

The effect of feeding sequence on fat concentration in milk*

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Summary — Eighteen multiparous dairy cows in mid-lactation were used in an experiment with a 2 x 2 latin-square design. During each 3-week period, the cows were offered a diet composed of a restricted amount of hay supplemented with a mixture of barley and soya-bean meal (ground and pelleted), given 1 h either after (HC treatment) or before the hay (CH treatment). The concentrate represented 50% of the total dry matter in the diet. The feeding sequence had no effect on the resultant milk yield (20.2 and 20.4 kg/day, respectively, in HC and CH treatment) or milk composition (respectively, 38.2 and 38.2 g/kg for fat concentration and 28.7 and 28.8 g/kg for protein concentration). Except for some slight modifications in ruminal digestion, the feeding sequence had no significant effect on the dairy cow performances. It is possible that the concentrate proportion in the diet was not high enough to elicit a low fat concentration under the CH treatment.

dairy cow / feeding sequence / milk composition

Résumé — Effet de l'ordre de distribution des aliments sur le taux butyreux du lait. Dix-huit vaches Holstein et Montbéliardes en pleine lactation ont été utilisées dans un schéma en inversion avec deux périodes successives de 3 semaines. Au cours de chaque période, les vaches ont reçu un foin de prairie naturelle distribué en quantité limitée 1 heure avant (traitement HC) ou 1 heure après (traitement CH) la distribution du concentré (présenté sous forme broyée et agglomérée). Celui-ci a représenté en moyenne 50% de la ration. La séquence de distribution des aliments n'a eu d'effet significatif ni sur la production laitière (respectivement 20,2 et 20,4 kg/jour pour les traitements HC et CH) ni sur la composition chimique du lait (respectivement 38,2 et 38,2 g/kg pour le taux butyreux et 28,7 et 28,8 g/kg pour le taux protéique). Malgré quelques légères modifications digestives, la séquence de distribution des aliments de la ration à l'auge a donc eu peu d'effet sur les performances zootechniques des vaches laitières. Il est possible que la proportion de concentré dans la ration n'ait pas été suffisamment élevée pour que le traitement CH entraîne une baisse du taux butyreux.

vache laitière / séquence de distribution / composition du lait

* The results of this work were presented during the first days of the 3R in Paris, 1 December 1994.

INTRODUCTION

Milk fat concentration varies in response to a number of dietary factors (Journet and Chilliard, 1985; Sutton, 1989; Hoden and Coulon, 1991), the main ones being the type of feed and the ratio of forage to concentrate in the diet. When the proportion of concentrate in the diet (on a dry matter basis) exceeds 50%, its type (percentage of starch), physical form (ground, rolled or pelleted), method of distribution (alone or combined with forage) and the number of daily meals may affect the milk fat concentration (Gibson, 1984; Coulon et al, 1989; Robinson, 1989). The effects of these factors are linked to: i) changes in the molar percentage of volatile fatty acids (VFA) in the rumen (Sutton, 1981), from which acetate and butyrate are the precursors for the short- and medium-chain fatty acids of milk fat; ii) increased lipids in the diet, which with body lipids are the only source of the long-chain fatty acids in milk; iii) changes in the plasma insulin postprandial peak, induced by a significant variation in VFA supply from the rumen (Sutton and Morant, 1989). These factors generally explain most of the variation in milk fat concentration observed among farms (Agabriel et al, 1993b). Nevertheless, such differences are, in some cases, not always accounted for. Agabriel et al (1993a) observed wide variations in milk fat concentration in a group of top genetic level farms that were homogeneous for the main variation factors of fat concentration. These variations were linked to particular feeding practices, especially the grain processing and feeding sequence (Coulon et al, 1994). In practice, feeding hay before the concentrate has been a recommended strategy to prevent low milk fat results. The aim of this strategy is to promote salivation, to increase the ruminal buffering capacity and to limit the changes in ruminal pH that are associated with the ingestion of rapidly fermented feeds (McLeod et al, 1994). However, the experimental data are limited and contradictory (Voight et al, 1978;

Giacomini et al, 1985; Nocek, 1992; McLeod et al, 1994). The aim of the study was to determine the effects of the feeding sequence (between hay and concentrate) on milk fat concentration in dairy cows fed diets containing 50% concentrates.

