazing dairy cows when comparing low N sward with 1.7 t OM·ha^{-1} to high N sward with 2.3 t OM·ha^{-1} of green leaf mass. In the present study, green leaf mass on both swards, HN and LN, was higher (2.1 and 2.6 t OM·ha^{-1} for LN and HN respectively) than the level reported for the LN sward by these authors, which might partly explain the lack of difference between N fertilisation levels upon herbage intake.

In our study, ADF digestibility and cellulolytic activity of rumen juice were not affected by the reducing N fertiliser rate. This may be related to the ruminal ammonia concentration that always remained higher than the values reported to limit cellulolysis [20]. This level of ammonia was in turn directly related to the relatively high content in crude protein of grass harvested by the cows. On the contrary, Peyraud et al. [31] and Delagarde et al. [9] observed a lower fibre digestibility (6 percentage units lower) and very low ruminal ammonia levels when feeding low N grass to dairy cows. This indicates that the crude protein content in the diet might have had a limited intake in the study of Delagarde et al. [9]. This was confirmed by the positive effect of feeding supplement protein to the cows grazing on low N sward reported by these authors whereas such an effect was not observed in our study.

Lowering N fertilisation did not modify in vivo OM digestibility, which is in agreement with the data reported by Demarquilly [11] on sheep and by Van Vuuren et al. [42], and Peyraud et al. [32] on dairy cows. Therefore, total digestible consumed OM was similar for both HN and LN treatments, which is in good agreement with the milk yields measured.

4.3. Protein supplementation

Supplementation did not modify herbage intake of the dairy cows. This result agreed with the defoliated volume which did not vary between the LN and LN+S treatment. The behaviour variables that should be more sensitive to management changes than intake [18], also confirmed the lack of an effect of supplementation on herbage intake, since the time spent grazing and biting rate for the LN+S treatment did not differ from those of unsupplemented treatments.

These results contrasted with the data usually reported when supplementing with cereal-based concentrates that leads to a depression in herbage intake that can vary from 0.2 to 0.8 kg per kg concentrate offered [40]. On the contrary, the response in herbage intake to protein supplementation appears rather variable according to the N content of the forage. Increased voluntary intake with protein supplementation was classically reported [8, 16] on forages having a low crude protein content (< 80 g·kg^{-1} DM). Similarly, Delagarde et al. [9] reported a slight positive effect of feeding protected soybean meal in dairy cows grazing on sward with a crude protein content lower than 120 g·kg^{-1}. On the contrary, for pastures with a protein content higher than 140 to 160 g·kg^{-1} DM, there is little benefit obtained by feeding protein unless it is in a form that resists degradation in the rumen [15, 34, 37, 46]. Several authors have reported no or little effect on herbage intake and (or) digestibility when feeding protected protein concentrate on pastures [10, 28].

An unprotected supplement may depress grazing time and possibly intake because of a high increase in the production of volatile fatty acids in the rumen [13]. In our study, protein supplementation did not alter ruminal fermentation. Neither mean pH, nor total concentration of VFA and osmolarity differed between the LN+N and

LN treatment. Additionally, supplementation did not substantially modify diurnal ruminal pH as compared to the LN treatment, since the range of fluctuation after feeding the supplement was very small. These results might be related to the fact that the rumen degradability of the supplement was low (half of the supplement was protected) and the amount offered daily to the cows was relatively small. The increase in milk yield on LN+S treatment was clearly associated with an increase in total DOM intake and thus in digestible energy. According to the French Net Energy System [22], the increase in net energy with supplementation was 2.1 UFL·d^{-1} (1.17 UFL·kg^{-1} DM soybean meal). Therefore, milk yield response represents an efficiency of utilisation of supplemental energy of 52%. A higher energy uptake may in itself be an explanation for the change in milk yield rather than the protected protein supplement overcoming a protein deficiency in ryegrass [34, 45]. Indeed, plasma NEFA and glucose contents might support the suggestion that supplemented cows are probably on a higher plane of nutrition than unsupplemented cows.

