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Influence of carbohydrate or protein supplementation on intake, behaviour and digestion in dairy cows strip-grazing low-nitrogen fertilized perennial ryegrass

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Abstract — The effect of concentrate composition on intake, grazing behaviour and ruminal fermentation was studied on dairy cows strip-grazing on perennial ryegrass swards receiving a low level of nitrogen (N) fertilization. Four treatments were compared in spring 1994 on eight cows according to a 4 × 4 Latin square design: 1) control with no supplement (NO), 2) rapidly fermentable wheat concentrate (WHE), 3) slowly fermentable maize and soya-bean hulls concentrate (MSH), and 4) protected soya-bean meal (SBM). The supplementation level was 2.5 kg organic matter (OM)·d^{-1}. Individual herbage intake was calculated by estimating faecal output (using chromic oxide) and herbage digestibility (faecal N and ADF as indicators). Herbage intake was 13.7, 13.4, 13.8 and 15.8 kg OM·d^{-1} for NO, WHE, MSH and SBM, respectively. The intake level for unsupplemented cows was low and appears to have been mainly limited by a strong deficiency in ruminally degradable protein (ruminal ammonia: 0.8 mmol·L^{-1}; blood urea: 1.2 mmol·L^{-1}). WHE and MSH had no effect on the level of herbage intake, while total digestible OM intake was increased by 2.0 kg. Carbohydrate supplementation had no effect on grazing behaviour or on the intensity of ruminal fermentation. The SBM concentrate clearly increased herbage intake by way of improved ruminal digestion due to the input of N. Supplementation with SBM increased ruminal ammonia (+0.7 mmol·L^{-1}), total volatile fatty acids (VFA) (+14 mmol·L^{-1}), as well as blood urea (+2.5 mmol·L^{-1}), estimated total neutral detergent fibre (NDF) digestibility (+0.05) and OM digestibility (OMD) of herbage (+0.02). A stimulatory effect of nutritional status, protein mainly, on intake cannot be excluded, as suggested by behavioural data. (© Elsevier / Inra)

dairy cow / grazing / nitrogen / supplementation / herbage intake / ruminal digestion / grazing behaviour

Résumé — Influence de la complémentation énergétique ou azotée sur l'ingestion, le comportement alimentaire et la digestion des vaches laitières pâturant du ray-grass anglais peu fertilisé. L'effet de la nature du concentré distribué à des vaches laitières au pâturage sur l'ingestion, les

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fermentations ruminales et le comportement alimentaire a été étudié au printemps 1994 sur un ray-grass anglais peu fertilisé. Un témoin sans concentré (NO), deux concentrés énergétiques, à dégradation rapide (WHE : blé) ou lente (MSH : maïs + coques de soja), et un concentré azoté (SBM : tourteau de soja tanné), apportés à raison de 2,5 kg MO par vache et par jour, ont été comparés sur huit vaches en production selon un schéma en carré latin 4 × 4 pendant 8 semaines. Les quantités ingérées d'herbe ont été calculées en mesurant la quantité de fèces (oxyde de chrome) et en estimant la digestibilité de l'herbe ingérée à partir de la composition chimique des fèces (N et ADF). Les quantités ingérées d'herbe ont été de 13,7, 13,4, 13,8 et 15,8 kg MO·j^{-1} pour les traitements NO, WHE, MSH et SBM respectivement. Le niveau d'ingestion des vaches non complémentées a été faible, sans doute à cause de la faible teneur en azote de l'herbe ingérée (11 % MAT) et d'une digestion ruminale limitée par une forte carence en azote dégradable (NH_3 ruminal : 0.8 mmol·L^{-1}; urémie : 1.2 mmol·L^{-1}). Dans ces conditions, l'ingestion d'herbe n'a pas été réduite par l'apport de concentré énergétique WHE ou MSH et l'ingestion totale de MO digestible a été accrue de 2,0 kg. L'apport de concentré énergétique n'a eu aucune influence sur l'intensité des fermentations ruminales ni sur le comportement alimentaire. En revanche, les quantités ingérées d'herbe ont augmenté fortement quand les vaches ont reçu le concentré SBM. Cet effet positif du SBM sur l'ingestion d'herbe semble clairement lié à une amélioration de la digestion ruminale. En effet, avec ce traitement, les teneurs ruminales en NH_3 (+ 0.7 mmol·L^{-1}) et en AGV totaux (+ 14 mmol·L^{-1}), mais aussi l'urémie (+ 2.5 mmol·L^{-1}), la digestibilité totale estimée du NDF (+ 0.05) et la dMO de l'herbe (+ 0.02) ont été fortement augmentées par rapport au témoin. Un effet positif de l'état nutritionnel azoté sur l'ingestion ne peut pas être exclu au regard des données de comportement alimentaire. (© Elsevier / Inra)

vache laitière / pâturage / azote / complémentation / ingestion / digestion ruminale / comportement alimentaire

1. INTRODUCTION

In the current context of dairy production, reducing the level of nitrogen (N) fertilization of grazed swards is a possible way to economize N inputs. The direct consequences are a fall of productivity per hectare and a decrease in the N content of grass, with possible effects on the nutrition of the dairy cows [36, 56]. For instance, decreasing the N fertilization of the swards can involve a strong reduction in the level of herbage intake even when the herbage allowance is maintained [10]. Under these conditions of double potential deficit in N and energy, it is important to know the effect of the nature of the concentrate on herbage intake as well as the concerned mechanisms.