MATERIALS AND METHODS

Cows and treatments

Ten Holstein and eight Montbeliarde cows at on average 56 days of lactation at the beginning of the trial were used in this study. These 18 cows (4 primiparous and 14 multiparous) were housed in individual litter stalls and milked in the milking parlor at 6:00 and 15:30. In early lactation, all cows received a diet containing hay from native mountain grassland (*ad libitum*) supplemented with a concentrate according to INRA (1989) recommendations. The trial started on 21 February 1994. The quantities of concentrate given were determined for the whole experiment so as to cover the maintenance and milk production requirements determined by monitoring over the 2 weeks preceding the trial, according to INRA (1989) recommendations. This concentrate was ground and pelleted and composed of soya bean (15%) and barley (85%). To maintain a concentrate:forage ratio close to 50:50 for each cow, the quantities of hay offered (composition given in table I) were adjusted to those of concentrates. High yielding cows received 12 kg dry matter (DM)/day of hay, and low yielding cows received 10 kg DM/day of hay. The diets remained unchanged throughout the experiment. Hay and concentrate were distributed in two meals per day, in the same trough, but not mixed. During a preexperimental period (PP), one-third of the hay was given at 7:30 and the rest at 17:30. The concentrate portion was distributed evenly in each feeding at 8:30 and 16:30. The first experimental period (P1, 3 weeks) started on 7 March. Two groups of nine cows were formed based on parity, date of calving and milk yield and composition over the preceding 2 weeks. During P1, nine cows received the concentrate at 7:30 and 16:30 and the hay 1 h after (CH treatment), while nine other cows received the hay at 7:30 and 16:30 and the concentrate 1 h after (HC treatment). During the second period (P2, following 3 weeks), the treatments were reversed. The feeding sequences were

Table 1. Food composition.

	<i>Hay</i>	<i>Barley</i>	<i>Soya meal</i>
DM (%)	86.0	86.9	88.3
DM basis			
Organic matter (%)	92.8	97.4	92.7
Crude protein (%)	10.4	12.1	52.0
Crude fibre (%)	35.1	5.0	7.0
Organic matter digestibility (%)	54	na ¹	na ¹
Energy value (UFL ² /kg DM)	0.60	1.16	1.17
PDIN value (g/kg DM) ³	65	79	371
PDIE value (g/kg DM) ⁴	70	102	254

¹ Not available; ² UFL: feed unit for lactation (INRA, 1989); ³ PDIN: truly digestible protein in the small intestine when energy in the rumen is not limiting (INRA, 1989); ⁴ PDIE: truly digestible protein in the small intestine when the amount of degraded N in the rumen is not limiting (INRA, 1989).

changed abruptly in 1 day. Throughout the experiment, the cows received 200 or 300 g/d (according to their yield) of a mineral additive (6% P and 22% Ca) containing trace elements.

Measurements

The quantity of milk produced was weighed individually at each milking and samples were taken individually for fat and protein concentration from both the morning and evening milkings, 2 days/week every week during the trial, and 5 days/week during the last week of P1 and P2, and the first week of P2. Ruminal fluid from every cow was sampled at 11:00 during the last week of periods P1 and P2, by abdominal puncture with a transcutaneous probe. The pH was measured immediately and a sample was stored frozen for VFA measurement (Jouany, 1982). The individual daily consumption of concentrate and hay were determined during all phases of the trial. On 2 days during the second week of periods P1 and P2, the hay intakes in HC treatment were measured just before the morning concentrate distribution. Feeding behaviour was observed on 2 days during the last week of periods P1 and P2, for 3 h following distribution of the first meal, in the morning and in the afternoon. Individual observations on each cow were made every 5 min to determine the time needed to eat all of the concentrate offered. The DM content of the foods was determined every

day for hay and once a week for concentrates. The chemical composition of the hay was determined twice in the course of the trial. The DM and organic matter digestibilities of the hay were determined twice using six wether sheep during 1-week measurement periods after a 2-week adaptation period. The characteristics of the foods are shown in table 1. Their nutritional value was computed according to the INRA equations as established by Andrieu and Demarquilly (1987).

