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Persistence of pasture feeding volatile biomarkers in lamb fats

Guilhem Sivadier, Jérémy Ratel, Erwan Engel *

INRA UR370 Qualité des Produits Animaux, 63122 Saint-Genès-Champanelle, France

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ABSTRACT

Recent studies have evidenced volatile biomarkers in ruminant tissues that distinguish between exclusive pasture and exclusive concentrate diets. As ruminants usually alternate these diets, we set out to monitor the persistence of volatile tracers of pasture diet in perirenal fat and caudal subcutaneous fat in lambs ($n = 28$) fed on pasture and then fattened at increasing levels with concentrate: Four groups of lambs ($n = 7$) were stall-finished to achieve a final weight gain of 0, 4, 8, and 12 kg, respectively. Thirty nine pasture diet tracers including terpenes, 2,3-octanedione and toluene were found that distinguish between the four different diets in both tissues. According to their clearance rates monitored in the adipose tissues of lambs fattened with different amounts of concentrate, different types of persistence were evidenced. Most of the compounds exhibited a "short" persistence, e.g. 2,3-octanedione and terpenes, while some displayed a "medium" or "long" persistence. Finally, performing discriminant analysis on ratios of tracers from the two adipose tissues enabled the correct differentiation of the four different diets.

1. Introduction

There is an increasing demand from consumers, commercial entities and bodies operating product certification systems for the development of robust analytical tools to authenticate ruminant feeding based on meat products. Numerous studies have found the analysis of the volatile fraction of dairy (Engel et al., 2007) and meat products (Engel & Ratel, 2007; Sebastian, Viallon-Fernandez, Berge, & Berdague, 2003; Vasta & Priolo, 2006) to allow the construction of robust models for animal diet authentication. These studies were conducted on samples from animals fed exclusively either pasture or concentrate. However, ruminants are usually fed diets in which pasture and concentrate periods alternate. Some authors (Aurousseau, Bauchart, Faure, et al., 2007; Aurousseau, Bauchart, Galot, et al., 2007; Larick & Turner, 1990; Larick et al., 1987; Noziere et al., 2006) have studied the variations in levels of diet biomarkers in meat or milk samples from animals fed with alternating diets consisting of an exclusive pasture feeding period followed by a concentrate-finishing period of variable duration. Monitoring the level of carotenoids (Noziere et al., 2006) or fatty acids (Aurousseau, Bauchart, Faure, et al., 2007; Aurousseau, Bauchart, Galot, et al., 2007) allowed only the differentiation of diets exhibiting marked differences in the concentrate-finishing levels. By analysing the volatile fraction of beef samples, Larick et al. (1987) showed that volatile compounds could segregate diets into graduated concentrate-finishing durations. However, the list of the feeding tracers identified differs from other literature reports and may not be reliable, given the harsh operating conditions used by these authors. Recent developments in volatile compound analysis such as instrumental drift correction (Arvanitoyannis & Van Houwelingen-Koukaliaroglou, 2003; Deport, Ratel, Berdague, & Engel, 2006) and gentle operating conditions limiting the occurrence of artefacts caused by heat (Vasta, Ratel, & Engel, 2007) identified dozens of relevant discriminant compounds with identifiable metabolic origins.

The objective of this three-step study was to determine the persistence of volatile tracers of pasture diet in adipose tissues of lambs initially fed on pasture and stall-fattened to achieve a final weight gain of 0 kg (P), 4 kg (PC4), 8 kg (PC8), or 12 kg (PC12). Two adipose tissues, the perirenal fat (PRF) and the caudal subcutaneous fat (CSCF), were analysed in each animal, as we had previously found in preliminary study that the parallel analysis of volatile diet tracers from the two tissues improved the power of differentiation between exclusive pasture and exclusive concentrate diets (Sivadier, Ratel, Bouvier, & Engel, 2008). The aim of the first step was to identify the volatile pasture diet tracers by comparing the volatile compound composition in the two tissues of lambs fed on pasture and then fattened for either 0 or 12 kg with concentrate. The second step aimed at characterising the persistence rates of these tracers by determining their relative abundances in adipose tissues of lambs stall-finished for a final weight gain of 0, 4, 8, or 12 kg. The third step

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* Corresponding author. Tel.: +33 (0)473624589; fax: +33 (0)473624731.
E-mail address: erwan.engel@clermont.inra.fr (E. Engel).

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aimed at assessing the relevance of these findings concerning the persistence of the pasture diet tracers for distinguishing between different concentrate fattening degrees in grazing lambs on the basis of conventional GC–MS analysis of their adipose tissues.

2. Materials and methods

2.1. Animal products

A herd of 28 male INRA401 lambs born at the INRA research centre (Theix, France) during a 6-week period were divided, after a 45-day weaning period, into four groups of seven lambs each. The first group (P) was fed exclusively at pasture (consisting predominantly of Graminaceae) with no other additional feed. The animals were slaughtered according to the conventional EU procedures at the targeted weight of 40 kg, corresponding to a mean age of 186 days. The other three groups were initially fed at pasture until they reached the target weights of 28 kg (PC12), 32 kg (PC8), and 36 kg (PC4). Each of these three groups was installed in a large stall where they were offered a pelleted concentrate diet consisted of (w/w) 30% hay and 70% commercial concentrate containing barley (20%), wheat bran (15%), wheat (15%) and sugar beet (15%) as main constituents (THIVAT Nutrition Animale, Saint-germain-de-salles, France). The animals were all slaughtered at the target weight of 40 kg weight, corresponding to a mean age of 163 days.

