



## Diet authentication in sheep from the composition of animal tissues and products<sup>1</sup>

Sophie Prache<sup>1</sup>

<sup>1</sup> Institut National de la Recherche Agronomique, INRA, UR1213 Herbivores, Site de Theix, F-63122 Saint-Genès-Champanelle, France.

**ABSTRACT** - There is currently an increased consumer demand for information on herbivore production factors, particularly animal diet. To meet these demands, producers and commercial entities develop specifications via quality certifications. There is therefore a need for analytical tools that may guarantee that the specification commitments have been fully met or to help with constructing them. The present paper reviews the current state of knowledge concerning diet authentication in sheep meat and milk, the different approaches that have been investigated, some leading examples concerning the discrimination of contrasting feeding situations, together with the persistence of some diet markers in the event of changes in animals' diet. The nature of the diet strongly influences the composition of the animal tissues and products, which is due to specific compounds that are directly transferred from the feed to the end product or that are transformed or produced by rumen micro-organisms or the animal's metabolism under the effect of specific diets. Some of these compounds can therefore be used as diet markers. Compounds such as carotenoids, phenolic compounds, fatty acids, volatile compounds and ratios of oxygen, hydrogen, carbon and nitrogen stable isotope are potential tracers in meat and milk or animal tissues of animal feeding diets. Moreover, differences in meat and milk composition induce differences in their optical properties, and therefore in their spectral features, which can also be used for diet authentication. These techniques have already allowed discrimination among products obtained in contrasting feeding conditions. Intermediate situations, for example in case of modification of the animal's diet, may be less easily recognized and may require a combination of tracing methods. In particular, the persistence of tracers when animals are stall-fed a concentrate-based diet after pasture and its implications for traceability are discussed. Finally, further directions for research are highlighted.

Key Words : authentication, diet tracers, meat, NIR spectroscopy, pasture-feeding, reflectance

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**RESUMO** - Atualmente, há um aumento crescente na procura dos consumidores por informações dos fatores produtivos em herbívoros, particularmente na dieta animal. Para elucidar essa procura, produtores e entidades comerciais desenvolvem especificações através de certificações de qualidade. Há ainda uma necessidade de ferramentas analíticas que podem garantir que os comitês de especificações tenham total conhecimento ou ajuda para a construção desses. Este trabalho é uma revisão do estado atual de conhecimento a respeito da autenticação da dieta na carne e no leite de ovinos. Das diferentes abordagens que têm sido investigadas, os principais exemplos dizem respeito à discriminação do contraste de situações alimentares, juntamente com a persistência de alguns marcadores na dieta com o objetivo de mudanças da alimentação animal. A natureza do alimento influencia fortemente a composição dos tecidos e dos produtos de origem animal. Isso é devido aos componentes específicos que são diretamente transferidos a partir do produto final ou que são transformados ou produzidos pelos microorganismos ruminais ou então pelo metabolismo de dietas específicas. Alguns desses componentes podem ainda serem usados como marcadores da dieta. Componentes, como os carotenóides, compostos fenólicos, ácidos graxos, compostos voláteis e os isótopos estáveis de oxigênio, hidrogênio, carbono e nitrogênio são marcadores potenciais na carne e no leite ou então nos tecidos dos animais. Além do mais, diferenças na composição da carne e do leite induzem às diferenças em suas propriedades ópticas e, além disso, e em suas características espectrais, que também pode ser usadas para a autenticação da dieta. Essas técnicas já permitem a discriminação entre produtos obtidos com as condições alimentares. Situações intermediárias, por exemplo, em caso de modificação da dieta animal, pode ser menos fácil reconhecer e pode requerer uma combinação de métodos utilizando marcadores. Em particular, a persistência de marcadores quando os animais são confinados com a base alimentar a partir de concentrado após permanência na pastagem e sua implicação para a traçabilidade é discutida. Finalmente, direções adicionais para pesquisa são destacadas.

