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Riboflavin secretion in cow's milk varies according to diet composition and season

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Abstract. In ruminants, B-vitamin supply from the diet and synthesis by the rumen microflora is considered sufficient to avoid deficiency. Nevertheless, factors affecting their concentrations in milk have never been studied in-depth, even if dairy products are among the best contributors of several vitamins (especially riboflavin) in human nutrition. We compared, during a year, riboflavin concentrations in bulk tank milks from 20 groups of 5 commercial farms divided among production systems mainly characterized by the forage nature (grass or maize silage). Riboflavin concentrations were greater in milks from grass-based than maize silage systems (1.91 *vs* 1.70 mg.L^{-1}, P<0.001). Moreover, they increased during summer (from 1.71 to 1.99 mg.L^{-1},P<0.001) whatever the feeding system. These differences were largely explained by a dilution effect due to the level of the milk yield but the mean amount of riboflavin secreted in milk per cow per day was higher when cows were fed a diet rich in maize silage by comparison to a herb-based diet (42.5 *vs* 33.4 µg.d^{-1}, respectively, P< 0.001). Conversely to what should be thought, riboflavin concentrations in milk are submitted to variations, and more interestingly regulations, that influence the global nutritional quality of milk.

Keywords. Cow milk – Vitamin – Feeding system – Season.

La sécrétion de riboflavine dans le lait de vache varie selon la composition de la ration et la saison

Résumé. *En raison de leur double origine alimentaire et microbienne ruminale, la disponibilité en vitamines B chez les ruminants est considérée pouvoir couvrir les besoins de ces animaux. C'est la raison pour laquelle, la variabilité de leurs concentrations dans le lait n'a jamais été explorée, même si le lait et les produits laitiers sont parmi les premiers contributeurs de certaines vitamines (dont la riboflavine) en nutrition humaine. Nous avons déterminé les concentrations en riboflavine dans des laits de tournées simulées correspondant à 20 groupes de 5 fermes commerciales caractérisés par leur système fourrager (basé sur l'herbe ou l'ensilage de maïs). Les concentrations de riboflavine étaient supérieures dans les laits issus des systèmes à base d'herbe par rapport à ceux utilisant préférentiellement le maïs (1,91* vs *1,70 mg.L^{-1}, P<0,001). Elles ont augmenté également pendant l'été quel que soit le système considéré (de 1,71 à 1,99 mg.L^{-1}, P<0,001). Ces différences s'expliquent principalement par un effet de dilution lié à la quantité de lait produite ; cependant, la quantité moyenne de riboflavine sécrétée dans le lait par vache et par jour était supérieure lorsque les vaches recevaient une ration à base d'ensilage de maïs que lorsqu'elles recevaient une ration à base d'herbe (respectivement 42,5* vs *33,4 µg.j^{-1}, P<0,001). Contrairement au concept couramment admis, la concentration de riboflavine du lait est variable, et de manière encore plus intéressante, elle semble être régulée, ce qui impacte la qualité nutritionnelle globale du lait et des produits laitiers.*

Mots-clés. *Lait de vache – Vitamine – Système d'alimentation – Saison.*

I – Introduction

For decades, availability of B vitamins has been globally considered as sufficient to cover the estimated requirements of dairy cows (except vitamins B_5 and B_9) thanks to supply from the diet and/or from synthesis by rumen microorganisms (National Research Council, 2001). However, as milk production levels considerably increased through bovine genetic selection and optimised feed efficiency, it is obvious that cow requirements for B vitamins, which are involved in the main metabolic pathways (cell respiration, energy production, nucleic acid synthesis, amino-acid metabolism and protein synthesis, neoglucogenesis...), increased proportionally (Girard *et al.*, 2010). To date, the relationship between performance levels and B vitamins requirements has not been studied. Proofs that B vitamin availability to lactating cows would be lower than needs are insufficient even though some experimental results suggested it. For example, increasing B vitamin supply (especially B_9 and B_{12}) to lactating dairy cows has beneficial effects, including on milk performance (Girard *et al.*, 2010). Moreover, it was also observed that their dietary supplementation raised their concentration in milk (Graulet *et al*., 2007). This latter observation was interesting from a nutritional point of view, because milk and dairy products are good sources of vitamin A, B_{12} (cobalamins) and B_2 (riboflavin) (Coudray, 2011). However, the factors regulating concentration of B vitamins in milk have not been fully characterized. In a first publication, we reported that the highest levels of milk vitamin B_9 and vitamin B_{12} were generally associated with grass- and maize silage-based diets, respectively (Chassaing *et al*., 2011). In the present work, we compared riboflavin secretion in milks obtained from different production conditions varying in term of diet composition, season or altitude.

