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Milk characterization: effect of the breed

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Abstract

It has previously been shown that it is possible to distinguish milks from different geographical locations and feeding diets. The ^{18}O enrichment values measured by isotope ratio mass spectrometry appeared to be the most discriminant parameter for both the geographic origin and the diet. The aim of this study is to examine the influence of the breed of cow on the isotopic enrichments of milk water when the animals were kept in the same environment and fed on the same diet. The present results indicate that the breed of cow influences the isotopic enrichments of milk. The effect is, however, of small magnitude and unlikely to diminish the capacity of $\delta^{18}O$ measurements to discriminate between different diets and production sites.

Keywords: Milk; Lipids; Water; Isotope ratio mass spectrometry (IRMS); Authentication; Breed; Geographic origin; Feeding diet

1. Introduction

Consumers are, for many reasons, asking for information about the food they are purchasing. This is especially the case for animal products, where BSE has raised fears. The producers also want to be able to prove that Protected Designation of Origin (PDO) procedures have been respected.

We have previously shown that it is possible to discriminate between the sites where animals were raised and the food they consumed on the basis of the isotopic enrichment of body water and the ^{13}C NMR spectrum of fat (Renou et al., 2004). These parameters are influenced by the geographic origin of the animal, which combines the influences of the type of food it was fed and the isotopic enrichment of its drinking water. Using such tools, it was possible to classify 100% of animals according to the production site.

One further question is whether the breed of the animal influences the isotopic enrichment of body and milk water. Although no information is available about any such effect, the isotopic enrichment of body water could theoretically be modified by factors other than food and drinking water (Ritz, Cole, Couet, & Coward, 1996); (Ritz, Cole, Davies, Goldberg, & Coward, 1996). Indeed, energy expenditure and the relative contributions of carbohydrate, fat and protein to energy production influence the isotopic composition of body water. This water is in isotopic equilibrium with CO_2 (Coward, Ritz, & Cole, 1994) and CO_2 production varies with energy expenditure and the respiratory quotient (a measure of

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the relative contributions of carbohydrate and fat oxidation to energy metabolism). As CO_2 passes through the alveoli, fractionation of ^{18}O results in a progressive enrichment of the body water as the energy expenditure and respiratory quotient increase.

Therefore, the aim of this study was to examine the influence of the breed of cow on the isotopic enrichment of milk water when the animals were kept in the same environment and fed on the same diet.

2. Materials and methods

2.1. Milk samples

Cows in mid-lactation were kept under the same conditions at the INRA (Institut National de la Recherche Agronomique) site of Marcenat in the Massif Central. The cows (6 per breed) were fed on a hay-based diet and were of 5 breeds: Holstein, Montbéliarde, Tarentaise, Limousine and Salers. Milk was collected each week for 4 weeks in April 1999.

The 120 milk samples (20 ml) were immediately frozen and stored at −20° prior to analysis. After thawing, rennet was added to full milk and the samples were left overnight at room temperature. Milk water was collected by filtration and its ^{18}O enrichment was determined by gas chromatography-isotope ratio mass spectrometry (IRMS) (VG Isochrom-μgas, VG Isotech, Cheshire, UK). Samples (1 ml) were placed in 10 ml vacutainers (100 × 16 mm, Becton Dickinson sterile vacutainers with no additive), previously filled with a 5% CO_2/He gas mixture at atmospheric pressure. The vacutainers were then placed in a shaker and equilibrated at 25 °C for a minimum of 10 h, by which time at least 99% equilibrium had been reached. Results were expressed as the isotope ratio in ppm relative to that of International Standard Vienna Mean Ocean Water (SMOW) :

$$\delta = \left(\frac{R_S}{R_{SMOW}} - 1\right) \times 10^3, \quad (1)$$

where R_S and R_{SMOW} are the heavy to light isotope ratios in the sample and SMOW, respectively. The $^{18}O/^{16}O$ isotope ratio in SMOW is 2005.2 ppm.

2.2. Data analysis and statistics

The software STATISTICA V.5.5 (Statsoft, France) was used for data analysis.