Palavras-chave: alimentação a pasto, autenticação, carne, marcadores na dieta, NIRS, reflectância

## Introduction

Diet is one of the most important production factors affecting the composition of milk and meat from cattle, sheep and goats. The sensory and nutritional properties of meat from pasture-fed animals differ from those of grain-fed animals in flavour, colour and fatty acid composition (Rousset-Akrim et al., 1997; Priolo et al., 2002a; Aurousseau et al., 2004), and studies have demonstrated the nutritional advantages of meat produced from pasture-animals (Enser et al., 1998; Aurousseau et al., 2004; Wood et al., 2003). These effects are due to specific compounds that are directly transferred from the feed to the end-product or that are transformed or produced by ruminal micro-organisms or the animal's metabolism under the effect of specific diets. Some of these compounds can therefore be used as diet markers (Prache et al., 2005, Prache et al., 2007). There is currently a frequent demand in Europe for clear information on herbivore production factors, particularly the feeds supplied to animals, with an increasingly positive image attached to grassland-based food production, which is considered as natural, healthy and respectful of animal welfare. To meet these demands, producers and commercial entities have developed specifications for quality certifications (see Prache et al., 2007 for examples in dairy products and beef and lamb meat). The organizations managing product certification systems therefore have a need for control tools to be able to guarantee that the specification commitments have been fully met. The French lamb brand of "Agneau Pré-Salé" (salt-marsh lambs) provides a pertinent illustration of such specification commitments. The meat from these lambs is renowned for its particular flavour, which stems from the specific flora of these salt-marsh pastures which are regularly flooded by seawater. Producers commit to raise the lambs on the 'prés salés' for a minimum duration, to keep the concentrate supplementation below a predefined ceiling, and not to finish the lambs on concentrate diets indoors for longer than a certain maximum time. For scientists, these commitments lead to research questions such as identifying specific markers of these pastures, and more generally of pasture-feeding, investigating their latency of appearance and persistence in the animal tissues, and studying the dose-response curves relating marker concentrations in the animal tissues with intake levels. The ability to authenticate the feed given to animals has therefore become a major challenge in Europe for scientists, monitoring bodies, commercial entities and producers alike. Efforts have recently been made to develop analytical tools to quantify

specific compounds in the products or animal tissues that can act as diet markers. This review of recent developments deals mainly with authentication of feeding diet, although it also provides some insight into authentication of geographical origin. To date, much of this research in sheep has focused on pasture-based vs. concentrate-based diets.

### *Analytical tools for diet authentication*

Efforts have recently been made to develop analytical tools to authenticate the diet. The first approach investigated is the quantification of specific compounds in the animal's products or tissues whose presence or proportions are characteristics of the diet. The second approach is the use of fingerprints: actually, differences in the meat and milk composition induce differences in their optical properties, and therefore in their spectral features, which can also be used for diet authentication. To date, much of this research in sheep has focused on pasture-based vs. concentrate-based diets.

### *Molecular and atomic biomarkers*

Plant biomarkers are compounds that are not synthesized by animals and whose occurrence in the animal products or tissues is unambiguously due to the food they have eaten. Carotenoid pigments and terpenes, for example, are lipophilic plant micronutrients that are transferred into animal tissues and products after absorption and then recovered in milk and fat. Water-soluble micronutrients such as phenolic compounds have also proved useful tools for authenticating animal diet via milk.

### *Carotenoids pigments*

Carotenoids form the main group of natural pigments. Lutein is the only carotenoid stored in the fat of sheep, whereas cattle also (and mainly) store carotenes (Prache et al., 2003b; Nozière et al., 2006a). Carotenoid content in the animal tissues and products is highly dependent on dietary supply (Dian et al., 2007b). Carotenoid pigments are present at high levels in green leafy pasture (430 to 700 mg/kg DM for total carotenoids, 250 to 415 mg/kg DM for lutein, Prache et al., 2003a). The forage content decreases with drying and conservation duration proportionally to the degree of light exposure, since carotenoids are highly UV-sensitive. Sun-drying in the field therefore strongly decreases the carotenoid content of the forage: compared with the initial level in pasture, the concentration observed for wilted silage is about 60% (at 28% dry matter) to 30% (at 35% dry matter), 30% for haylage and 20% for hay (Nozière et al., 2006a). Dehydrated alfalfa contains 40 to 65% of fresh green