On well N-fertilized swards, an increase in the protein content of the concentrate modifies only slightly the level of herbage intake by grazing the dairy cow [17, 23, 24] and generally involves a weak impact on dairy production [8]. On the other hand, on poorly N-fertilized swards, there is a more significant increase in zootechnical performances following the introduction of protein into concentrate [8]. Such an effect may arise directly from the increase in the nitrogenous supply to the animal, but perhaps it may also be due to an increase in the intake level and the digestibility of the grass [5, 21, 35].

At grazing, some recent trials on lactating dairy cows [10, 37] showed that the supply of 2 kg of protected soya-bean meal did not reduce herbage intake even at high herbage allowance. Such an effect is rarely observed with carbohydrate concentrates, with which the substitution rate is generally close to 0.4–0.6 [32, 34, 48]. However, there are very few comparisons in the literature between N-rich and carbohydrate-rich concentrates for grazing dairy cows, and it is not possible to discern any positive effect of protein in the concentrate on herbage intake. The advantage of providing N input via the concentrate may be more relevant

with low N grass. In this context, more studies have been conducted on rangeland, including grassland poor in N but also low in energy content [28, 46]. To our knowledge, there are no data concerning the response of intake by supplementing dairy cows on poorly N-fertilized swards characterized by low levels of crude protein content, but which nevertheless maintain a high energy content in grass [36].

Therefore, the present investigation was carried out to study the influence of the nature of the concentrate, mainly protein content, on the intake and digestion of dairy cows grazing low N-fertilized perennial ryegrass swards.

2. MATERIALS AND METHODS

2.1. Treatments, cows and experimental design

The experiment was carried out at the Méjusseaume Inra experimental farm near Rennes (France) from 6 May to 30 June 1994. It was conducted on eight ruminally cannulated Holstein dairy cows at pasture, with the aim of comparing four supplementation treatments. Cows either received no supplement (control: NO) or were fed 3 kg of either a concentrate with rapidly rumen degradable carbohydrates (wheat: WHE), or a concentrate with slowly rumen degradable carbohydrates based on maize grain and soya-bean hulls (MSH), or a protein-rich concentrate based on protected soya-bean meal (SBM). All supplements were formulated to be isoenergetic.

Table I. Components, chemical composition and nutritive value of the experimental concentrates and of herbage ingested.

Feeds	Concentrates			Herbage ingested			
	WHE	MSH	SBM	P1[a]	P2	P3	P4
Component composition (g·kg^{-1})							
Wheat	965	–	–				
Soya-bean hulls	–	495	–				
Maize	–	470	–				
Protected soya-bean meal	–	–	965				
Sugar beet molasses	20	20	20				
Fat	10	10	10				
Salt	5	5	5				
Chemical composition (g·kg^{-1} DM)							
DM (g·kg^{-1} fresh matter)[b]	888	893	891	185	204	236	255
OM	971	954	928	912	927	924	927
CP	146	116	469	126	104	103	101
NDF	126	380	251	427	413	402	446
ADF	37	227	91	223	217	204	223
ADL	9	13	11	13	11	13	14
Starch	612	354	40	–	–	–	–
Nutritive value (per kg DM)							
UFL	1.20	1.09	1.19	0.98	1.00	0.94	0.93
PDIN (g)	100	82	362	79	67	69	67
PDIE (g)	114	117	307	91	89	86	87

[a] P1 to P4: period 1 to period 4 (respectively 6–19 May, 20 May–2 June, 3–16 June, 17–30 June); [b] DM content of herbage determined on herbage cut by motorscythe (5 cm to ground level). WHE: rapidly fermentable wheat concentrate; MSH: slowly fermentable maize and soya-bean hulls; SBM: protected soya-bean meal; DM: dry matter; OM: organic matter; CP: crude protein; NDF: neutral detergent fibre; ADF: acid detergent fibre; ADL: acid detergent lignin; UFL: fill unit for lactating dairy cows; PDIN: protein digested in the intestine when microbial proteins are limited by rumen-degraded protein; PDIE: protein digisted in the intestine when microbial proteins are limited by rumen-fermented feed energy.

The components and chemical compositions of concentrates are given in *table I*. Concentrates were fed individually to cows in two equal meals per day during milking times. Cows were milked twice daily, at 06:30–08:00 h in the morning and 16:30–18:00 h in the afternoon.

Animals were multiparous lactating dairy cows, each fitted with a large rumen cannula (internal diameter 123 mm). The eight cows were paired at the beginning of the experiment according to milk production (28.2 ± 5.3 kg FCM per day), live weight (674 ± 57 kg) and time from calving (156 ± 43 days) determined at pasture between 25 April and 1 May, when they received 3 kg daily of a mixed concentrate. Each pair was then assigned to a treatment sequence according to a 4 × 4 Latin square arrangement balanced for residual effects of the treatments. Experimental periods lasted 14 days, including 2 days for concentrate transition, 6 days for adaptation to the treatment and 6 days for measurements.

