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Latency and Persistence of Diet Volatile Biomarkers in Lamb Fats

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Several studies have shown that volatile compounds are particularly well-suited for the authentication lamb diet by gas chromatography−mass spectrometry (GC-MS) analysis of adipose tissue. The aim of the present work was to use dynamic headspace-GC-MS to study the variations in the amounts of volatile diet tracers in perirenal fat (PRF) and caudal subcutaneous fat (CSCF) in lambs ($n = 24$) that were fed with concentrate and then allowed to graze for 0, 17, 51, or 85 days. Twenty-six volatile compounds were found to distinguish between the four diets ($p < 0.05$) in both PRF and CSCF. Of these diet tracers, 16 were found to be related to the pasture diet and increased at different rates according to the time spent at pasture (latency), while 10 were found in higher amounts in tissues of lambs fed with exclusive concentrate and exhibited different rates of clearance (persistence). Twenty-four of these discriminant compounds, including alkanes, ketones, terpenes, and 2,3-octanedione, were previously stated as pasture diet tracers in several earlier studies, suggesting their potential universality. All degrees of latency or persistence were exhibited by the pasture and concentrate diet tracers, respectively. A principal component analysis performed on ratios of selected diet tracers from both adipose tissues evidenced successful differentiation of the four feeding situations.

KEYWORDS: Authentication; meat product; GC-MS; diet tracers; volatile compounds; adipose tissues; latency; persistence

INTRODUCTION

In recent decades, there has been increasing consumer awareness of the marked influence of how farm animals are reared on the quality of their meat and meat products (*1, 2*). After several recent food crises, there is now a strong demand from consumer and commercial entities for analytical tools to objectively guarantee that specification commitments have been fully met, in addition to the traditional document-based traceability systems.

Numerous previous studies have reported that the composition of the diet fed to ruminants strongly influences the composition of biomarkers such as fatty acids (*3*−*6*), pigments (*7*−*9*), vitamins (*10, 11*), stable isotopes (*12*), and volatile compounds (*11, 13*−*19*) in their tissues. These studies have dealt with exclusive diets, whereas ruminants are usually fed with diets alternating pasture and concentrate intakes. Some authors have monitored the levels of these biomarkers on volatile compounds (*13*), carotenoids (*20, 21*), and fatty acids (*22, 23*) in tissues of animals fed on pasture and then fattened with concentrate-based diets.

In previous work (*13*), volatile compounds were found to be particularly relevant biomarkers for successfully distinguishing between different types of diets consisting of pasture feeding followed by increasing concentrate-finishing periods. This may be due to the wide diversity of these lipophilic compounds, especially in animal adipose tissues, and to their multiple origins: They can arise from a direct transfer from feeds or from animal metabolism (endogenous synthesis) or ruminal microorganisms (*24*). We have also previously established that simultaneously processing the discriminant compounds identified in different adipose tissues, especially perirenal fat (PRF) and caudal subcutaneous fat (CSCF), strongly increased the discriminative power for the diets (*19*).

Previous studies (*11, 18, 19*) have shown that dynamic headspace−gas chromatography−mass spectrometry (DH-GC-MS) is suitable for the complexity of volatile compounds for food authentication, particularly when gentle operating conditions are used to avoid the formation of artifacts caused by heat (*19*) and when chemometric tools are applied to data to correct instrumental drifts (*18, 25, 26*).

The aim of this three-step study was to investigate the time course of the pasture and concentrate diet tracer composition in two fat tissues of lambs fed concentrate and then allowed to graze for fattening in four increasing periods: 0, 17, 51, or 85 days. The first step aimed to identify the pasture and concentrate volatile diet tracers in lamb fat tissues. The second step was dedicated to characterizing the degree of latency and persistence of these diet tracers in fats of concentrate lambs finished with pasture for various durations. Finally, the third step aimed to use the informative contents of these tracers to distinguish between the different pasture-finishing periods of lambs.