II – Materials and methods

The study was carried out in 20 groups of five farms divided among four production systems mainly characterized by their forage system and altitude: feeding systems based on grass in Mountain (GM) or in Lowland (GL) and maize silage feeding systems located in Mountain (MM) or in Lowland (ML). In GM and GL, forages were mainly hay during the wintering period and fresh grass during the grazing period. The proportion of maize in cow diets was lower in MM than in ML whatever the period of the year. It was particularly marked in summer when the proportion of maize silage was extremely reduced in MM at the benefit of pasture / grass silage or grass silage / hay combination. In each group of farms, bulk tank milk was sampled five times in the course of the year 2008 at key times in animal feeding patterns: twice in the over-wintering period with diets based on preserved forage (January and February), and three times during the grazing period: in May, July and September. During the week of each milk sampling, a survey was carried out to record herd characteristics, performance and feeding. Average diets for each group were described on the basis of proportions of forages and concentrate in the diet calculated from the declared quantities the farmers dispensed. When the amount of one feed ingested was unknown (such as pasture), the estimation of the amount ingested was based on the energy requirements. The energy balance was supposed equal to zero. Milk riboflavin was extracted in duplicate by successive acid hydrolysis and enzymatic treatments using papain and acid phosphatase. Vitamin concentrations were measured in the extracts using a Acquity UPLC equipped with a 150 x 2.1 mm HSS T3, 1.8-µm column (Waters, France) and fluorescence detection (λ_{exc} = 400 nm; λ_{em} = 520 nm). Data were processed by Anova using the MiniTab16 software introducing the production system, period, and the interaction into the model as fixed effects. Because the characteristics of the production systems changed between periods, especially the diet composition, period was not considered a repeated factor.

III – Results and discussion

Riboflavin concentrations in the hundred milks studied varied from 1.51 to 2.15 mg.L^{-1} with a mean value of 1.78 ± 0.02 mg.L^{-1}. As for vitamin B_9 (Chassaing *et al*., 2011), riboflavin concentrations were greater in milks from grass-based than maize silage systems (1.91 *vs* 1.70 mg.L^{-1}, P<0.001, Fig. 1A). However, the interaction between diet composition and altitude was significant (P<0.01) because riboflavin concentrations were the highest in milks produced in lowland grass-based systems whereas the lowest values were observed in maize silage based systems also in lowland. Whatever the production systems, riboflavin concentrations increased during summer (from 1.71 to 1.99 mg.L^{-1}, P<0.001). Values observed in maize silage systems were in agreement with food tables (Graulet *et al*., 2013). However, riboflavin concentrations in milks produced in a grass-based system were higher (+14%), reinforcing the nutritional value of these milks.