Statistical analysis

The analysis of the effect of the feeding sequence on cow performance was performed using the results of the final week of periods 1 and 2. These data were processed by analysis of variance (SAS, 1987). The fixed effects included in the model were treatment (concentrate before or after hay), breed, cow (nested within breed) and period. In the absence of any breed x treatment interaction, the results presented concern only the effect of treatment.

RESULTS

During the trial, no health disorders were observed, no acidosis in particular. Hay DM intakes for HC and CH treatments were

Table II. Data concerning milk production, food intake and rumen characteristics.

Group	HC	CH	RSD	Significance
Milk yield (kg/d)	20.2	20.4	1.2	NS
Fat concentration (g/kg)	38.2	38.2	3.0	NS
Protein concentration (g/kg)	28.7	28.8	0.6	NS
Hay intake (kg DM/d)	9.0	8.6	0.7	NS
Concentrate intake (kg DM/d)	9.1	9.1	0.1	NS
Energy supply (UFL/d)	14.3	14.1	0.5	NS
Energy balance (UFL/d)	0.7	0.4	0.4	*
Rumen pH	5.71	5.49	0.31	*
VFA (mol/100 mol)				
Acetic acid	67.1	67.7	2.8	NS
Propionic acid	17.0	14.3	3.2	*
Butyric acid	12.3	14.2	2.1	*
Isobutyric acid	0.68	0.58	0.08	**
Valeric acid	1.39	1.82	0.70	NS
Isovaleric acid	1.22	0.93	0.32	NS
Caproic acid	0.40	0.47	0.18	NS

HC: hay before concentrate; CH: concentrate before hay; RSD: residual standard deviation; VFA: volatile fatty acids; NS: not significant. * $P < 0.05$; ** $P < 0.01$.

similar (table II). DM concentrate intakes were 9.1 kg/day for both treatments. For the HC treatment, the hay DM intake during the first hour after distribution was 2.1 kg/day. The time needed to eat all of the concentrate offered was slightly longer in the CH than in the HC treatment (18 vs 15 min, respectively, $P < 0.01$). Five cows systematically refused more than 2 kg DM of hay per day in both treatments. During the first 2 h following hay distribution, the time these cows spent eating hay was very short (39 vs 64 min for the other cows).

The feeding sequence did not significantly influence either milk yield (20.2 and 20.4 kg/day, respectively, in the HC and CH treatments), or milk composition (38.2 and 38.2 g/kg fat concentration, and 28.7 and 28.8 g/kg protein concentration, in the HC and CH treatments, respectively) (table II). The milk fat concentration did not differ

between the five cows that systematically refused the hay and the others (38.2 vs 38.0 g/kg), although the proportion of concentrate in their diet was higher (58 vs 49%). Between the end of period P1 (the last 4 days) and the beginning of period P2 (the first 4 days), the change in the feeding sequence was not associated with any significant modifications of milk yield and composition.

The pH of ruminal fluid was low for both treatments, particularly for the CH treatment (5.49 vs 5.71, $P < 0.05$). Whatever the treatment, the longer the time the cow spent eating the concentrate, the lower the pH of the ruminal fluid ($P < 0.01$) (fig 1).

The treatment had no important effect on the VFA concentrations (table II). Acetic acid (C2) concentration was high and similar in the two treatments (67%). During the HC treatment, propionic acid (C3) concentration

was higher (17.0 vs 14.3, $P < 0.05$) and butyric acid (C4) was slightly lower (12.3 vs 14.2, $P < 0.05$) than during the CH treatment.