2.2. Grazing and pasture management

Four paddocks of 2-year-old perennial ryegrass pasture (*Lolium perenne* L. cv Belfort) were used. They were cut two to three times during the winter and spring prior to the experiment. Paddocks received fertilization levels of 100 kg P and 175 kg K per ha in November 1993 and only 20 kg N per ha immediately after each preparation cut in 1994. A new paddock was provided for each of the four periods. Time of the final cut in each paddock was planned to achieve 32 days of herbage regrowth during each intake-measurement period, i.e., on day 12 of each period. The eight cows were divided into two groups of four, all treatments being in each group. The two groups strip-grazed in adjacent sub-paddocks separated only by electric fencing, in order to facilitate individual faecal sampling at pasture. Similar areas were offered daily to the two groups. Front fences were moved each morning with a daily herbage allowance of 17 kg organic matter (OM) per cow measured at 5 cm above ground level. Back fences were also moved each morning so that the cows were allowed to graze the area of the previous day. Water and mineral block were always available at grazing and during milking. Daily time of access to pasture was approximately 21 h.

2.3. Sward measurements

The pre-grazing herbage mass was measured by motorscythe cutting at 5 cm above ground level on days 4, 8 and 12 of each period. On each of these days, four 0.5 × 5 m strips were cut, the total fresh weight per strip recorded and the dry matter (DM) content of the herbage determined for each strip by drying a subsample for 48 h at 80 °C. Fresh subsamples of strips cut on days 8 and 12 were frozen, then bulked at the same weight and freeze-dried for subsequent chemical analysis. Mean residual sward height was measured each day with a rising plate metre using 30 readings per group. Extended sward height was also measured before and after grazing on areas grazed on days 9 and 13. On both of these days, 60 tillers were taken at random and measurements were made of the extended height to ground level of the longest leaf and stem. The chemical composition of the herbage ingested by the cows was determined as previously described by Delagarde et al. [10]. Briefly, herbage handfuls cut to ground level were collected on days 8 and 12 and immediately frozen with the vertical sward structure preserved. Later, grass was cut at the mean residual extended sward height measured in other respects, and the upper part was freeze-dried for chemical analysis.

2.4. Animal measurements

Herbage intake was measured indirectly by estimating the faecal output attributable to herbage and predicting the digestibility of the selected herbage. Total faecal output was estimated from dilution in faeces of chromic oxide as an external marker assuming total recovery. Twice daily, during milking times, the cows were dosed via the ruminal cannula with 200 g of pelleted concentrate containing 5 % of Cr_2O_3 (i.e., 18 g Cr_2O_3 per cow per day) and with coloured plastic particles for dung identification [10]. Each morning, from days 11 to 15, all dung pats were sampled in the milking parlour and in the field. A first subsample of faeces was dried for 72 h at 80 °C, bulked per cow and per period and then ground for chromium, ash, N and ADF analysis. These analyses were performed on dried faeces, according to Comerón and Peyraud [7] to obtain measurements of herbage intake. A second subsample of faeces was frozen per cow and per period, and then freeze-dried and ground for neutral detergent fibre (NDF) analysis in order to determine NDF digestibility on the same freeze-dried basis as the herbage samples.

The OM intake from grass was calculated by estimating faecal OM attributable to grass and from estimated OM digestibility (OMD) of grass based on faecal excretion of N and ADF attri-

butable to grass. The faecal OM output attributable to herbage was calculated by subtracting the indigestible OM attributable to concentrates (182, 110, 150 and 100 g per kg OM for chromic oxide concentrate, WHE, MSH and SBM, respectively [22]) from the total estimated faecal OM output. For supplemented cows, the amounts of faecal N and ADF attributable to concentrates were calculated from the N and ADF contents of concentrates (*table I*), from the apparent N digestibility of concentrates given by Inra [22] and the apparent ADF digestibility of concentrates given by Demarquilly et al. [11]. The amounts of faecal N and ADF attributable to herbage were calculated by subtracting the amounts of N and ADF attributable to concentrates from the total N and ADF excreted in faeces. Faecal N and ADF contents derived from herbage were calculated by dividing the amount of N and ADF from herbage by the faecal OM output attributable to herbage. The herbage OMD was then calculated from the following equation [7]:

$$\text{OMD (g·kg}^{-1}\text{ OM)} = 0.791 + 0.0334\ \text{FN} - 0.0038\ \text{FADF}$$
$$(n = 24;\ R = 0.89;\ \text{RSD} = 0.013)$$

where FN is the faecal N content from herbage (g·kg^{-1} OM) and FADF is the faecal ADF content from herbage (g·kg^{-1} OM).

Grazing and rumination activities detected from jaw movements were recorded automatically for 24-h periods with a portable electronic device (APEC [4]). Behaviour was recorded for each cow on several consecutive days from day 7. On average, 4.1 daily records were completed per cow and per period. The mean daily biting rate was measured by visual observation on 1 day at the end of each period. Each cow was observed 2 min every 15 min during grazing in the morning (08:00–11:00 h), afternoon (13:30–16:00 h) and evening (18:00–22:00 h).