4.4. Urinary N output at grazing

Reducing the N fertiliser rate, greatly reduced N excreted in the urine since the amount of N consumed was decreased as compared to the HN treatment. Furthermore, the carrying capacities of the swards differed between HN and LN treatments, and when the results from a single animal were extrapolated to the concurrent field situation, the differences between treatments were further accentuated. Urinary N output decreased by 18% per cow but decreased by 40% when expressed per unit area.

With SBM supplementation, the efficiency of additional protein supply was very low (+ 87 g·d^{-1} protein in milk per 566 g supplemental digestible protein in the intestines calculated according to INRA

[22]). Poppi and McLennam [34] also reported a very low efficiency of extra-absorbed protein in temperate pastures. In consequence, amino acids absorbed in excess were catabolised with a dramatic increase in urinary N per cow (66% higher than for the LN treatment). The high level of urea in plasma and the relative similar level of ammonia in the rumen of cows given a soybean supplement compared to cows on the LN treatment, are in agreement with greater amounts of protein absorbed in the intestines and subsequently catabolised. The increase in the amount of N excreted per cow on the LN+S treatment, related to the number of animals carried by the low fertilised sward, led to a total urinary N output per unit area which was similar to the HN treatment although the origin of N was different. For the HN treatment, urine N excretion was primarily due to an excess of degradable N intake, whereas for the LN+S treatment most of the excretion of N in the urine came from the amino acids provided in excess over the milk secreting capacity.

5. CONCLUSION

These results confirmed that the N fertiliser rate and protein supplementation are efficient means to manipulate animal performances and animal N losses to the environment. Reducing the level of N fertilisation would appear to be an efficient mean of reducing N losses per cow, and what is more, per unit area, since the carrying capacity of the low fertilised sward is decreased.

Although supplementation with protein proved to be of a benefit at grazing since herbage intake was not modified and thus the response in milk production was substantially improved, protein supplementation should be used with care at grazing because urinary N losses increase quite rapidly even in the case of high producing cows.

ACKNOWLEDGEMENTS

REFERENCES

[1] Alais C., Science du lait. Principes des techniques laitières, SEPAIC (Ed.), Paris, 1988.

[2] Arriaga-Jordan C.M., Holmes W., The effect of concentrate supplementation on high yielding dairy cows under two systems of grazing, J. Agric. Sci. (Camb.) 107 (1986) 453–461.

[3] Blaser R.E., Symposium on forage utilization: effects of fertility levels and stage of maturity on forage nutritive value, J. Anim. Sci. 23 (1964) 246–253.

[4] Brun J.P., Prache S., Bechet G., A portable device for eating behaviour studies, in: Armstrong R., Doney J. (Eds.), 5th Meeting of European Grazing Workshop, Hill Farming Research Organisation, Midlothian, 1984.

[5] Chilliard Y., Bauchart D., Barnouin J., Determination of plasma non-esterified fatty acids in herbivores and man: a comparison of values obtained by manual or automatic chromatographic, titrimetric, colorimetric and enzymatic methods, Reprod. Nutr. Dev. 24 (1984) 469.

[6] Combellas J., Hodgson J., Herbage intake and milk production by grazing dairy cows. 1. The effects of variations in herbage mass and daily herbage allowance in a short-term trial, Grass Forage Sci. 34 (1979) 209–214.

[7] Comeron E.A., Peyraud J.L., Prediccion de la digestibilidad del pasto ingerido por vacas lecheras, Rev. Argent. Prod. Anim. 13 (1993) 23–30.

[8] Del Curto T., Cochran R.C., Nagaraja T.G., Conah L.R., Beharka A.A., Vanzant E.S., Comparison of soyabean meal/sorghum grain, alfalfa hay and dehydrated alfalfa hay pellets as supplemental sources for beef cattle consuming dormant tallgrass prairie forage, J. Anim. Sci. 68 (1990) 2901–2915.