Individual variations in milk fat concentration were linked to the time spent eating the concentrate ($R = -0.41$, $P < 0.05$) and to the ratio of (C2 + C4) to C3 ($R = 0.50$, $P < 0.01$). These variables together explained 39% of the variability in the milk fat concentration.

DISCUSSION

In this trial, the feeding sequence had no effect on animal performance, which is in agreement with the results of similar studies (Giacomini et al, 1985; Nocek, 1992; McLeod et al, 1994). In fact, the high milk fat concentration under the CH treatment was more surprising than the lack of effect of the HC treatment.

Although our experimental conditions were favourable to a decrease in milk fat concentration under CH treatment (high proportion of concentrate based on barley, ground and pelleted, and offered in two

meals), such a decrease did not occur. Thus, the absence of effect of the HC treatment is not surprising. Indeed, the effects of feeding practice are generally sensitive only in the presence of low milk fat concentration (Gibson, 1984; Sutton and Morant, 1989) because of a high proportion of concentrate in the diet (Sutton et al, 1985; Coulon et al, 1989), a low fibre content or an excessive grinding of the ration (Grant et al, 1990). It is possible that in our trial, the concentrate proportion in the diet was not high enough (and the crude fibre content of the ration not low enough) to elicit a low milk fat concentration under the CH treatment.

Even if some differences appeared between treatments in the ruminal fermentations, it is likely that they were not of great biological importance. The time of ruminal sampling did not correspond exactly at the same digestion stage in the two treatments, and it is possible that, over 24 h, the quantities of acetic, propionic and butyric acid were similar for both treatments. This might explain the lack of effect of experimental treatments on the milk fat concentration. Thus, the ratio of (C2 + C4) to C3, which is considered to be a good indicator of variation in milk fat concentration (Journet and Chilliard, 1985; Oldham and Sutton, 1979), was high, greater than 3.5 in 33 of the 36 samplings. In our study, the milk fat concentration variations did not seem to be affected by this ratio (Journet and Chilliard, 1985) as confirmed by the wide variability of milk fat concentration when this ratio was higher than 4 (fig 2). The low values of rumen pH were consistent with the time of ruminal sampling (3 h 30 min after the distribution of the first feed, corresponding with low pH [Sutton, 1981]) and with the sampling location in the rumen: in the dorsal sack, where pH is lower than in the ventral sack (-0.3 to -0.4 units) (Lampila and Poutianen, 1966; Brugère et al, 1990). The feeding sequence sometimes modifies the ruminal fluid pH, as in our experiment, or the molar percentages of VFA (Voight et

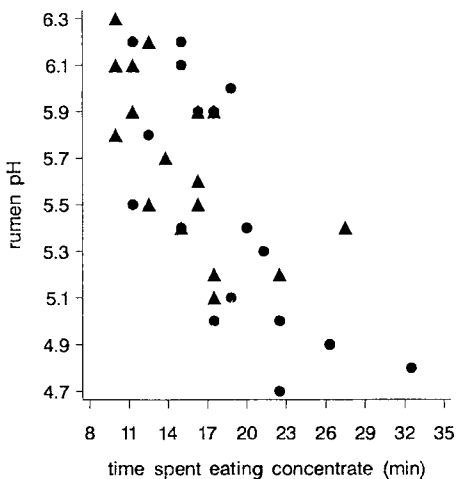


Fig 1. The relationship between ruminal pH and time spent eating the concentrate. HC treatment (▲), CH treatment (●).

al, 1978; Nocek, 1992) without altering the fat concentration of the milk. Therefore, other factors are necessarily involved (Sutton and Morant, 1989). The negative link between ruminal fluid pH (or fat concentration) and the time spent eating the concentrate illustrates the difficulty of interpreting this type of results. These relationships are surprising. In this case, peculiar digestive or metabolic characteristics may result in both a low pH (and low fat concentration), and a long time spent eating the concentrate, as observed by Rémond (1969) with high concentrate rations. It is also possible that a longer time spent eating involves a more rapid liberation of highly degradable compounds in the rumen, because of a higher grinding of particules during mastication, and a rapid decrease in pH.