On day 11, ruminal fluid was assayed for ammonia (NH_3), pH and volatile fatty acids (VFA). Ruminal fluid was sampled during morning milking time before concentrate allocation at 06:45 h, and then at 07:45 h (at milking), 09:30, 11:00, 13:30, 17:30 (at milking) and 21:30 h. At each time, a 50-mL sample was taken and the pH was immediately measured. After straining, two subsamples were frozen according to the procedure described by Mambrini and Peyraud [29] for NH_3 and VFA analysis. Mean daily ammonia, pH and VFA concentration were calculated as the mean of the seven samples over the day. No adjustment was made for differences in time interval between samplings. Ruminal cellulolytic activity was estimated for day 9 from in sacco DM disappearance of soya-bean hulls after 9 and 24 h incubation in the rumen in duplicated nylon bags (dimensions 6 × 11 cm, pore size 0.046 mm, 3 g sample). Blood samples were taken, also on day 11, for urea and glucose analysis from each cow via the caudal vein during morning milking time. Plasma was separated by centrifugation, deproteinized by adding 2 mL of $HClO_4$ per mL of plasma and then frozen.

Milk production was measured at each milking, and milk fat and protein contents were determined each week on eight consecutive milkings. The live weight of the cows was also measured on the last day of each period.

2.5. Chemical analysis

Dry matter content was determined by drying for 48 h at 80 °C (72 h for faeces). All samples of herbage, concentrates and faeces were ground to a 0.8-mm screen before analysis. OM, N, NDF, ADF, NH_3 and VFA were analyzed as previously described by Mambrini and Peyraud [29]. OMD derived from the pepsin-cellulase method was determined on herbage offered and ingested, using the technique described by Aufrère and Demarquilly [3]. Chromic oxide in faeces was determined by the method of Matheson and Davidson [30] as modified for an auto-analyser (Technicon) by Poncet and Rayssiguier [42]. Glucose was determined according to Trinder [51] with an auto-analyser (Isamat), while blood urea was determined by a colorimetric procedure with a continuous flux analyser (Technicon) [33].

2.6. Statistical analysis

The effect of the different groups of cows on the herbage data sets was tested by simple variance analysis [43], taking both period and group effects into consideration. Intake, behavioural and digestive data were analyzed as a 4 × 4 Latin square design using the general linear models procedure of SAS [43]. The model included effects due to the individual cow, period and treatment. Orthogonal contrasts were conducted to assess the following effects: 1) no concentrate vs. concentrate, 2) carbohydrate vs. protein supplements, 3) WHE vs. MSH. Significance and tendency were declared at $P < 0.05$ and $0.05 < P < 0.10$, respectively. One cow was ill

during period 1 and animal measurements were not achieved for this cow in period 1. Statistical analysis was carried out on 31 sets of animal data.

3. RESULTS

3.1. Sward characteristics

Herbage mass, height and chemical composition as well as daily herbage allowance and mean residual sward height were not significantly different between the two separated groups of cows. Thus, only mean results are presented here. Herbage mass above 5 cm averaged 2 690 kg OM·ha^{-1} for a mean plate metre height of 15.4 cm. Mean extended tiller and stem height before grazing were 35.3 and 14.9 cm, respectively. Each cow was offered an average grazing area of 64 m^2 per day, and the consequent herbage allowance was 16.7 kg OM/cow/d (above 5 cm). Mean residual sward height measured from the rising plate metre was fairly high, with a mean value of 8.3 cm. Mean extended tiller and stem height after grazing were 15.5 and 11.5 cm, respectively.

The herbage cut by motorscythe contained an average of 929 g OM, 95 g crude protein (CP), 427 g NDF and 228 g ADF per kg DM. Its OMD, as estimated from the pepsin-cellulase method, averaged 0.767. The herbage selected by the cows was slightly higher in CP (109 g per kg DM) than the herbage offered as a result of setting the residual sward height higher than 5 cm. However, the CP content of herbage selected remained low for all periods (*table I*). The NDF and ADF contents did not differ between offered and selected herbage. The herbage is characterized by a very low N value (91 g PDIE/UFL; PDIN–PDIE = –19 g/UFL; *table I*).

3.2. Animal data

Supplementation increased total faecal OM output, especially with MSH compared to WHE (*table II*). Supplementation tended to increase faecal OM output attributable to herbage without any effect due to the type of supplement. The OMD of herbage selected by unsupplemented cows – estimated from faecal indicators – was 0.785, 0.779, 0.768 and 0.758 for periods 1, 2, 3 and 4, respectively. On average, herbage OMD was 3.1 units (U) higher for SBM than for the two carbohydrate supplements and 1.9 U higher than the control. Herbage OM intake was similar for NO, WHE and MSH treatments. The type of carbohydrate supplement had no effect on the herbage OM intake. However, this was increased by 2.1 kg OM when cows received SBM, and the carbohydrate vs. protein supplement contrast was highly significant. As a result, total OM intake was much higher for all supplemented cows, and even more for SBM compared with carbohydrate supplements. Compared with the control, total digestible OM intake was increased by 2.0 kg when using WHE and MSH, and increased by 4.2 kg with SBM.

Total NDF intake was higher when using SBM than with the two carbohydrate supplements. However, faecal NDF content and total NDF excreted in faeces were much lower on SBM than on WHE or MSH (*table II*). Estimated total NDF digestibility was, on average, 7.7 U higher on SBM than on the two other supplements, being 5.5 U higher than on the control. The nature of the carbohydrate supplement did not affect the total NDF digestibility.

The daily time spent grazing or ruminating and the mean rate of biting were not significantly affected by the treatments (*table II*). Grazing activity ceased at dusk between 22:00 and 23:00 h, and grazing time during the night (23:00 to 07:00 h) was only 22 min. On average, grazing during the daytime represents 96 % of the total grazing activity.

