**Original article**

# Effect of forage type on milk yield, chemical composition and clotting properties of milk

JB Coulon [1], P Pradel [2], I Verdier [3]

[1] *Laboratoire adaptation des herbivores aux milieux, INRA, Theix, 63122 Saint-Genès-Champanelle;*
[2] *Domaine de la Borie, INRA, 15190 Marcenat;*
[3] *Laboratoire de recherches fromagères, INRA, 36, rue de Salers, 15000 Aurillac, France*

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**Summary** — Fifty-four lactating multiparous cows were used in a 3 x 3 latin square trial. During each 3-week period cows were offered a diet composed either of perennial ryegrass silage (11 kg dry matter/d), or second-cutting cocksfoot hay (13 kg dry matter/d) or maize silage (11 kg dry matter/d). Each diet was supplemented with a mixture of barley, soya-bean meal and urea in proportions calculated to keep the overall rations isocaloric and isonitrogenous. The cows fed grass silage yielded, respectively, 0.4 ($P > 0.05$) and 1.9 kg/d ($P < 0.01$) more milk than those fed maize silage or hay, but their milk was significantly poorer in proteins and caseins. This result is linked to a distribution of ingested energy in favour of milk yield rather than the deposition of body reserves with the grass silage-based diets. The casein/protein ratio, calcium concentration, pH and rheological characteristics of the milk were not significantly different between the types of forages.

**dairy cow / coagulation property / milk composition / feeding**

**Résumé — Effet de la nature de la ration de base sur la composition chimique du lait et son aptitude à la coagulation.** *Cinquante-quatre vaches laitières multipares en milieu de lactation ont été utilisées dans un essai en carré latin 3 x 3. Pendant chaque période de 3 semaines, les animaux ont reçu une ration de fourrage isoénergétique, à base d'ensilage de ray-grass anglais (11 kg de matière sèche/j), de foin de seconde coupe de dactyle (13 kg de matière sèche/j) ou d'ensilage de maïs (11 kg de matière sèche/j). Chaque ration a été complémentée avec la même quantité d'un mélange d'orge, de tourteau de soja et d'urée dont les proportions ont été calculées de manière à ce que les apports azotés soient voisins et non limitants dans les 3 traitements. Les vaches recevant de l'ensilage d'herbe ont produit respectivement 0,4 (P > 0,05) et 1,9 kg/j (P < 0,01) de lait de plus que celles recevant de l'ensilage de maïs ou du foin, mais leur lait a été significativement plus pauvre en protéines et en caséines. Ce résultat est lié à un partage de l'énergie ingérée plus favorable à la production de lait* *qu'à la reconstitution des réserves corporelles avec la ration à base d'ensilage d'herbe. Le rapport caséines/protéines, la teneur en calcium, le pH et les caractéristiques rhéologiques du lait n'ont pas été significativement différents selon le type de ration de base.*

***vache laitière / aptitude à la coagulation / composition du lait / nature de l'alimentation***

## INTRODUCTION

The cheesemaking ability of milk is a complex notion encompassing different aspects, in particular chemical composition, clotting properties and capacity to produce a ripened cheese. The influence of production factors on this cheesemaking ability is currently a subject of much debate, particularly in the case of cheeses with "Appellation d'Origine Contrôlée" (AOC), for which the possibilities of modifying the raw material during processing are limited, if not forbidden. The influence of production factors (feeding in particular) on the chemical composition of milk (essentially fat and protein concentration) has been studied extensively (Journet and Chilliard, 1985; Rémond, 1985; Sutton, 1989; DePeters and Cant, 1992; Murphy and O'Mara, 1993). Amongst these studies, a certain number demonstrated that the type of forages could lead to differences in animal performances. However, in the majority of these studies, the effects could be attributed to modifications in nutrient supply, particularly energy, linked to a change in forage type (Roffler *et al*, 1967; Holter *et al*, 1976; Nicks *et al*, 1979; Bertilson and Bursted, 1983; Broderick, 1985; Coulon *et al*, 1985). Nevertheless, several trials revealed that for a same nutritive supply, grass silage-based rations lead, comparitively to hay-based or maize silage-based rations, to higher milk yields, due to a distribution of ingested energy more favourable to milk secretion than to buildup of body reserves (Vérité and Journet, 1971; Spörndly, 1989). Under these conditions, due to a dilution effect, the fat and protein concentrations are subsequently lower with grass silage-based rations (Rémond, 1978; Coulon and Garel, 1993). These modifications can affect both the ability of milk to coagulate, for which protein concentration is a determining factor (Remeuf *et al*, 1991; Macheboeuf *et al*, 1993; Martin and Coulon, 1995), and cheese characteristics.