pasture levels (Dian et al., 2008). Maize silage is poor in these pigments (70-80 mg/kg DM), and zeaxanthin, the carotenoid present in maize seed, is not stored by ruminants. Most concentrates are very low in these pigments. Carotenoids have therefore been successfully proposed as markers of pasture-feeding in sheep (Prache and Theriez, 1999) and more generally in herbivore meat and milk (Prache et al., 2002; Serrano et al., 2006; Prache et al., 2007).

#### *Phenolic compounds*

Forages are also rich in cellular phenolic compounds (flavonoids and other phenolic compounds, FPC), which have recently proved their usefulness for diet authentication. Besle et al. (2005) compared six diets in cows: concentrates-rich (65% of the diet), based on maize silage, ryegrass silage, ryegrass hay, native mountain hay and native mountainous grazed pasture (+15% concentrates). The FPC content of the forages, as determined by HPLC, ranged from 0.8 to 8 g kg DM<sup>-1</sup>, with the native pasture by far the richest (Fraisie et al., 2007). About 60 FPC were detected in the milks; half were found in all the milks, but other compounds, that were specific to one diet, may be used for authenticating the animal diet. Experimental evaluation of these compounds is warranted for meat.

#### *Fatty acid composition*

Since animal diet can strongly affect the fatty acid composition of meat and milk (Wood & Enser, 1997; Wood et al., 2003; Aurousseau et al., 2004, in lamb; Martin et al., 2005 in dairy cows), the fatty acid composition of milk and meat may be a useful source of relevant information on the animal's diet. Alpha-linolenic acid (C18:3n-3) is a characteristic fatty acid of forage lipids and is not synthesized by mammals. Green pasture is rich in  $\alpha$ -linolenic acid, which typically ranges between 50-70% of total lipids (Aurousseau et al., 2004), and part of the dietary linolenic acid escapes ruminal hydrogenation. Grain-based diets contain high proportions of linoleic acid (C18:2n-6). Feeding pasture therefore results in higher concentrations of C18:3n-3 (and other n-3 PUFA) and lower concentrations of C18:2n-6 (and other n-6 PUFA) in muscle lipids than grain-based diets (Enser et al., 1998). The C18:2n-6/C18:3n-3 ratio in muscle phospholipids could therefore be of interest to discriminate pasture-fed from concentrate-fed animals. However, it should be noted that linseed is also very rich in  $\alpha$ -linolenic acid, and its inclusion in grain-based concentrates could affect the reliability of the discrimination based on the milk or meat fatty acid composition (Scollan et al., 2001, in beef; Cooper et al., 2004, in lambs). Further experiments are required to address this question.

#### *Volatile compounds*

The nature and the concentration of volatile compounds that are present in ruminant tissues and products are strongly influenced by the animal's diet (Vasta and Priolo 2006). These compounds come from a direct transfer from the diet or from the animal's metabolism or ruminal microorganisms. They are extracted using dynamic headspace, then analysed by gas chromatography-mass spectrometry (DH-GC-MS) (Priolo et al., 2004b).

Among these volatile compounds, numerous studies have focused on terpenes. Terpenes form a large class of molecules almost exclusively synthesized by plants, and they are transferred from the feed to the animal tissues and products (Priolo et al., 2004b, in lambs; Serrano et al., 2007, in beef; Martin et al., 2005, in dairy products). The terpene profile of the animal product is therefore modulated by the terpene profile of the diet, which in turn varies greatly according to the botanical family of the plants forming the diet (Mariaca et al., 1997). Terpenes contents in grassland plants actually vary widely according to botanical family, and dicotyledons are typically more terpene-rich than monocotyledons. Since natural grassland flora is a component of the "terroir" (and thus any relevant quality label), its terpene profile together with that of the corresponding animal product can therefore be characteristic of the geographical area. A number of studies have reported higher amounts and wider diversities of terpenes in the tissues and products of grazing animals than animals given conserved forages or concentrates (Suzuki & Bailey, 1985; Young et al., 1997; Priolo et al., 2004b and Prache et al., 2009 in lambs; Martin et al., 2005 in dairy products; Serrano et al., 2006 in beef), and terpenes have been used successfully in meat and milk as markers of animal diet (Cornu et al., 2001, in beef; Priolo et al., 2004b; Prache et al., 2009 in lamb; Martin et al. 2005, in milk). Moreover, these compounds have also been successfully used in meat from pasture-fed beef to discriminate between three geographical origins in France (Cornu et al., 2001); it should be noted, however, that these promising results have been obtained using animals that remained in the same geographical location from birth to slaughter at 24 months of age.