Although the mean ruminal ammonia content was not affected by the supply of WHE or MSH (0.7 mmol·L^{-1} on average), it was twofold higher on SBM (+ 0.7 mmol·L^{-1}; *table III*). Supplementation reduced the mean daily ruminal pH, the decrease being

Table II. Effect of type of supplement on organic matter (OM) and neutral detergent fibre (NDF) excretion, digestibility and intake, as well as on behaviour of strip-grazing dairy cows.

Parameters	Treatments				RSD[a]	Contrasts[b] ($P <$)		
	NO	WHE	MSH	SBM		(1)	(2)	(3)
Total faecal OM output (kg)	3.14	3.54	3.76	3.56	0.198	0.001	0.321	0.034
Faecal OM output from herbage (kg)	3.08	3.21	3.32	3.26	0.198	0.057	0.959	0.250
Herbage OM digestibility	0.774	0.762	0.761	0.793	0.0081	0.527	0.001	0.716
Herbage OM intake (kg)	13.7	13.4	13.8	15.8	0.83	0.073	0.001	0.353
Total OM intake (kg)	14.0	16.3	16.7	18.6	0.83	0.001	0.001	0.295
Total digestible OM intake (kg)	10.8	12.7	12.9	15.0	0.68	0.001	0.001	0.519
NDF intake (kg)	6.3	6.6	7.4	8.0	0.38	0.001	0.001	0.001
Faecal NDF content (% OM)	61.9	61.7	63.7	56.5	2.87	0.345	0.001	0.169
Faecal NDF (kg)	1.96	2.19	2.41	2.02	0.158	0.003	0.001	0.013
Estimated total NDF digestibility	0.691	0.661	0.677	0.746	0.0189	0.498	0.001	0.282
Grazing time (min)	517	480	494	502	39.3	0.161	0.399	0.502
Ruminating time (min)	545	544	556	538	23.6	0.919	0.244	0.315
Biting rate (bites/min)	52.3	53.5	52.4	53.0	2.00	0.460	0.944	0.277

[a] RSD: residual standard deviation; [b] Contrasts: (1) no concentrate vs. concentrates, (2) carbohydrate vs. protein concentrates, (3) WHE vs. MSH. See *table I* for abbreviations.

Table III. Effect of type of supplement on ruminal fermentations and cellulolytic activity, blood urea and glucose in strip-grazing dairy cows.

Parameters	Treatments				RSD[a]	Contrasts[b] ($P <$)		
	NO	WHE	MSH	SBM		(1)	(2)	(3)
Ammonia (mmol·L^{-1})	0.8	0.8	0.6	1.5	0.43	0.323	0.001	0.572
pH	6.2	6.1	6.2	5.9	0.11	0.021	0.001	0.078
VFA (mmol·L^{-1})	97	101	100	111	5.3	0.014	0.001	0.759
acetate (mol%)	62.4	60.2	61.2	60.4	1.25	0.005	0.542	0.150
propionate (mol%)	20.7	22.1	21.4	22.3	1.32	0.039	0.330	0.351
butyrate (mol%)	13.4	13.5	13.5	13.7	0.94	0.660	0.641	0.946
isoacids (mol%)	2.0	2.1	2.0	1.7	0.18	0.411	0.001	0.321
minors (mol%)[c]	1.8	2.2	1.9	2.0	0.36	0.108	0.845	0.155
Ketogenic/glycogenic ratio[d]	3.7	3.4	3.5	3.4	0.26	0.024	0.346	0.314
Cellulolytic activity 9 h (%)[e]	32.7	29.8	32.1	34.1	2.9	0.558	0.023	0.127
Cellulolytic activity 24 h (%)[e]	51.6	51.6	54.1	53.8	5.0	0.486	0.675	0.324
Blood urea (mmol·L^{-1})	1.2	1.5	1.1	3.7	0.36	0.001	0.001	0.076
Blood glucose (mmol·L^{-1})	3.63	3.74	3.67	3.88	1.154	0.063	0.017	0.310

[a] RSD: residual standard deviation; [b] Contrasts: (1) no concentrate vs. concentrates, (2) carbohydrate vs. protein concentrates, (3) WHE vs. MSH; [c] valeric plus caproic acids; [d] acetate + butyrate/propionate; [e] disappearance of soya-bean hulls (% DM) after in sacco incubation in the rumen (9 or 24 h). VFA: volatile fatty acids; see *table I* for other abbreviations.

entirely due to the effect of SBM (–0.3 U). Moreover, pH tended to be lower with WHE than with MSH. Mean total VFA in the ruminal fluid was higher on SBM than on the three other treatments (+12 mmol·L^{-1}). The mean effect of SBM on ammonia is noticeable for each sampling time, and not only after concentrate meals at milkings (*figure I*). The contrast between carbohydrate and protein supplements is significant at each sampling time. With SBM, pH was lower and VFA higher than other treatments for the three first sampling times in the morning (*figure I*), in particular at 06:45 h before the first concentrate allocation and at least 8 h after the last grazing meal.

For all supplemented cows, there was a decrease of the molar proportion of acetate, an increase in the molar proportion of propionate and, subsequently, a decrease of the ketogenic/glycogenic ratio (*table III*). These effects did not differ with the type of supplement. The molar proportion of isoacids was lower on SBM than on carbohydrate supplements. Ruminal cellulolytic activity slightly decreased for carbohydrate supplements compared to SBM after 9 h incubation in the rumen. No effect due to treatments was observed after 24 h incubation.