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MATERIALS AND METHODS

Animal Products. The animals used in the experiment were lambs reared at the INRA Research Center (Theix, France) in controlled conditions. A flock of 28 male lambs born during a 2 week period were divided, after a 40 day weaning period, into four groups of seven lambs each according to the type of diet that they were fed. The first group (C) was kept indoors and fed exclusively with a pelleted concentrate mixed with 30% hay, with no other additional feed. The main constituents of the commercial concentrate were barley (20%), wheat bran (15%), wheat (15%), and sugar beet (15%) (THIVAT Nutrition Animale, Saint-germain-desalles, France). The animals were then slaughtered at the target age of 150 days, corresponding to a mean weight of 37 kg, in accordance with the conventional EU procedures. The three other groups were first fed concentrate for a target period of 65 (CP85), 99 (CP51), and 133 days (CP17). They were then allowed to graze ad libitum (90% rye grass) until age 150 days for slaughtering, corresponding to a mean weight of 37 kg. No significant difference was found between the final weight of lambs fed C (mean = 36 kg), CP17 (mean = 36 kg), CP51 (mean = 38 kg), and CP85 (mean = 38 kg) according to one-way analysis of variance (ANOVA) ($p < 0.05$). Four lambs died during rearing: one in the C group, one in the CP51 group, and two in the CP85 group.

Concerning the nature of the samples, the volatile profiles of the PRF and CSCF were chosen for the study in light of our previous results (*19*). We found that simultaneously considering the discriminant diet information of these two adipose tissues improved diet authentication.

Preparation of Samples. At 1 h post mortem, mean weights of 80 g of PRF and 16 g of CSCF were excised from the lamb carcasses. PRF was the adipose tissue directly covering the left kidney, whereas CSCF was taken 10 cm from the tail. The adipose samples were trimmed free of all traces of muscle and immediately immersed in liquid nitrogen, wrapped in aluminum 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 (model Dangoumeau, Prolabo, Nogent-Sur-Marne, France) to a fine homogeneous powder. Three grams of powder was 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 quantity of 1.2 g of liquid lipid phase was obtained, placed in glass vials sealed under a nitrogen flow, and stored at −20 °C until analysis. Just before analysis, the frozen extracts were thawed for 7.5 min at 70 °C.

Addition of the Standards. The comprehensive combinatory standard correction (CCSC) was used to correct instrumental drifts, according to Deport et al. (*25*). As previously stated (*18*), six standards were chosen according to various criteria such as (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%), 1-bromo-butane (S2; purity 99.7%), fluoro-benzene (S3; purity 99.7%), bromo-benzene (S4; purity 99.5%), 1-fluoro-naphthalene (S5; purity 99.0%), and 1-phenyl-nonane (S; purity 99.8%) (Sigma-Aldrich Chimie, Saint-Quentin-Fallavier, France). A mixture of the six standards was made and coanalyzed with the lipid liquid extract at a final concentration of approximately 50 ppm for each standard (w/w).

DH-GC-MS Analysis. A plug of 0.2 g of glass wool (VWR International, Fontenay-sous-Bois, France) was introduced into a glass extraction cartridge (diameter 28 mm, length 100 mm, Ets. Maillière, Aubière, France). An aliquot of 1 g 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 DH using a purge-and-trap device (model 3100 Sample Concentrator, Tekmar, Cincinnati, OH). After a prepurge 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) 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 analyzed by GC (model 6890, Hewlett-Packard, PA); the interface was heated at 225 °C for 2 min followed by injection of the compounds in splitless mode into the nonpolar phase of the capillary column (SPB5, 60 m × 0.32 mm × 1 μm, Sigma-Aldrich, St. Louis, MO). The oven temperature was held at 40 °C for 5 min and then increased to 230 °C with a gradient of 3 °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 33−230 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 75K, Wiley 275 L, or Masslib (MSP Kofel, Zollikofen, Switzerland) and (ii) comparison of linear retention indices (LRI) with published LRI values (*29*). Terpenes were identified on the basis of laboratory previous work (*27*). For confirmation purposes, commercial compounds were purchased and analyzed with the same DH-GC-MS device: The commercial compounds analyzed comprised a mix of C8−C20 linear alkanes in hexane, toluene (purity 99%; CAS 108-88-3), hexan-2-one (purity 99.5%; CAS 591-78-6), acetophenone (purity 99%; CAS 98-86-2), nonan-2-one (purity 99%; CAS 821-55-6), decan-2-one (purity 99.5%; CAS 693-54-9), pyridine (purity 99%; CAS 110-86-1), 2,3-butanediol (purity 98%; CAS 513-85-9), heptan-2-one (purity 99%; CAS 110-43-0), 50% 1-octen-3-one in 1-octen-3-ol (product W351504), undecan-2-one (purity 99%; CAS 112-12-9), tridecan-2-one (purity 99%; CAS 593-08-8), and pyrazine (CAS 290-37-9) (Sigma-Aldrich Chimie). The peak area of the tentatively identified compounds was integrated from the specific ion for each of the molecules to avoid coelution problems. The integrations were performed with the Enhanced ChemStation software (version D.01.02.16, Hewlett- Packard).