The aim of this study, conducted with 3 different genetic types, was to describe and analyse performances in production, chemical composition and clotting properties of milk in cows fed, at identical levels of nutritive supply, a diet based on hay, grass silage or maize silage. The milk yielded by these animals was then used in the manufacturing of Saint-Nectaire cheeses. The results concerning the characteristics of these cheeses figure in another publication (Verdier *et al*, 1995).

## MATERIALS AND METHODS

### *Animals and feeding*

Fifty-four multiparous dairy cows, belonging to the breeds Holstein ($n$ = 18), Montbéliarde ($n$ = 18) and Tarentaise ($n$ = 18), were used. These animals had calved between 11 November and 10 January (5 December on average). In early lactation, all cows were fed a diet of grass silage supplemented according to INRA recommendations (1989). Three weeks prior to the beginning of the experiment, all animals were fed, in restricted quantities, a diet composed of second-cutting cocksfoot hay (H), perennial ryegrass silage (GS) and maize silage (MS), in equal proportions (*ie* around 4 kg dry matter [DM]/d of each forage). The characteristics of the different feeds are specified in table I. The trial began on 12 February. Three groups of 6 cows per breed were

**Table I.** Composition and nutritive value of forages.
*Composition chimique et valeur nutritive des fourrages.*

| | *Ryegrass silage* | *Cocksfoot 2nd cut hay* | *Maize silage* |
|---|---|---|---|
| Dry matter (DM, %) | 21.7 | 85.2 | 32.1 |
| Organic matter (g/kg DM) | 913 | 911 | 949 |
| Crude protein (g/kg DM) | 163 | 104 | 92 |
| Crude fiber (g/kg DM) | 291 | 303 | 214 |
| Organic matter digestibility | 0.74 | 0.67 | 0.70 |
| UFL (/kg DM) | 0.92 | 0.77 | 0.86 |
| PDIN (g/kg DM) | 98 | 65 | 55 |
| PDIE (g/kg DM) | 83 | 80 | 65 |
| pH | 3.9 | | 4.0 |
| $NH_3$-N (% total N) | 6.5 | | 8.7 |

formed on the basis of production performances of the 3 pre-experimental weeks and of the genetic variant of the κ casein. Three experimental periods, each of 3 weeks, were then conducted, during which each group was successively fed the 3 types of forage. To avoid digestive disorders, 1 kg of hay was incorporated into the maize silage-based ration. Throughout each period, the quantities of each forage provided were calculated so that energy supplies were identical. They were, respectively, 11, 13 and 11 kg DM/d for groups GS, H and MS. The various diets were supplemented with a mixture of barley, soya-bean meal and urea. The quantities of concentrate given were determined for the whole experiment, so as to cover the maintenance and milk production requirements monitored over the preexperimental period, according to INRA recommendations (1989). These quantities remained unchanged for any given animal throughout the experiment, and from one group to the other. The respective proportion of each ingredient in the concentrate differed according to the type of forage, so that the nitrogen supply levels were as close as possible and non-restrictive. On average, concentrate was composed of 90% barley and 10% soya-bean meal in GS and H treatments, and of 75% barley and 25% soya-bean meal in MS treatment. One hundred and fifty g/d of urea was added in H and MS treatments, and 100 g/d in GS treatment. Changes from the different diets were carried out over 3 d. On 25 April, all animals were again fed the preexperimental mixed diet, for a 2-week period. Throughout the entire experiment, the cows were given 200 g/d of a mineral additive (6P-22Ca).

### *Samples and measurements*

The quantity of milk produced was individually weighed after each milking and the fat and protein concentrations were determined 5 d a week, at each milking (infrared method). Concentrate and forage intakes were measured individually every day. The dry matter content of the forages was measured 5 d a week. DM content of silage was corrected to allow for DM losses during oven-drying according to Dulphy and Demarquilly (1981). The chemical composition of the forages was determined 3 times during the trial, as were the digestibilities of dry and organic matter, by using groups of 4 wether sheep per forage type during measurement periods of 1 week after a 2-week adaptation period. These data enabled the calculation of the nutritive value of the forages (feed unit for lactation [UFL] and protein digested in the small intestine when energy [PDIE] or nitrogen [PDIN] is not limiting in the rumen), according to INRA recommendations (Andrieu and Demarquilly, 1987) (table I). The cows were weighed once a week, on the Tuesday, at the beginning of the afternoon.