Blood urea concentration in the control was very low (1.2 mmol·L^{-1}), being unaffected by the supply of either WHE or MSH. However, uremia was much higher when cows were given SBM (+2.5 mmol·L^{-1}) (*table III*). Similarly, glycemia was not changed when cows were supplemented with WHE or MSH (3.68 mmol·L^{-1} on average) but increased when SBM was given (+0.20 mmol·L^{-1}).

Supplementation sharply increased milk production (*table IV*). On average, the production increased by 2.5 kg milk per day with carbohydrate supplements, whatever their nature, and 5.2 kg milk per day with SBM. The carbohydrate vs. protein supplement contrast is highly significant. Milk fat content tended to be lower (–1.3 g·kg^{-1}) when concentrate was given, without any effect from the supplement type. Milk protein content was higher (+0.9 g·kg^{-1}) on SBM than on the three other treatments. Cows showed a much higher live weight when given SBM (+22 kg) (*table IV*).

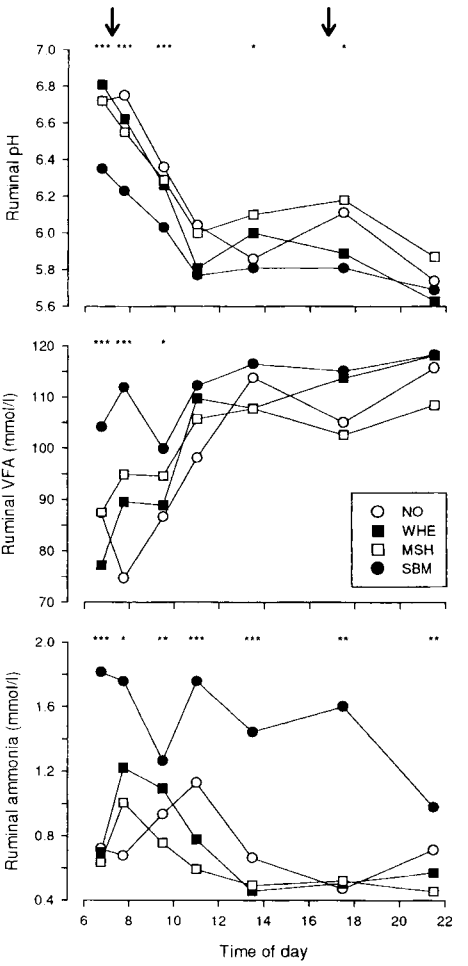

Figure 1. Effect of type of supplement on diurnal kinetics of ruminal pH, volatile fatty acids (VFA) and ammonia in strip-grazing dairy cows (arrows indicate concentrate allocation at milkings; asterisks indicate significance level of carbohydrate vs. protein contrast: * $P < 0.10$; ** $P < 0.05$; *** $P < 0.01$). NO: control with no supplement; WHE: rapidly fermentable wheat concentrate; MSH: slowly fermentable maize and soya-bean hulls; SBM: protected soya-bean meal.

Table IV. Effect of type of supplement on animal performance in strip-grazing dairy cows.

Parameters	Treatments				RSD[a]	Contrasts[b] ($P <$)		
	NO	WHE	MSH	SBM		(1)	(2)	(3)
Milk production (kg)	19.6	22.0	22.2	24.8	1.14	0.001	0.001	0.814
FCM production (kg)	19.4	21.1	21.3	23.8	1.19	0.001	0.001	0.720
Fat production (g)	770	819	830	929	53.1	0.002	0.001	0.687
Protein production (g)	566	648	641	740	32.0	0.001	0.001	0.673
Milk fat content ($g \cdot kg^{-1}$)	39.4	38.0	38.4	38.0	1.38	0.052	0.696	0.619
Milk protein content ($g \cdot kg^{-1}$)	29.5	29.9	29.6	30.6	0.71	0.119	0.015	0.412
Live weight (kg)	651	651	652	673	8.4	0.062	0.001	0.746

[a] RSD: residual standard deviation; [b] Contrasts: (1) no concentrate vs. concentrates, (2) carbohydrate vs. protein concentrates, (3) WHE vs. MSH. See *tables I* and *III* for abbreviations.

4. DISCUSSION

4.1. Grass intake without supplementation

In the present trial, the unsupplemented cows ingested 13.7 kg OM from herbage, which is a low intake level compared with previous trials carried out at the same experimental site. Intake levels of 15–17 kg OM have been reported by Peyraud et al. [37, 38] and Delagarde et al. [10] for unsupplemented cows having similar milk production level and offered fertilized grass under ad libitum conditions. The drop in milk production observed for unsupplemented cows during the present trial (8.8 kg FCM in 5 weeks) is compatible with the low level of intake. Their energy balance, as calculated according to the Inra [22], is only slightly negative (–0.3 UFL·d^{-1}), which fails to reflect the considerable energy deficit of these cows. This is because the milk production adapted itself very rapidly to the drastic fall in inputs during the trial when concentrate was removed from the diet.