Data Treatment. Data were processed using the Statistica Software release 7.1 package (Statsoft, Maisons-Alfort, France) and the R software version 2.1.4 (*28*). In a previous study (*19*), 125 compounds were found to differentiate the lipid liquid phases extracted from PRF and CSCF of lambs fed exclusively at either pasture or on concentrate. These compounds were semiquantified in the same adipose tissues of the present study, and the instrument drifts were corrected by CCSC. DH-GC-MS raw data were processed with CCSC according to Engel and Ratel (*18*): The mixture of the six selected standards was analyzed together with the sample, and the abundance of each compound specific ion was normalized by the sum of the abundances of the specific ion of standards, selected among the $\sum_{p=1}^{6} C_6^p$ possible sums, where p represents the number of standards involved in a given sum, enabling the best product differentiation.

To determine which compounds distinguished between the exclusive concentrate diet (C) and the concentrate then 85 days pasture-finishing period diet (CP85), a one-way ANOVA was performed according to the model: CCSC pretreated abundance of compound specific ion = type of feeding ($p < 0.05$). Principle component analyses (PCA) were performed on the CCSC pretreated abundances of the discriminant compound specific ions to visualize the structure of the data.

To monitor the latency and persistence of the specific tracers of the four types of diet in PRF and CSCF, the abundance of the compounds distinguishing between C and CP85 was determined in the liquid lipid extract of the adipose tissues of the lambs fed the other alternating diets. Their abundances were then filtered by one-way ANOVA (model: CCSC pretreated abundance of compound specific ion = type of feeding, $p < 0.05$) according to the four modalities: C, CP17, CP51, and CP85. For a given compound, its relative amounts over the four groups were considered to establish its variation rate, and a Newman−Keuls mean comparison test was performed to evaluate the

Table 1. Compounds Tentatively Identified as Lamb Feeding Tracers in Perirenal and Caudal Subcutaneous Lamb Fat Tissues