Other volatile compounds such as 2,3-octanedione and 3-methylindole (skatole) are typically higher in pasture-fed than in concentrate-fed lambs (Vasta & Priolo, 2006). Suzuki & Bailey (1985) found that the fat from pasture-fed lambs contained much more 2,3-octanedione than the fat from corn-fed lambs, and this result was confirmed in subsequent experiments (Young et al., 1997; Sebastian et al., 2003; Priolo et al., 2004b). Young et al. (1997) hypothesized that this

compound may originate from the action of lipoxygenase on linoleic and linolenic acids, both the enzyme and its substrate being abundant in green leafy tissue. Young et al. (1997) also reported that skatole was present at higher levels in fat from pasture-fed lambs than concentrate-fed lambs. This has been attributed to enhanced ruminal degradation of tryptophan, due to the typically high protein content and high protein/non-fibrous carbohydrate ratio of green pasture. However, it should be noted that condensed tannins are responsible for reduced protein degradability in the rumen, and may, as a consequence affect skatole biosynthesis (Roy et al., 2004).

#### *Isotopic composition*

The proportions of stable isotopes of some elements, such as oxygen ( $^{18}\text{O}/^{16}\text{O}$ ), hydrogen ( $^2\text{H}/^1\text{H}$ ), carbon ( $^{13}\text{C}/^{12}\text{C}$ ) and nitrogen ( $^{15}\text{N}/^{14}\text{N}$ ) are subject to natural variation. The reasons for these variations are well known and some of them may be of interest for authenticating the animal's diet or the geographical origin.

These variations may actually be linked to climatic and environmental conditions, to altitude and latitude, such as for  $^{18}\text{O}/^{16}\text{O}$  and  $^2\text{H}/^1\text{H}$  in water, which enables using the isotopic composition of the water in meat and dairy products to obtain useful information on the geographical origin of the corresponding products (Rossmann et al., 2000; Renou et al., 2004a, b). For example, Boner and Förstel (2004) discriminated beef samples originating from Argentina and Germany, by using  $^{18}\text{O}/^{16}\text{O}$  and  $^2\text{H}/^1\text{H}$  in meat water. Nevertheless, it should be noted that the isotopic composition of the water in herbivores products is subject to seasonal variations (Kornexl et al., 1997).

The natural variations of some stable isotopes proportions may also be linked to the vegetal's or the animal's physiology. For example, terrestrial  $\text{C}_4$  plants present a higher  $^{13}\text{C}$  proportion than  $\text{C}_3$  plants, because of different metabolic pathways during photosynthesis (Gebbing et al., 2004; Schmidt et al., 2005). In Europe, the utilization of maize, which is a  $\text{C}_4$  plant, in the diet, therefore results in high relative  $^{13}\text{C}$  contents in the animal tissues, whereas animal products from temperate grassland-based diets show lower  $^{13}\text{C}$  contents (Piasenter et al., 2003; Gebbing et al., 2004; Moreno-Rojas et al., 2008). However, the C isotope signature cannot reveal intensive fattening by use of other cereals due to similar photosynthetic pathways of most grassland plants and cereals, and it should be noted that tropical grasslands may also contain  $\text{C}_4$  plants. As far as nitrogen is concerned, the N stable isotope composition of plants is modulated by both the

botanical family, with less  $^{15}\text{N}$  enrichment of plant nitrogen compounds in leguminous plants that use nitrogen in the air as a nitrogen source and the mineral fertilisation, N fertilizer application increasing the  $^{15}\text{N}$  level of the nitrogen compounds in plants (Schmidt et al., 2005; Prache et al., 2009). The stable isotope ratios of nitrogen ( $^{15}\text{N}/^{14}\text{N}$ ) in the meat may therefore give some information on the intensification level of the pastures and crops used for animal feeding, and present some interest for identifying animal products coming from low-input systems (Schmidt et al., 2005).