The amount of grass offered in the present trial (17 kg OM·cow^{-1}·d^{-1}) is probably not limiting for intake [38] and cannot on its own explain the low intakes measured for the control treatment. Moreover, the high residual height of refusals (15.5 cm extended height, 8.3 cm measured with rising plate metre) suggests that the animals were not constrained to graze the lower strata of the sward, as would occur if the availability of grass was low and limiting for intake.

On the other hand, the herbage offered to the animals was characterized by particularly low protein content throughout the course of the trial (an average of 95 g CP·kg^{-1} DM above 5 cm). All digestive and metabolic measurements carried out on the animals reveal a very strong deficit in degradable N with the unsupplemented diet, a feature that is rarely observed in temperate climate with cows grazing on perennial ryegrass. The ruminal ammonia content of 0.8 mmol·L^{-1} observed here is evidently limiting for cellulolytic activity. This level is below the critical threshold of 1.4 mmol·L^{-1} proposed by Clark et al. [6]. Similarly, Vérité et al. [55] showed that a uremia level of 1.2 mmol·L^{-1} is indicative of considerable undernutrition in N that could lead to a drop in performances. In the present trial, the cellulolytic activity of the rumen was estimated at 52 %, based on the degradation of soya-bean hulls DM after 24 h residence in the rumen. This value is far below the estimates of 60 and 70 % obtained by Delagarde et al. [10] on unfertilized swards (ruminal ammonia: 2.7 mmol·L^{-1}) and fertilized swards (ruminal ammonia: 10.4 mmol·L^{-1}), respectively. The low cellulolytic activity is reflected in the low digestibility of NDF (0.69). In a stall-feeding experiment with lactating dairy cows fed on fresh perennial ryegrass, Peyraud et al. [39] also observed a decrease of NDF digestibility (78 to 72 %) by lowering N fertilization and CP content of grass (15 to 11 %).

4.2. Effect of carbohydrate supplementation

The carbohydrate supplementation (wheat or maize–soya-bean hulls) had no influence on the amount of herbage ingested. Such additivity between herbage and concentrates is not observed when grazed herbage of good quality is offered ad libitum, grass intake being reduced by the supply of concentrate [2, 34]. The additivity between herbage and concentrate observed in the present study could be linked to the low level of DM, energy and N intake, below intake capacity and energy and protein requirements [13, 15]. By modifying the amounts of herbage offered, many authors have shown that the degree of substitution increases as a function of the intake level of unsupplemented cows [15, 20, 27, 32]. In all the previously mentioned studies, the intake capacity of unsupplemented cows receiving low amounts of herbage was not attained because these animals ingested 2–3 kg OM less than cows receiving large

amounts of herbage. Phillips et al. [41] obtained similar results with lambs during two indoor trials. In their study, these authors reported no effect of concentrate input on herbage intake during the winter when intake levels were low (65 $g \cdot kg^{-1}$ $LW^{0.75}$). Nevertheless, they reported a reduction in herbage intake with concentrate supplementation during the spring when intake levels were high (80 $g \cdot kg^{-1}$ $LW^{0.75}$) despite the lowering of herbage quality.

In the present study, the herbage intake did not vary with the nature of the carbohydrate concentrate. This result is in agreement with the data obtained from dairy cows indoors [44, 47] and at pasture [14]. In contrast, Meijs [31] and Kibon and Holmes [27] observed a lower herbage intake when feeding concentrate based on rapidly degradable starch than with a concentrate rich in cell-wall material. According to stall-based studies carried out by Stakelum et al. [49] and Dillon et al. [12], this effect is associated with a modification of fermentations in the rumen, with generally lower OM and NDF digestibility [1, 53, 54]. A decrease of ruminal pH, an increase in starch availability in the rumen and competition between amylolytic and cellulolytic bacteria for essential substrates can occur [16]. However, other studies have shown that the supplementation of fresh grass with degradable cereals at small doses (3 kg [9]) or even at high doses (8 kg [34]) does not necessarily produce digestive perturbations in the rumen. In the present trial, wheat or maize–soya-bean hulls supplementation caused no modification of ruminal fermentations with respect to the unsupplemented control and no effect on NDF digestibility. This indicates that the digestive interactions were very weak with both of these concentrates with no consequence on fibre digestion.

4.3. Effect of protein supplementation

The replacement of carbohydrate concentrates by protected soya-bean meal has made it possible to increase the amount of ingested herbage. This positive effect of N supplementation on grass intake is clearly attributable to the low CP content of the grass. It has been observed previously with poor quality forages [18, 19, 25, 50], but only to a small extent with green forage, probably because the CP content in fresh grass is currently higher than in conserved forages.