The results of this trial did not confirm the farm observations (Coulon et al, 1994), possibly because of the experimental conditions in which it was carried out (McLeod et al, 1994). In our trial, the two groups of

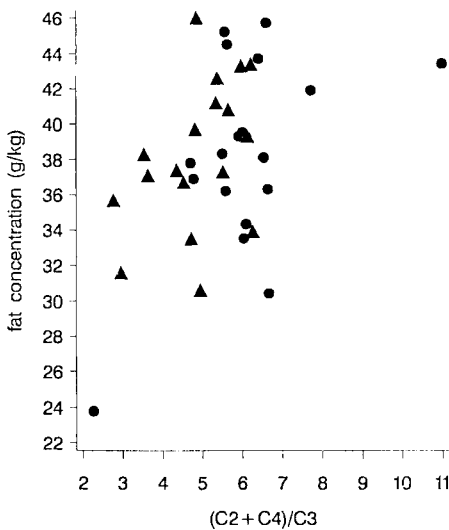


Fig 2. The relationship between milk fat concentration and the ratio of (C2 + C4) to C3 in the ruminal fluid. HC treatment (▲), CH treatment (●).

cows were housed near each other and one group could have influenced the eating behaviour of the neighbouring cows (Dulphy et al, 1980). Cows may be stimulated to salivate when feed is offered to adjacent cows, as has been observed for sheep (Denton, 1957). This illustrates the difficulty of experimentally reproducing certain conditions observed on farms (Robinson, 1989).

Finally, except under extreme conditions that are rarely observed in farms, feeding practices have no systematic effects on milk fat concentration. Such effects are difficult to analyse because of the great complexity of variation factors (and of interactions with animal characteristics) that could influence the milk fat concentration (Sutton, 1989). Contradictory results on the effects of feeding frequency (Gibson, 1984; Robinson, 1989; Yang and Varga, 1989; Klusmeyer et al, 1990) or of the presentation of concentrate (Coulon and Agabriel, 1995) on milk fat concentration have already illustrated this complexity. Further experimental studies should be conducted in order to explain the variations of milk fat concentration around medium values usually observed in practice (36–42 g/kg) and to establish reliable recommendations.

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REFERENCES

- Agabriel C, Coulon JB, Marty G, Bonaïti B (1993a) Changes in fat and protein concentrations in farms with high milk production. *J Dairy Sci* 76, 734-741
- Agabriel C, Coulon JB, Marty G, Bonaïti B, Boniface P (1993b) Effets respectifs de la génétique et du milieu sur la production et la composition du lait de vache. Étude en exploitations. *INRA Prod Anim* 6, 213-223