The diet and the site where the animals are raised may thus influence the isotopic composition of the water, fat and protein of their tissues and products. In return, the analysis of the stable isotope composition of the animal tissues and products by isotope ratio mass spectrometry (IRMS) may give pertinent informations on the animal's diet and geographical origin. However, it should be noted that animals may consume mixed diets and undergo changes in the nature of their diet, together with changes in their geographical location, all issues that may make the use of such markers more complex.

#### *Fingerprint approaches*

##### *Methods based on the optical properties of tissues and products*

Differences in the meat and milk composition induce differences in their optical properties, therefore in their spectral features, which can be used for diet authentication. These approaches offer numerous advantages, being rapid, non-destructive and chemical-free. Also, some of the spectral methods developed to date can be implemented on-line in the industry. For example, visible reflectance spectroscopy has been used successfully to discriminate between carcasses from pasture-fed and stall concentrate fed lambs (Dian et al., 2007a). In this study, the perirenal fat reflectance spectrum was measured using a portable MINOLTA CM-2002 spectrophotometer, which measures the proportion of light reflected at 10-nm intervals at wavelengths between 400 and 700 nm, and records the corresponding reflectance spectrum. A discriminant analysis is then performed on the optical data using a partial least squares discriminant analysis approach (PLS-DA), to discriminate feeding treatments and identify regions of the visible spectrum involved in the discrimination. This study confirmed the importance of carotenoid pigments in discriminating between perirenal fat from pasture-fed and concentrate-fed lambs and suggested that haeminic

pigments could also be involved. Moreover, a spectrophotometric index ( $I_{450-510}$ ) has been proposed by Prache & Theriez (1999), which enables quantifying the 'signature' of carotenoid pigments from the mathematical analysis of the reflectance spectrum of the fat. This patented technique is of practical utility because the apparatus is portable and measurements can be made rapidly; it can thus be integrated into slaughter lines without disturbing output. This method was further generalized to beef and dairy products (Prache et al., 2002; Priolo et al., 2003; Serrano et al., 2006; Nozière et al., 2006b). Although less simple, as it necessitates a non-portable laboratory spectrophotometer, near infrared reflectance spectroscopy (NIRS) further enhances the reliability of the discrimination by enlarging the optical information, the reflectance spectrum of the tissue or the end-product being measured at 2-nm intervals at wavelengths between 400 and 2500 nm. On a database composed of 120 pasture-fed and 139 concentrate-fed lambs, Dian et al (2008) correctly classified 97.5% and 97.8% of the pasture-fed and concentrate-fed lambs respectively, using multivariate analysis over the full set of reflectance data obtained on perirenal fat. In the same way, Cozzolino et al (2002) investigated the spectral characterization of the meat using visible and near infrared reflectance spectroscopy to discriminate beef meat from two feeding systems (pasture vs. maize silage-based diet). Analysis of the optical information showed differences in muscles resulting from the two different feeding systems, which allowed to correctly classify 81% of the meat samples. These spectral methods, that are relatively easy to implement in the meat and milk industry, probably have important potential applications.

#### *Functional genomics*

There are also promising breakthroughs in the development of functional genomics. Given that nutrients regulate metabolic activity by modifying gene expression, it is likely that gene expression will find use in the development of new methods to authenticate feeding diets in herbivores. Cassar-Malek et al. (2005) observed gene expression modifications in response to production system. They performed transcriptome studies using a multi-tissue bovine cDNA repertoire to compare gene expression profiling in pasture-fed vs. maize silage-fed Charolais steers. They found that Selenoprotein W was under-expressed in the *Rectus abdominis* of pasture-fed animals and could therefore be further investigated as a potential indicator of a pasture-based system. This approach is however only beginning to emerge and will require further experimental evaluation.