At pasture, with high CP content of herbage, CP content of the concentrate has generally no effect on herbage intake. Jennings and Holmes [23] showed no effect of 14 vs. 9 % digestible CP in the concentrate on the herbage intake by dairy cows grazing on perennial ryegrass–white clover swards (25 % CP). Jones-Endsley et al. [24] also reported no significant effect on grass intake comparing 16 and 12 % CP in the concentrate in high-yielding dairy cows grazing on alfalfa–orchardgrass swards (24 % CP). Similar results where obtained by Hamilton et al. [17] on kikuyu pasture (15 % CP) comparing 0 and 30 % of cottonseed meal in a barley-based concentrate given at the rate of 3 kg daily. However, the range of CP content in the concentrate studied by these authors was quite low. Delagarde et al. [10] did not observe any reduction in herbage intake by providing 2 kg of protected soya-bean meal to cows grazing N-fertilized grass (21 % CP), even though a slight increase occurred in the amount of ingested herbage with low level of N fertilization (grass with 14 % CP). With lactating ewes stall-fed with fresh grass (16 % CP), Penning et al. [35] obtained the same degree of substitution by providing either barley, soya-bean meal or fish meal. In the above trial, the OMD of the diet was, on average, higher by 3–4 U in ewes receiving high-protein concentrates than in ewes receiving either barley or no supplement at all. On the basis of three trials carried out on young bullocks at pasture and indoors (grass with 20 % CP), Vadiveloo and Holmes [52] demonstrated that increasing the CP content of the concentrate from 13 to 21 % has no effect on herbage intake, but that supplementation with a

slowly degradable protein-rich concentrate with 30 % CP tends to raise the herbage intake when compared with a cereal-based concentrate.

Most of these studies at pasture were undertaken with the same method for estimating the amount of herbage intake, which involved using chromic oxide as a marker, and evaluating a single value for herbage digestibility and applying it to all the treatments (determined in vitro or based on faecal indicators). This assumption for the purposes of calculation implies the absence of digestive interactions. However, when a protein-rich concentrate is used to supplement a diet deficient in N, numerous studies have shown a clear improvement in forage digestibility, as confirmed more recently by Hannah et al. [18] and Peyraud et al. [40]. To our knowledge, only a few studies at pasture have taken account of the possibility of positive digestive interactions in calculating the herbage intake [5, 28]. This was done by measuring the in vitro digestibility for each treatment, using the rumen juices of animals during trials as an inoculum. Using young bullocks showing no deficiency in ruminal degradable N (ammonia: 7.1 $mmol \cdot L^{-1}$), Krysl et al. [28] were unable to observe any effect of soya-bean meal supplementation either on the in vitro digestibility of grass (11 % CP), on the ruminal pH, or on the ruminal and total digestibilities of NDF. On the contrary, Caton et al. [5], using grass with 8 % CP under conditions of strong deficiency in ruminal degradable N (ammonia: 1.2 $mmol \cdot L^{-1}$), observed that supplementation with cottonseed meal brought about a significant rise (+3 U) in the in vitro digestibility of herbage as well as an increase in the rate of disappearance of NDF in bags. For supplementation studies at pasture, the use of internal markers [14, 24] may also be a relevant method. In the present trial, the calculation method made it possible, at least in part, to take account of the effect of concentrate on the digestibility of herbage. According to the assumptions made for the purposes of calculation, the OMD of herbage was increased by 1.9 U when cows received protected soya-bean meal. This result is in good agreement with the calculated variation of 5.5 U in NDF digestibility.

The whole set of digestive measurements in the rumen (ammonia, VFA, pH and cellulolytic activity) support the increase in the intensity of ruminal fermentations with SBM compared with the two other concentrates. An analysis of the kinetics of ruminal parameters reveals that fermentations were more intense for SBM throughout the day, but particularly in the morning before the arrival of animals into the paddock, i.e., a long time after cessation of grazing at 22:00–23:00 h. Thus, the SBM concentrate did not show a short-term action after each distribution, but it rather enabled an increase in the base level of ruminal fermentary activity. It is likely that the N in protected soya-bean meal, being slowly degradable, is only slightly released into the rumen during the meal. More probably, this N could be re-released in a continuous way into the rumen by the recycling of blood urea.

Behavioural data broadly support the additivity between herbage and concentrate. In fact, neither the time spent grazing nor the rate of biting show any variation as a function of treatment. However, it should be noted that the increase in herbage intake with SBM is not reflected by any increase in the grazing time or in the rate of biting. Therefore, the rise in herbage intake with SBM could be linked with an increase in the intake rate via the bite weight. This result suggests a stronger motivation of the animal and stimulation of intake at the metabolic level via amount and quality of absorbed amino acids in addition to the positive effect of N on ruminal digestion [26, 45, 50].

The short-term milk production response to the treatments is in close agreement with the herbage intake results. In comparison with carbohydrate concentrates, the particularly well-marked effect of SBM on milk

production level, milk protein content, live weight and glycemia can be explained chiefly by the increase in nitrogenous inputs (+ 712 g PDIE·d^{-1}, corresponding to +43 %), but also by the increase in energy inputs (+2.5 UFL·d^{-1}, corresponding to +14 %). The positive effect of SBM on herbage intake found in the present trial on poorly fertilized swards can partly explain the relationship between milk production response and supplementation with concentrate containing protected soya-bean meal described in the study of Delaby et al. [8]. These latter authors have shown that, on poorly fertilized perennial ryegrass swards, the milk production response follows a strongly curved relation at low contents of protected soya-bean meal in the concentrate. The response is weak and linear in the case of well-fertilized swards.

5. CONCLUSION

The level of herbage intake by lactating dairy cows strip-grazing on N-poor perennial ryegrass swards was low mainly because of a strong ruminal degradable N deficiency. In such a situation, supplementation with carbohydrate concentrates did not reduce herbage intake with no modifications on ruminal fermentation parameters. The use of a protein-rich concentrate – even if slowly degradable – under such N-deficient conditions clearly involves an increase in ruminal fermentation activity, herbage OM digestibility and level of herbage intake.

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