Once in each period, in an average sample of the evening milking of the 6 cows belonging to each group (whose milk would be used in the manufacturing of Saint-Nectaire cheese [Verdier *et al*, 1995]), milk pH (at 20°C), milk protein concentration and urea concentration (colorimetric method using dimethyl amino benzaldehyde) were measured. Clotting properties (rennet clotting time, curd firming time, curd firmness) were also measured, using a Formagraph apparatus according to the method proposed by McMahon and Brown (1982). Milk (10 ml) was added with 200 μl of a 13 mg activated chymosin/l rennet solution. Measurements were performed at the milk natural pH, then after pH standardization at 6.60 by addition of a few drops of a 10% lactic acid solution. All measurements were duplicated. Total bacterial count (method NF V08051), somatic cell count (Somacount, Bentley), butyric spore count (Cerf and Bergère, 1968) and coliform count (identified on desoxycholate lactose agar [Leifson, 1935]) were measured in each of the average milk samples. As the levels of these different counts were always low, none of the samples were discarded. During the last week of each period, individual milk samples were taken at the morning milking for the analysis of casein (Rowland's method [1938]) and calcium (flame emission method [International Organization for Standardization, 1987]) concentrations.

Energy and nitrogen balance were calculated from the difference between food input and the animals' requirements, according to INRA recommendations (Andrieu and Demarquilly, 1987; Vermorel, 1989; Vérité and Peyraud, 1989). Energy supply was corrected for the decrease in diet energy value resulting from the increased feeding level and higher proportion of concentrate in the diet (Vermorel, 1989). The energy content of the milk produced was calculated from its fat and protein concentrations according to the formula proposed by Sjaunja (1989). The energy value of live-weight gain was estimated as 4.5 UFL per kg (Chilliard *et al*, 1987).

### *Analysis of results*

The analysis of results was carried out using the mean data of the last week of each experimental period. These data were processed by analysis of variance (SAS, 1987). Fixed effects included in the model were the type of forage, breed, animal (nested within breed) and period, as well as breed x treatment interaction. In the absence of significant breed x treatment interaction, only results concerning the type of forage are presented.

## RESULTS

As planned, the energy and nitrogen supplies were never limiting (table II). Throughout the trial, the animals showed positive energy and nitrogen balances. The energy and nitrogen supplies were, however, slightly higher in groups MS and H because of a slight overestimation of the PDIE value of grass silage and a lower ingestion than forecasted (10 *vs* 11 kg DM/d), essentially due to a slight overestimation of DM content of the offered feed. The passage from one type of diet to another was not accompanied by marked refusals of feed.

Cows fed grass silage (GS) yielded 0.4 ($P > 0.05$) and 1.9 kg/d ($P < 0.01$) more milk than those fed maize silage (MS) or hay (H), respectively. Their milk was significantly poorer in proteins and caseins ($P < 0.01$) than those belonging to groups MS and H. The proportion of caseins in the proteins was identical between all 3 rations (82%). The animals fed maize silage presented higher fat concentration than those fed grass silage or hay (+1.4 and +1.7 g/kg, respectively, $P < 0.01$). Overall, the yield of milk solids was similar for groups MS and GS, and about 10% lower for group H (table II). The calcium concentration of the milk was similar between all 3 treatments. The urea concentration of the milk was high, and greater ($P < 0.01$) in group GS in comparison to MS and above all H.

The pH of the milk was identical in all 3 treatments, as were the majority of its rheological characteristics (table III). The clotting time of the milk at natural pH was, however, slightly shorter with the hay-based diet ($P < 0.05$), and the firming time at standardized pH slightly shorter with the maize silage-based diet ($P < 0.05$).

The cows fed hay gained more weight than those fed grass silage or maize silage (+284, +48 and −57 g/d, respectively, $P < 0.05$).