#### *Discrimination of contrasting diets*

The first step in an experimental evaluation of potential diet markers is to investigate their discrimination reliability in contrasting conditions. Over recent years, we have undertaken research to discriminate pasture-fed from concentrate-fed lambs and authenticate pasture-feeding in lamb meat. We have compared lambs fed either green leafy pasture (P) or a diet containing 80-85% concentrate and 15-20% hay (C). The feeding level of C lambs was adjusted to achieve similar growth patterns in both treatments. C lambs ingested only 2-3% of the carotenoid intake level of P lambs (Prache et al., 2003a). In these studies, carotenoid pigments in plasma and fat, volatile compounds (terpenes and 2,3-octanedione) in the fat, and the fatty acid composition of the meat were all able to distinguish between P and C lambs (Priolo et al., 2002b; Prache et al., 2003a et b; Priolo et al., 2004b; Aurousseau et al., 2004).

Plasma and perirenal fat carotenoid content were respectively 5-fold and 3-fold higher in P than in C lambs (63.3 vs. 11.6 µg/l and 24.6 vs. 8.7 ng/g, Prache et al., 2003a and b). In this experiment, the spectrophotometric index ( $I_{450-510}$ ), which was calculated from the reflectance spectrum of perirenal fat in the area of light absorption by carotenoid pigments (450 to 510 nm), enabled to perfectly discriminate P and C lambs (Priolo et al., 2002b). It should be noted that is preferable to measure the reflectance spectrum on perirenal rather than subcutaneous fat, since perirenal fat stores more carotenoids (Priolo et al. 2002b, Dian et al 2007b, in lambs; Serrano et al., 2006, in beef). It is worth noting also that a wide variability between animals, particularly between pasture-fed animals, is generally observed in plasma, milk and fat carotenoid concentrations and in the spectrophotometric index.

The concentration of 2,3-octanedione in subcutaneous fat was 25-fold higher in P than in C lambs (Priolo et al., 2004b). In these experiments with lambs, marked differences were also found in the sesquiterpene profiles of fat from P vs. C lambs (Priolo et al., 2004b). This result was obtained with lambs grazing pastures containing mainly graminaceae. Hence, it did not support the hypothesis that a generic discrimination of pasture-fed vs. concentrate-fed animals by means of a terpene profile may be difficult because pastures rich in monocotyledons have low terpene concentrations (Mariaca et al., 1997).  $\beta$ -caryophyllene, trans-cadina-1(6),4-diene were present at significant levels in the lambs fed pasture until slaughter, but only at basal levels or even undetectable in C lambs. These compounds may therefore be proposed as biomarkers of pasture-feeding in lamb meat, particularly  $\beta$ -caryophyllene. Alpha- and  $\beta$ -

cubebene contents were also 4-fold higher in P lambs than in C lambs, although their discrimination potential may be lower than  $\beta$ -caryophyllene. As for carotenoids, the variability in terpene concentrations in the fat from P lambs was generally high. However, not all the terpenes detected in lamb fat were positively related to pasture diets, since  $\beta$ -gurjunene was found at 80-fold higher levels in fat from C lambs than fat from P lambs.