[9] Delagarde R., Peyraud J.L., Delaby L., The effect of nitrogen fertilisation level and protein supplementation on intake, feeding behaviour and digestion in grazing dairy cows, Anim. Feed Sci. Technol. 66 (1997) 165–180.

[10] Delagarde R., Peyraud J.L., Effet de la nature de concentré sur la digestibilité d'un régime à base d'herbe deshydratée chez la vache laitière, Renc. Rech. Ruminants 5 (1998) 264.

[11] Demarquilly C., Fertilisation azotée et qualité du fourrage, Fourrages 69 (1977) 61–81.

[12] Dewhurst R.J., Thomas C., Modelling of nitrogen transactions in the dairy cow and their environmental consequences, Livest. Prod. Sci. 31 (1992) 1–16.

[13] Faverdin P., Aubert T., Peyraud J.L., Widyobroto B., Effects of the addition of water-filled bladders or volatile fatty acids to the rumen on the feeding behaviour in lactating dairy cows at different stages of lactation, Ann. Zootech. 42 (1993) 186.

[14] Germon J.C., Management systems to reduce impact of nitrates, Commission of the European Communities, Elsevier, London, UK, 1989, 274 p.

[15] Hamilton B.A., The response of grazing dairy cows to protein supplements, in: O'Toole D.K., Baigent D.R. (Eds.), Biennal Research Report 1984-1986, North Coast Agricultural Institute, Wollongbar, 1986, pp. 18–19.

[16] Hannah S.M., Cochran R.C., Vanzant E.Z., Harmon D.L., Influence of protein supplementation on site and extend of digestion, forage intake and nutrient flow characteristics in steers consuming dormant bluestem range forage, J. Anim. Sci. 69 (1991) 2624–2633.

[17] Hodgson J., The relationship between the digestibility of a sward and the herbage consumption of grazing calves, J. Agric. Sci. (Camb.) 70 (1968) 47–51.

[18] Hodgson J., Ingestive behaviour, in: Leaver J.D. (Ed.), Herbage intake handbook, British Grassland Society, 1982, pp. 113–138.

[19] Hodgson J., Grazing behaviour and herbage intake, in: Frame J. (Ed.), Grazing, British Grassland Society, Occasional Symposium No. 19, 1986, pp. 51–63.

[20] Hoover W.H., Chemical factors involved in ruminal fiber digestion, J. Dairy Sci. 69 (1986) 2755–2766.

[21] Hurtaud C., Rulquin H., Vérité R., Effect of infused volatile fatty acids and caseinate on milk composition and coagulation in dairy cows, J. Dairy Sci. 76 (1993) 3011–3023.

[22] INRA, Jarrige (Ed.), Ruminant Nutrition: Recommended Allowances and Feed Tables, INRA, John Libbey Eurotext, Paris, 1989.

[23] Krysl L.J., Branine M.E., Cheema A.U., Funk M.A., Galyean M.L., Influence of soyabean meal and sorghum grain supplementation on intake, digesta kinetics, ruminal fermentations, site and extent of digestion and microbial synthesis in beef steers grazing blue grama rangeland, J. Anim. Sci. 67 (1989) 3040–3051.

[24] Mathieson J., Davidson D., The automated estimation of chromic oxide, Proc. Nutr. Soc. 20 (1970) 30–31.

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

[26] Minson D.J., Effects of chemical and physical composition of herbage eaten upon intake, in: Hacker J.B. (Ed.), Nutritional Limits to Animal Production from Pastures, Commonwealth Agricultural Bureaux, Farnham Royal, 1982, pp. 167–182.

[27] Moore J.J., Sax S.M., A revised automated procedure for urea nitrogen, Clin. Chem. Acta 11 (1965) 475–476.

[28] Penning P.D., Orr R.J., Treacher T., Responses of lactating ewes, offered fresh herbage indoors and when grazing, to supplements containing different protein concentrations, Anim. Prod. 46 (1988) 403–415.