3. Results and discussion

The ^{18}O enrichments of milk according to the breed and sampling time are shown in Table 1 and the mean value for all milks agrees with previous results (Renou et al., 2004). The highest enrichments were observed for the Salers and Limousine breeds, while the Tarentaise displayed the lowest values during these 4 weeks. According to a *Newman–Keuls* test, the *Tarentaise* breed differed significantly from the *Limousine* and *Salers* breeds over this period, whereas there was no significant difference between the latter two breeds, or between the *Montbéliarde* and *Holstein* breeds (Table 2). Although the enrichment values for week 2 were significantly higher than those for weeks 1, 3 and 4, these differences were small (maximum 2 ppm between Salers and Tarentaise cows).

Table 1
$\delta^{18}O$ values for milk samples from the different breeds during the 4 week study

	Tarentaise	*Montbéliarde*	*Limousine*	*Salers*	*Holstein*	All breeds	Drinking water
Week 1	−9.79 ± 0.29	−9.61 ± 0.41	−9.28 ± 0.18	−9.13 ± 0.17	−9.44 ± 0.46	−9.45 ± 0.38	−10.57
Week 2	−9.60 ± 0.37	−9.49 ± 0.31	−8.67 ± 0.46	−8.78 ± 0.25	−9.14 ± 0.35	−9.13 ± 0.50	−10.71
Week 3	−9.92 ± 0.56	−9.71 ± 0.31	−9.07 ± 0.30	−9.00 ± 0.34	−9.65 ± 0.35	−9.47 ± 0.52	−10.75
Week 4	−10.17 ± 0.49	−9.77 ± 0.21	−9.01 ± 0.38	−8.80 ± 0.18	−9.41 ± 0.25	−9.43 ± 0.59	−10.61
Weeks 1–4	−9.87 ± 0.46	−9.64 ± 0.32	−9.01 ± 0.39	−8.93 ± 0.27	−9.41 ± 0.38	−9.37 ± 0.51	

Table 2
Newman–Keuls test for $\delta^{18}O$ values

	F	*p*	*Tarentaise*	*Montbéliarde*	*Limousine*	*Salers*	*Holstein*
Week 1	3.9	0.01000000	a	ab	b	b	ab
Week 2	8.1	0.00020000	a	a	b	b	ab
Week 3	6.8	0.00070000	a	a	b	b	a
Week 4	17.8	0.00000050	a	c	b	b	c
Weeks 1–4	28.6	0.00000002	a	c	b	b	d

Different letters indicate a significant difference between breeds ($p < 0.05$). Fisher's, *F*; Probability *p*.

These results were compared to those obtained for milk from *Holstein* cows fed on silage diet during the same period (Renou et al., 2004). The $\delta^{18}O$ for a silage diet was -7.85 ± 0.37 and was always significantly different from the values for milks from cows fed on a hay-based diet. Hence, the diet effect was more important than the breed effect, although still of small magnitude here (maximum 2 ppm between the two diets for the same breed). We have previously shown that the diet of cows of the same breed can alter the ^{18}O enrichment of their milk by 6–10 ppm, while the location where they are kept can modify it by 3–9 ppm (Renou et al., 2004).

These cows consumed the same diet for 4 weeks and drank the same water and the isotopic enrichments of food and water are the major determinants of the isotopic composition of body water (Ritz et al., 1996). On the other hand, the animals were of the same age but differed in the time elapsed since their last pregnancy. The Salers and Limousine cows still had calves aged 8–12 weeks, whereas the other breeds were milked twice a day (time from delivery 4.5 months). These small differences in physiology could influence the isotopic enrichment of the milk, notably through isotopic fractionation along the pathways of CO_2 production and/or water evaporation. Variations in milk production rate can induce small metabolic changes, due to behavioural adaptation (a changing energy expenditure) and/or adaptation of the energy metabolism (affecting fat mass, fat oxidation and hence the respiratory quotient). Changes in the respiratory quotient will affect CO_2 production, resulting in more or less fractionation of ^{18}O during passage through the alveoli. Changes in energy expenditure can not only directly affect CO_2 production but also alter metabolic water production and, as water is evaporated through the mucosa, skin or lungs, there is isotopic fractionation of ^{18}O. Finally, it may be speculated that ^{18}O is fractionated in the udder between body and milk water and that different milk production rates could directly modify the ^{18}O enrichment.

4. Conclusion

This study shows that the breed of cow can influence the isotopic enrichments of milk, even in circumstances where the food and water consumed are similar. The effect however, is of small magnitude and unlikely to diminish the capacity of $\delta^{18}O$ measurements to discriminate between different diets and production sites. These results now have to be confirmed by a study involving different geographic sites and diets of the animals and the IRMS data will need to be compared with physiological measurements.

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