Perirenal and subcutaneous fats were chosen for the study according to a preliminary work (Sivadier et al., 2008), in which it was established that the simultaneous data treatment of the volatile fraction of these two adipose tissues improved diet authentication. In addition, Vezinhet, Prud'hon, and Benevent (1974) reported different growth rates: perirenal fat is developed early in the lamb's life, while subcutaneous fat starts growing in the post-natal period. Diet information may thus have different implications in both tissues.

2.2. Preparation of samples

At 1 h *postmortem* a sample of each adipose tissue was excised from the lamb carcass: perirenal fat (PRF) was adipose tissue directly covering the left kidney, and caudal subcutaneous fat (CSCF) was tissue 10 cm from the tail. The adipose samples were trimmed free of all traces of muscle and immediately immersed in liquid nitrogen, wrapped in aluminium foil, vacuum packed and stored at −80 °C until the next preparation step.

Two days before the analysis each adipose tissue was immersed in liquid nitrogen, cut into small cubes (less than 0.1 g) and ground in liquid nitrogen with a crushing machine (Dangoumeau, Prolabo, Nogent-sur-Marne, France) to a fine homogeneous powder. Three grams of powder were then placed in glass vials (Wheaton Science Products) under a nitrogen flow and heated for 15 min at 70 °C in a 100–800 oven (Memmert, Schwabach, Germany). A mean of 1.2 g of liquid lipid phase was obtained, placed in glass vials sealed under nitrogen flow, and stored at −20 °C until analysis. Just before analysis, the frozen extracts were thawed for 7.5 min at 70 °C.

2.3. Addition of the standards

The comprehensive combinatory standard correction (CCSC) was used to correct instrument drifts, according to Deport et al. (2006). As previously stated by Engel and Ratel (2007), six standards were chosen according to various criteria including: (i) boiling point compatibility with the experimental conditions, (ii) stability, (iii) absence in samples before analysis, (iv) purity of commercially available solutions, (v) relative specificity of mass spectrometry fragmentation and (vi) safeness. The standards used were 2-methyl-pentane (S1; purity 99.5%), fluoro-benzene (S2; purity 99.7%), 1-bromo-butane (S3; purity 99.7%), bromo-benzene (S4; purity 99.5%), 1-fluoro-naphthalene (S5; purity 99.0%) and 1-phenyl-nonane (S6; purity 99.8%) (Sigma–Aldrich Chimie, Saint-Quentin-Fallavier, France). A mixture of the six standards was made and co-analysed with the lipid liquid extract at a final concentration of approx. 50 ppm for each standard (w/w).

2.4. Dynamic headspace–gas chromatography–mass spectrometry (DH–GC–MS) analysis

A plug of glass wool (0.2 g) (VWR International, Fontenay-sous-Bois, France) was introduced into a glass extraction cartridge (diameter 28 mm, length 100 mm, Ets. Maillière, Aubière, France). A 1 g aliquot of sample was placed on the glass wool and 10 μl of the mixture of standards was added on a second plug of 0.1 g of glass wool placed on the sample. The volatile fraction was extracted by dynamic headspace using a purge-and-trap device (3100 Sample Concentrator, Tekmar, Cincinnati, OH, USA). After a pre-purge of 5 min and a preheat of 15 min, the headspace of the sample was purged for 30 min under a 65 ml min^{-1} helium flow (He U quality, purity 99.995%, Messer, St.-Georges-d'Espéranche, France). The temperature of the sample during the DH extraction step was set at 70 °C. The volatiles were trapped by adsorption on a porous-polymer adsorbent Tenax trap column (Tenax TA, straight, 12″ × 30.5 cm, 24 cm of adsorbent, Supelco, Bellefonte, PA, USA) maintained at 36 °C. After a dry purge at 36 °C for 5 min, the volatile compounds were desorbed for 10 min at 230 °C under a helium flow (He N55, purity 99.9995%, Messer). Extracted compounds were then transferred to the head of a capillary column after cryoconcentration at −150 °C. After desorption, the Tenax trap was further heated for 30 min at 230 °C.

The compounds condensed at the head of the column were analysed by GC (model 6890, Hewlett–Packard, PA, USA); the interface was heated at 225 °C for 2 min followed by injection of the compounds in splitless mode into the non-polar phase of the capillary column (SPB5, 60 m × 0.32 mm × 1 μm, Sigma–Aldrich, St. Louis, MO, USA). The oven temperature was held at 40 °C for 5 min, and then increased to 230 °C with a gradient of 3 $°\text{C.min}^{-1}$, and maintained at this temperature for 10 min. The GC column was connected to a mass spectrometer (model 5973A, Hewlett–Packard). The temperature of the transfer line was set at 230 °C. The temperature was fixed at 180 °C in the MS source and at 150 °C in the MS quadrupole. The electron impact energy was set at 70 eV and data were collected in the range of *m/z* 33 to 230 at a scan range of 6.85 scans per second. Tentative identification of volatiles was based on: (i) mass spectra by comparison with MS spectra database including NBS 75 K, Wiley 275L or Masslib (MSP Kofel, Zollikofen, Switzerland) and (ii) comparison of retention indices (RI) with published RI values (Kondjoyan & Berdague, 1996) or with those of our internal data bank. The peak area of the tentatively identified compounds was integrated from the specific ion for each of the molecules to avoid co-elution problems. The integrations were performed with the Enhanced ChemStation software (version D.01.02.16, Hewlett–Packard).

2.5. Data treatment

Data were processed using the Statistica Software release 8.0 package (Statsoft, Maisons-Alfort, France) and the R software version 2.1.4. R development Core Team (2006). R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. ISBN 3-900051-07-0, URL http://www.R-project.org.