In the same experiments, Arousseau et al. (2004) found that the fatty acid composition in *longissimus thoracis* muscle phospholipids allowed perfect discrimination between P and C lambs. Proportions of C18:2n-6 and other n-6 PUFA were lower and proportions of C18:3n-3 and other n-3 PUFA were higher in the fatty acids of the *longissimus thoracis* muscle from P lambs compared with C lambs. These differences were significant in both triglycerides (TG) and phospholipids (PL), but were more noticeable in PL where higher proportions of PUFA accumulate. In muscle PL, the proportion of C18:3n-3 in total fatty acids ranged from 1.9 to 4.6% (3.1% on average) and from 0.6 to 1.4% (0.9% on average) in P and C lambs, respectively. Moreover, the C18:2n-6/C18:3n-3 ratio ranged from 2.44 to 4.77 and from 10.31 to 24.51 in P and C lambs, respectively. Milk fatty acid composition also proved to be very useful for discriminating feed diets in dairy cows (Martin et al., 2005), but there are no experimental data on dairy ewes. However, Priolo et al. (2004a) found that lambs fed exclusively maternal milk had a lower n-6 PUFA/n-3 PUFA ratio when the dams grazed at pasture than when they were fed concentrates indoors, with the ratios overlapping in only two lambs out of 20.

In these experiments with lambs, 2,3-octanedione in subcutaneous fat allowed to perfectly discriminate between P and C lambs, whereas skatole was not significantly different between the two groups (Priolo et al., 2004b). Young et al. (2003) reported higher levels of skatole in the fat of pasture-fed compared with concentrate-fed lambs (although the differences were not always significant). However, Sebastian et al. (2003) did not confirm this result. Although differences between studies may reflect differences in the site of fat measurement (perirenal fat in Young et al (2003) vs. subcutaneous fat in the other studies), the reliability of skatole in authenticating pasture-fed lamb meat probably requires further investigation.

The stable isotope ratios of carbon and nitrogen in lamb meat have also been used to authenticate the diet. Piasentier et al. (2003) analyzed the *longissimus thoracis* muscle by IRMS in lambs fed different diets during their finishing period (milk, pasture or concentrate containing maize grain).

A good discrimination (91.7% of samples) was obtained with the combination of  $\delta^{13}\text{C}$  of fat,  $\delta^{13}\text{C}$  of protein and  $\delta^{15}\text{N}$  of protein.

The nature of the diet ingested strongly influences the composition of milk and meat, and this composition may therefore be used to authenticate the diet. However, responses are sometimes highly variable between animals, and the extent to which this variability could impair the reliability of the authentication method needs to be addressed further. We have begun a validation procedure with a large cohort of animals, both in our experimental facilities (Dian et al., 2007a; Dian et al., 2008) and in private sheep farmhouses (Prache et al., unpublished). Questions regarding the effects of plant phenological stage on vegetal biomarker contents deserve further investigation, as well as the effects of sward availability and supplementation at pasture. Prache et al (2003a) reported that plasma carotenoid concentrations in pasture-fed lambs varied with time, with values in June being 1.5-fold higher than in July and 2.5-fold higher than in August. A plant's volatile compound content also varies with phenological stage (Mariaca et al., 1997), and Tornambé et al. (2006) observed that milk terpene contents from grazing cows were affected by both the phenological stage of the pasture and the grazing management system. More generally, future research may be directed towards investigating the animal's response to the marker intake level, as it has already been established for carotenoid pigments in sheep (Dian et al., 2007) and dairy cows (Calderon et al., 2007). Animal-specific factors, particularly breed and production level, may also modulate the animal's response (Fisher et al., 2000; Nozière et al., 2006a) and some of them are currently under evaluation (Macari et al., unpublished).

#### *Persistence of the diets effects and implications for diet authentication*

The research discussed so far has been focused on the development of potential markers, and has thus investigated the effects and discrimination of contrasting diets. However, it is frequently the case that grazing animals are finished indoors on concentrate diets due to pasture shortage or to increase their live weight gain.

The effect of concentrate finishing after grazing on the animal tissue and product concentrations of the compounds likely to serve as biomarkers of pasture-feeding, and its implications for authentication purposes, have recently been investigated in lambs (Prache et al., 2003a and b for carotenoid pigments; Priolo et al., 2004b for fat volatiles compounds). The authors compared the following

treatments: pasture-feeding (P), concentrate-based diet (C), and concentrate finishing for a long (PLC, 42 days on average) or a short (PSC, 30 days on average) period after pasture-feeding. Concentrate supply was regulated to achieve similar growth patterns across all treatments.