[29] Penning P.D., Parsons A.J., Orr R.J., Hooper G.E., Intake and behaviour response by sheep to changes in sward characteristics under rotational grazing, Grass Forage Sci. 39 (1994) 177–185.

[30] Peyraud J.L., Comeron E.A., Wade M., Lemaire G., The effect of daily herbage allowance, herbage mass and animal factors upon herbage intake by grazing dairy cows, Ann. Zootech. 45 (1986) 201–217.

[31] Peyraud J.L., Astigarraga L., Faverdin P., Digestion of fresh perennial ryegrass fertilised at two levels of nitrogen by lactating dairy cows, Anim. Feed Sci. Technol. 64 (1997) 155–171.

[32] Peyraud J.L., Astigarraga L., Review of the effect of nitrogen fertilisation on the chemical composition, intake, digestion and nutritive value of fresh herbage: consequences on animal nutrition and N balance, Anim. Feed Sci. Technol. 72 (1998) 235–259.

[33] Poncet C., Rayssiguier Y., Effect of lactose supplement on digestion of Lucerne hay by sheep. 1: Sites of organic matter and nitrogen digestion, J. Anim. Sci. 51 (1980) 180–185.

[34] Poppi D.P., Mc Lennan S.R., Protein and energy utilisation by ruminants at pasture, J. Anim. Prod. 73 (1995) 278–290.

[35] Reid D., The effect of frequency of defoliation on the yield response of a perennial ryegrass sward to a wide range of nitrogen application, J. Agric. Sci. (Camb.) 24 (1978) 615–625.

[36] Ribeiro J.M. de C.R., Brockway J.M., Webster A.J.F., A note on the energy cost of walking in cattle, Anim. Prod. 25 (1977) 107–110.

[37] Rogers G.L., Porter R.H.D., Clarke T., Stewart J.A., Effect of protected casein supplements on pasture intake, milk yield and composition of cows in early lactation, Aust. J. Agric. Res. 31 (1980) 1147–1152.

[38] SAS Institute, Inc., SAS/STAT Guide for Personal Computers, Version 6, Cary, NC, USA, 1987.

[39] t'Mannetje L., Frame J., Grassland and Society, Proc. 15th Gen. Meet. European Grassl. Fed., Wageningen, Netherlands, 1994, 618 p.

[40] Thomson D.J., The nitrogen supplied by and the supplementation of fresh or grazed herbage, in: Thomson D.J., Beever D.E., Gunn R.G. (Eds.), Forage Protein in Ruminant Nutrition, Animal Production Occasional Publication No. 6, British Society of Animal Production, London, 1982, pp. 53–66.

[41] Van Burg P.F.J., Prins W.H., den Boer D.J., Sluiman W.J., Nitrogen and intensification of livestock farming in EEC countries. Proceedings No. 199, The Fertiliser Society, London, 1981.

[42] Van Vuuren A.M., Krol-Kramer F., Van Der Lee R.A., Corbijn H., Protein digestion and intestinal amino acid in dairy cows fed fresh *Lolium perenne* with different nitrogen contents, J. Dairy Sci. 75 (1992) 2215–2225.

[43] Vérité R., Delaby L., Conduite alimentaire et rejets azotés chez la vache laitière. Interrelations avec les performances, Renc. Rech. Ruminants 5 (1998) 185–192.

[44] Wade M.H., Factors affecting the availability of vegetative *Lolium perenne* to grazing dairy cows with special reference to sward characteristics, stocking rate and grazing method, Thèse, Université de Rennes I, Rennes, France, 1991.

[45] Wilman D., Koocheki A., Lwoga A.B., The effect of interval between harvests and nitrogen application on the proportion and yield of crop fractions and on the digestibility and digestible yield and nitrogen content and yield of two perennial ryegrass varieties in the second harvest year, J. Agric. Sci. (Camb.) 87 (1976) 59–74.

[46] Wilson G.F., The influence of protein supplements on milk yield and composition, Proc. N.Z. Soc. Anim. Prod. 30 (1970) 123–127.