## DISCUSSION

This trial confirmed the beneficial effect of grass silage-based diets on milk production to the detriment of milk solid concentration, as already reported relative to hay (Journet and Dulphy, 1973; Burstedt and Lingvall, 1977; Spörndly, 1989; Coulon and Garel, 1993) or maize silage (Vérité and Journet, 1971). In this trial, differences between milk production of the grass silage diet and the

**Table II.** Food intake, milk production and live-weight change data.
*Quantités ingérées, production laitière et variations de poids vif.*

| | *Ryegrass silage* | *Cocksfoot 2nd cut hay* | *Maize silage* | *RSD*[1] |
|---|---|---|---|---|
| *Intake* | | | | |
| Forage intake (kg DM/d) | 10.0 | 12.4 | 11.4[2] | 0.6 |
| Concentrate intake (kg DM/d) | 4.9 | 4.9 | 5.0 | 0.2 |
| Energy supply (UFL/d) | 14.1[a] | 14.6[b] | 14.7[b] | 0.6 |
| PDIN supply (g/d) | 1635[a] | 1534[b] | 1623[a] | 51 |
| PDIE supply (g/d) | 1386[a] | 1536[b] | 1443[c] | 47 |
| *Balances* | | | | |
| Energy balance (UFL/d) | 0.8[a] | 2.1[b] | 1.3[c] | 0.6 |
| PDIN balance (g/d) | 326 | 316 | 315 | 60 |
| PDIE balance (g/d) | 77[a] | 318[b] | 135[c] | 59 |
| Live-weight change (g/d)[3] | 48[ab] | 284[a] | –57[b] | 673 |
| *Milk production* | | | | |
| Milk yield (kg/d) | 18.9[a] | 17.0[b] | 18.5[a] | 1.0 |
| Fat concentration (g/kg) | 40.0[a] | 39.7[a] | 41.4[b] | 1.8 |
| Protein concentration (g/kg) | 31.3[a] | 31.8[b] | 32.2[c] | 0.9 |
| Casein concentration (g/kg) | 25.4[a] | 26.2[b] | 26.2[b] | 1.0 |
| Casein:protein ratio [4] | 0.82 | 0.82 | 0.82 | 0.01 |
| Fat yield (g/d) | 756[a] | 676[b] | 766[a] | 43 |
| Protein yield (g/d) | 588[a] | 538[b] | 593[a] | 35 |
| Calcium concentration (g/kg) | 1.30 | 1.31 | 1.33 | 0.08 |
| Urea concentration (mg/100 ml)[5] | 41.3[a] | 30.2[b] | 34.0[c] | 3.5 |

[1] Residual standard deviation. [2] Included 0.8 kg DM of hay (see Materials and Methods). [3] Calculated between the 1st and the 3rd week of each period. [4] This ratio was computed by using the protein content of the milk sample used for casein content determination (30.8, 31.9 and 31.9 g/kg in groups GS, H and MS, respectively). This protein content was slightly different from that computed on the samples collected during the last week of each period, which figures in the table. [5] Measured on pooled samples. [abc] Values with different superscripts within rows differ significantly ($P < 0.05$).

*[1] Écart type résiduel. [2] Y compris 0,8 kg MS de foin (voir Matériel et méthodes). [3] Calculé entre la 1re et la 3e semaine de chaque période. [4] Ce rapport a été calculé en utilisant le taux protéique de l'échantillon utilisé pour la détermination du taux de caséine (respectivement 30,8, 31,9 et 31,9 g/kg dans les lots GS, H et MS). Ce taux protéique est légèrement différent de celui qui figure dans le tableau et qui est la moyenne des valeurs relevées au cours de la dernière semaine de chaque période. [5] Mesurée sur des échantillons poolés. [abc] À l'intérieur de chaque ligne, les valeurs suivies d'une lettre différente sont significativement différentes (P < 0,05).*

**Table III.** Clotting properties of milk.
*Aptitude à la coagulation du lait.*

| | *Ryegrass silage* | *Cocksfoot 2nd cut hay* | *Maize silage* | *RSD*[1] |
|---|---|---|---|---|
| Protein concentration[2] (g/kg) | 33.3[a] | 33.1[a] | 34.5[b] | 0.8 |
| pH[2] | 6.73 | 6.72 | 6.72 | 0.02 |
| *At natural pH* | | | | |
| Rennet clotting time (min) | 12.2[a] | 11.4[b] | 12.1[a] | 0.8 |
| Curd firming time (min) | 5.9 | 5.2 | 5.0 | 0.7 |
| Curd firmness (mm) | 35.2 | 36.2 | 34.6 | 5.5 |
| *At standardized pH (6.60)* | | | | |
| Rennet clotting time (min) | 10.1 | 9.9 | 10.0 | 0.6 |
| Curd firming time (min) | 4.4[a] | 4.4[a] | 4.1[b] | 0.4 |
| Curd firmness (mm) | 33.7 | 33.0 | 32.8 | 6.1 |