Carotenoid contents in plasma and fat and 2,3-octanedione content in fat all decreased with the interval from starting out on stall diet after pasture, according to an exponential model:  $Y = a \times e^{(-b \times \text{day})}$ , where  $b$  is the deceleration parameter (Prache et al., 2003a; Priolo et al., 2004b; Prache et al., 2005). Carotenoids show less persistence in blood than in fat (Prache et al., 2003a and b). After an average 8 days on the stall diet, the plasma carotenoid content of previously pasture-fed lambs fell to the values found in C lambs. The intensity of light absorption by lutein present in the fat is negatively correlated with the duration of the finishing period, this effect being mediated via a dilution of existing fat with whiter fat rather than through pigments coming out of the fat. The model we developed using live weight gain (LWG) during the finishing period and plasma carotenoid concentration at the end of the grazing period predicted that the spectrocolorimetric index ( $I_{450-510}$ ) would reach a level similar to that of the C lambs after 11 kg LWG on average on the stall diet, i.e. about 35 days when lamb average daily gain is 300 g/day. The persistence of carotenoid pigments is thus higher in fat than in plasma.

In this experiment, the terpene profile of the stall-finished grazing lambs did not differ markedly from that of the C lambs, suggesting that the persistence of these compounds is low.  $\beta$ -caryophyllene was found in all pasture-fed lambs, but only in two and one out of six PSC and six PLC lambs, respectively. The concentration of this compound was actually 15-fold higher on average in the fat from P compared with PSC lambs. Factorial discriminant analysis indicated that four of the 33 terpenes detected in the fat could discriminate P animals from all the others. Among the fat volatiles compounds, 2,3-octanedione proved to be a good provider of reliable information on the duration of the concentrate-finishing period. It is worth noting that the residual standard deviation of the semi-neperian logarithmic model of persistence of 2,3-octanedione in the fat was low, suggesting low interference of between-animal variability (Prache et al., 2005).

Furthermore, there is clear evidence that the combined use of different compounds and different tissues improves reliability in the discrimination of pasture-fed, concentrate-fed and concentrate-finished grazing lambs, by taking advantage of the differences in the persistence patterns (Prache et al., 2003b). Actually, lambs with low plasma

carotenoid concentration and high spectrocolorimetric index ( $I_{450-510}$ ) value at slaughter are concentrate-finished grazing lambs. This finding shows the refinement coming from the combined use of fat and blood measurements at slaughter, which takes advantage of the differences in the rate of reduction in carotenoid concentration in blood and fat. This method, however, does not enable to discriminate concentrate-fed lambs from concentrate-finished grazing lambs having a low absorption of carotenoids or an over-long finishing period.

The reliability of spectral methods, such as visible and near infrared reflectance spectroscopy for discriminating between pasture-fed, concentrate-fed and concentrate-finished grazing lambs is currently under evaluation (Macari et al., unpublished).

## Conclusions

Research on diet authentication in herbivore meat and milk is conducted within a general context of increasing consumer concern regarding the mode of animal production. Within this context, there is a need for authentication tools to guarantee that specification commitments have been fully met and to help constructing them. Recent developments in analytical methods made it possible to discriminate between contrasting diets in herbivores meat and milk, based on quantifying specific compounds in the product or the animal tissues or based on fingerprints. The results show that the combined use of different compounds and tissues is useful. Cost and ease of implementation vary among methods. The spectral methods developed to date are of particular interest, being reliable, rapid, non-destructive and chemical-free. Some of them can be easily implemented on-line in the industry and in abattoirs on a large number of animals. Other methods, that are more expensive and more difficult to implement, such as the analysis of volatile or phenolic compounds, can only currently be used on a small number of samples, but the possibility that they may be used can still deter fraud. Finally, these methods may be used in stages, the simplest one on a large number of animals, and the most expensive in the last resort. Some of these methods are currently being validated with large databases. A validation procedure is essential because between-animal variability can be high, and the ability to accumulate some of these compounds may have a genetic component. Further research will be directed at evaluating the capability of these methods to discriminate intermediate feeding conditions and investigating the interactions between feeding conditions and animal product characteristics.

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