- Andrieu J, Demarquilly C (1987) Valeur nutritive des fourrages : tables et prévisions. *Bull Tech CRZV Theix, INRA* 70, 61-74
- Brugère H, Champy R, Polack B, Rousseau JF (1990) Examen du jus de rumen et surveillance de l'alimentation des bovins. Apports de la titrimétrie. *Rec Méd Vét* 166, 133-142
- Coulon JB, Faverdin P, Laurent F, Cotto G (1989) Influence de la nature de l'aliment concentré sur les performances des vaches laitières. *INRA Prod Anim* 2, 47-53
- Coulon JB, Agabriel C, Brunschwig G, Muller C, Bonaïti B (1994) Effects of feeding practices on milk fat concentration for dairy cows. *J Dairy Sci* 77, 2614-2622
- Coulon JB, Agabriel C (1995) Effet de la forme de présentation de l'orge sur la production et la composition du lait de vache. *Ann Zootech* 44, 247-253
- Denton DA (1957) A gregarious factor in the natural conditioned salivary reflex of sheep. *Nature* 16, 341-344
- Dulphy JP, Rémond B, Thériez M (1980) Ingestive behaviour and related activities in ruminant. In: *Digestive physiology and metabolism in ruminants* (Y Ruckebusch, P Thivend, eds), MTP Press Ltd, Lancaster, UK, 103-122
- Giacomini DG, Clark JH, Vicini JL (1985) Effect of sequence of feeding on ruminal fermentation, milk yield and milk composition. *J Dairy Sci* 68 (suppl 1) 1342 (abstr)
- Gibson JP (1984) The effect of frequency of feeding on milk production of dairy cattle: an analysis of published results. *Anim Prod* 38, 181-191
- Grant RJ, Colenbrander VF, Albright JL (1990) Effect of particule size and rumen cannulation upon chewing activity and laterality in dairy cows. *J Dairy Sci* 73, 3158-3164
- Hoden A, Coulon JB (1991) Maîtrise de la composition du lait : influence des facteurs nutritionnels sur la quantité et les taux de matières grasses et protéiques. *INRA Prod Anim* 4, 361-367
- Institut National de la Recherche Agronomique (INRA) (1989) *Ruminant nutrition. Recommended allowances and feed tables* (R Jarrige, ed), INRA and John Libbey Eurotext
- Jouany JP (1982) Volatile fatty acid and alcohol determination in digestive contents, silage juices, bacterial cultures and anaerobic fermentor contents. *Sci Aliments* 2, 131-144
- Journet M, Chilliard M (1985) Influence de l'alimentation sur la composition du lait. 1. Taux butyreux : facteurs généraux. *Bull Tech CRZV Theix, INRA* 60, 13-23
- Klusmeyer TH, Cameron MR, McCoy GC, Clark JH (1990) Effects of feed processing and frequency of feeding on ruminal fermentation, milk production and milk composition. *J Dairy Sci* 73, 3538-3543
- Lampila M, Poutiainen E (1966) Systematic differences in the composition of the bovine rumen fluid between different parts of the rumen. *Ann Agric Fenn* 5, 279-297
- MacLeod GK, Colucci PE, Moore AD, Grieve DG, Lewis N (1994) The effects of feeding frequency of concentrates and feeding sequence of hay on eating behaviour, ruminal environment and milk production in dairy cows. *Can J Anim Sci* 74, 103-113
- Nocek JE (1992) Feeding sequence and strategy effects on ruminal environment and production performance in first lactation cows. *J Dairy Sci* 75, 3100-3108
- Oldham JD, Sutton JD (1979) Milk composition and the high yielding cow. In: *Feeding strategy for the high yielding dairy cow* (WH Broster, H Swan, eds), London, Granada, 114
- Rémond B (1969) Influence d'un apport croissant d'orge sur le comportement alimentaire de la vache laitière et la digestion. *Ann Zootech* 18, 55-64
- Robinson PH (1989) Dynamic aspects of feeding management for dairy cows. *J Dairy Sci* 72, 1197-1209
- Statistical Analysis System Institute (SAS) (1987) *SAS user's guide: statistics*. SAS Institute Inc, Cary NC, USA
- Sutton JD (1981) Concentrate feeding and milk composition. In: *Recent advances in animal nutrition* (W Haresign, ed), Butterworths, London, UK, 35-48
- Sutton JD (1989) Altering milk composition by feeding. *J Dairy Sci* 72, 2801-2814
- Sutton JD, Morant SV (1989) A review of the potential of nutrition to modify milk fat and protein. *Livest Prod Sci* 23, 219-237
- Sutton JD, Broster WH, Napper DJ, Siviter JW (1985) Feeding frequency for lactating cows. Effects on digestion, milk production and energy utilization. *Br J Nutr* 53, 117-130
- Voight J, Patkowski B, Krwielitzke R (1978) The effect of the order of roughage and concentrates in feeding on carbohydrates digestion and bacterial protein synthesis in the rumen of the dairy cow. *Arch Tierernaehr* 28, 67-76
- Yang CMJ, Varga GA (1989) Effect of 3 concentrate feeding frequencies on rumen protozoa, rumen digesta kinetics and milk yield in dairy cows. *J Dairy Sci* 72, 950-957