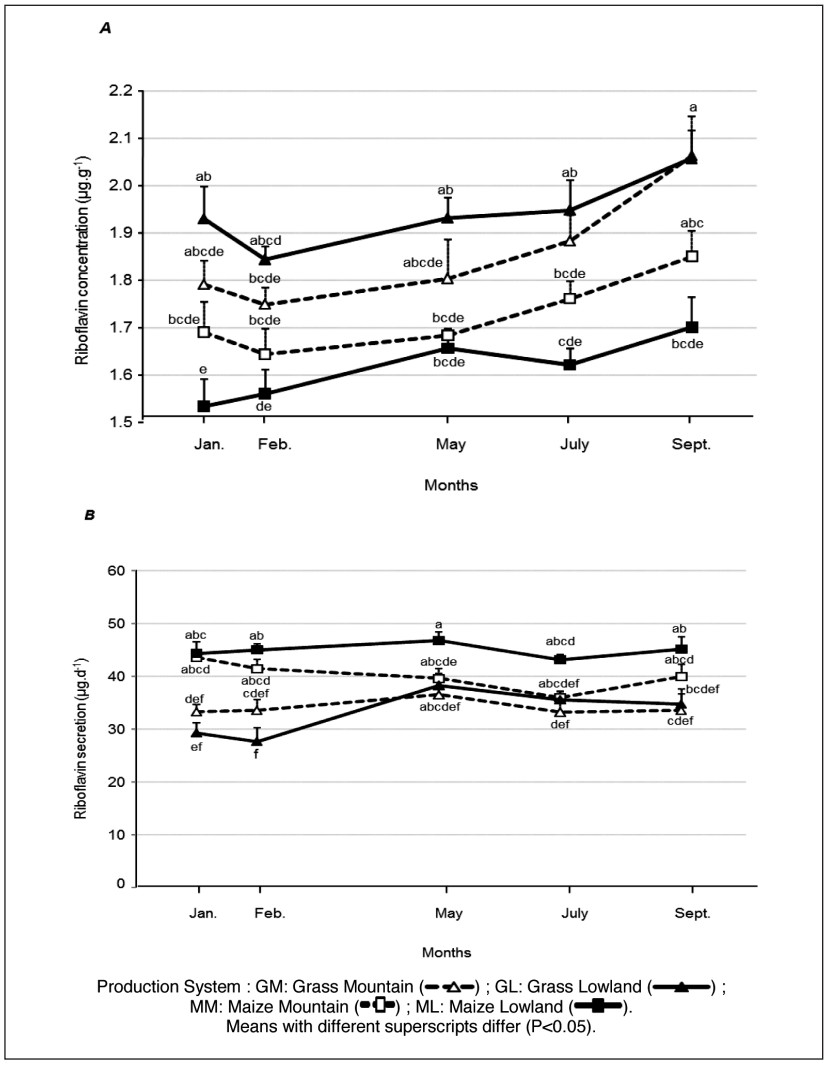

Fig. 1. Variations in milk riboflavin concentration (A) and secretion (B) according to feeding system, altitude and season.

Riboflavin secretion in milk per day and per cow was estimated for each system using the corresponding mean milk yield. Milk production levels were largely higher in maize-silage than in grass-based systems, especially in winter and in lowland. Consequently, the amount of riboflavin secreted in milk per day per cow was higher in maize- than in grass-based systems (42.5 *vs* 33.4 $\mu g.d^{-1}$, respectively, $P< 0.001$, Fig.1B). However, it was almost stable during the year and equivalent between lowland and mountain systems. Thus, variations in riboflavin concentrations in milk were largely explained by a dilution effect due to the higher milk yield in maize based-systems. The effects of season and altitude on the total amount of riboflavin secreted in milk are negligible. However these data suggest that riboflavin supply is greater for cows fed maize silage than for cows fed grass-rich diets, as illustrated by the amounts of vitamin secreted by the mammary gland. Of course, riboflavin could be provided to cows directly by the diet or indirectly by the influence of the diet on rumen fermentations, that is to be studied through complementary experiments. Finally, this last observation raise a concern about the adequacy of riboflavin supply to cows fed grass-rich diets and its role as a limiting factor for production efficiency.

IV – Conclusions

These results complete those obtained on folates (B_9) and vitamin B_{12} (Chassaing *et al*., 2011) confirming that milk concentrations of B vitamins vary according to diet and seasons even though complementary studies are needed for the other B vitamins and to better understand the underlying mechanisms. More interesting is the fact that riboflavin concentrations in milks produced by cows fed grass-rich diets especially in summer are higher than reference values reported in tables, reinforcing the interest of milk as a riboflavin source in human nutrition in general and giving an added value to milks produced in grass -based systems.

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