[1] Residual standard deviation. [2] Of the mean sample on which rheological characteristics were measured. [ab] Values with different superscripts within rows differ significantly ($P < 0.05$).
*[1] Écart type résiduel. [2] De l'échantillon moyen sur lequel les caractéristiques rhéologiques ont été mesurées. [ab] À l'intérieur de chaque ligne, les valeurs suivies d'une lettre différente sont significativement différentes (P < 0,05).*

other 2 diets were probably underestimated, and that of protein concentration overestimated, because of the slightly lower energy supply in the grass silage ration, relative to hay and maize silage rations. Indeed, energy supply is an important factor of variation of milk protein concentration, even when requirements are covered (Coulon and Rémond, 1991). This effect of the type of diet on animal performances can be explained 1) by a dilution effect of milk solids secreted; this is the case between the maize silage-based and the grass silage-based diets, for which the yields of secreted solids are identical, although the concentrations are significantly different; 2) by a different distribution of energy between milk and body reserves. Although it is always difficult to calculate variations in liveweight over short periods, our results showed that animals fed hay gained more weight than those fed grass or maize silage. In the grass silage group, 58% of the energy intake was excreted into milk, compared with 51% in the hay group and 56% in the maize silage group.

The reasons for the differences in the dilution of milk solids and in energy distribution according to type of forage have not yet been clearly established. They may be linked first to the balance in end products of ruminal digestion (Saama *et al,* 1993): diets producing a high acetic/propionic ratio in the rumen are generally beneficial to milk yield, and detrimental to weight gain and protein concentration (Ekern and Reid, 1963; Vérité and Journet, 1971; Thomas, 1984a). The reverse is true when this ratio is low (Rémond and Journet, 1972). Diets with a high acetic + butyric/propionic ratio are generally beneficial to fat concentration (Journet and Chilliard, 1985). Secondly, the observed differences may originate from differences in nitrogen supply of the diets. A high level of nitrogen supply may favour milk production (Vérité and Peyraud, 1989) without modifying fat and protein concentration (Rémond, 1985). Such a hypothesis is unlikely in this trial where PDIE supplies were slightly lower in group GS than in group H. Thirdly, the metabolic utilization of the nutriments could be different, due to nitrogen nutrition of cows (Vérité and Journet, 1975). A low proportion of essential amino acids (lysine and methionine) in total PDIE can affect protein concentration without modifying milk yield and fat concentration (Rulquin *et al*, 1993). However, in this trial, this hypothesis cannot be retained: methionine supply, computed according to INRAtion software (Coulon and Faverdin, 1995) was similiar in the 3 diets (1.84% PDIE) and lysine supply was higher in the GS group (6.94% PDIE) than in the H (6.89% PDIE) or MS (6.91% PDIE) groups. Finally, a specific effect of the species cropped might have occurred (Dionne *et al*, 1981; Murphy and O'Mara, 1993) with or without altering the composition of the volatile fatty acid mixture in the rumen (Thomas, 1984b). The beneficial effect of the maize silage-based diet on milk fat concentration is thus well-established (Hoden *et al*, 1985). It is linked both to the relative richness in fatty acids of this forage and to the ruminal fermentations which it induces, favouring the synthesis of short or medium chain milk fatty acids. In the same way, an increase in milk yield to the detriment of body reserves and fat concentration was observed in the changeover from a ryegrass-based diet to a cocksfoot-based (Demarquilly, 1963) or white clover-based diet (Thompson *et al*, 1985).

The type of basal diet supplied had no significant effect on either the pH of milk or its rheological characteristics, in agreement with our former observations (Coulon and Garel, 1993; Coulon, 1995). This lack of effect between the GS and H groups is partly due to the small differences in milk protein concentration of the average samples on which the rheological measurements were carried out. Milk protein concentration is, indeed, an essential factor of the variation in firming time and in curd firmness (Remeuf *et al*, 1991; Martin and Coulon, 1995). When the change in diet type is accompanied by a large modification in milk protein concentration, its clotting ability is also modified (Grandison *et al*, 1985). Nevertheless, in our study, the maize silage-based diet, whose protein concentration was the highest, did not show a much greater ability to coagulate than the others.

Considering the forages selected, our study does not allow the differentiation of the respective effects of storage method (silage or hay) and of type of flora (ryegrass or cocksfoot) on the characteristics of the milk. It does reveal, however, that besides the well-known quantitative factors (nutritive supply level of the diet), qualitative factors can significantly modify these characteristics. Comparisons conducted with identical forages cropped in the form of silage or hay should enable the clarification of the roles of these different factors.

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