LRI[a]	compounds[b] ($n = 49$)	ID[c]	diet[d] PRF[e] ($n = 37$)	diet[d] CSCF[e] ($n = 28$)	ref[f]
488	pent-1-ene	1	C		(*19*)
593	butanal	1		CP85	(*19*)
600	hexane	2	C		(*19, 31*)
623	2-methyl-propanenitrile	1	C	C	(*19*)
653	3-methyl-butanal	1	CP85		(*19*)
657	3-methyl-butan-2-one	1		C	(*19*)
684	1-penten-3-ol	1	CP85		(*19*)
726	1-methyl-cyclohexane	1		C	(*18*)
734	pyrazine	2		C	(*19*)
748	pyridine	2	CP85		(*19*)
755	(*E*)-2-pentenal	1	CP85		(*18, 19*)
771	toluene	2	CP85	CP85	(*18, 19*)
789	2,3-butanediol	2	C	CP85	(*18, 19*)
789	hexan-2-one	2	C	CP85	(*19*)
796	(*Z*)-4-octene	1	CP85		(*18, 19*)
800	octane	2		C	
806	(*E*)-4-octene	1	CP85		(*18, 19*)
810	(*Z*)-2-octene	1	CP85		(*18, 19, 30*)
815	(*E*)-2-octene	1	CP85		(*18, 19, 30*)
826	3-cyclohepten-1-one	1	CP85		(*19*)
873	heptan-4-one	1	C	CP85	(*19, 31*)
890	heptan-2-one	2	C		(*19, 31*)
900	(*Z*)-4-heptenal	1	CP85	CP85	(*18, 31*)
916	dimethyl-sulfone	1	CP85	CP85	(*17−19*)
944	α-pinene	3	CP85	CP85	(*19*)
962	1-chloroheptane	1	CP85	CP85	(*19*)
979	1-octen-3-ol	2		C	(*19*)
979	1-octen-3-one	2		C	(*18, 19*)
982	2,3-octanedione	2	CP85	CP85	(*17−19, 31*)
987	6-methyl-5-hepten-2-one	1		C	(*19*)
1000	decane	2		C	
1034	*p*-cymene	3	CP85	CP85	(*17−19, 31*)
1036	1,3,5-trimethyl-benzene	1	CP85	CP85	(*18, 19*)
1040	limonen	3		CP85	(*19*)
1066	1-chlorooctane	1	C	C	(*19*)
1074	3,5-octadien-2-one	1	CP85		(*18, 19*)
1079	acetophenone	2	C		(*18, 19*)
1093	nonan-2-one	2		CP85	(*18, 19, 31*)
1195	decan-2-one	2		CP85	(*19*)
1200	dodecane	2	CP85		
1297	undecan-2-one	2	CP85		(*18, 19, 31*)
1300	tridecane	2	CP85		(*18, 19, 31*)
1378	2,6,10-trimethyl-dodecane	1	C		(*19*)
1463	2,3,7-trimethyl-decane	1	C		(*19, 31*)
1488	1-ethyl-2-methyl-cyclododecane	1	CP85	CP85	(*19*)
1495	1-pentadecene	1	CP85	CP85	(*19*)
1500	pentadecane	2	C		(*19, 31*)
1500	tridecan-2-one	2	C		(*19, 31*)
1554	*trans*-cadina-1(6),4-diene	3	CP85	CP85	(*17, 19*)

[a] Linear retention indices on a SPB5 capillary column. [b] Compounds found to distinguish between the C and the CP85 diets. [c] Method used for the identification of the volatile compounds: 1 = tentative mass spectrum + LRI from the literature (*17−19, 31*), 2 = 1 + LRI of purchased commercial compounds, and 3 = 1 + LRI of laboratory database (*27*). [d] Type of lamb diet related to the compound considered in this study: C = exclusive concentrate and CP85 = concentrate followed by a finishing period of 85 days at pasture. [e] Adipose tissues: PRF and CSCF. [f] Literature references for compounds stated as pasture diet tracers.

significance of the amount variations between the four diets with increasing pasture-finishing durations.

To establish the possibility of distinguishing between the different types of diet on the basis of the volatile compounds from the lipid liquid extracts of the two adipose tissues, the most robust volatile tracers distinguishing between the four diets were obtained by filtering this new data set of PRF and CSCF with a one-way ANOVA (model: CCSC pretreated abundance of compound specific ion = type of feeding, $p < 0.05$) followed by a leave-one-out cross-validation procedure ($p < 0.05$). A PCA was then performed on the filtered tracers from each adipose tissue and from the two pooled together in the same data set ($n = 23$), to visualize the structure of the data. A Newman−Keuls mean comparison test was performed on the coordinates of the observations on the two principal components constituting the first factorial plane to assess its significance in the differentiation of the four diets. The gain in discriminative power obtained by processing the two adipose tissue volatile tracers in parallel was assessed by performing a discriminant analysis (DA) carried out on the PRF, CSCF, and the two data sets simultaneously. The Wilks' λ values, which give the quality of differentiation between the four diets, were determined for the combinations of four tracers (quartets) from one or the two adipose tissues and were built up according to the "best subset" algorithm.

The optimization of the differentiation of the four diets was obtained using ratios of tracer abundances according to Engel et al. (*11*). On the basis of the tracers from the two tissues, previously selected ($n = 23$), the A_{23}^2 possible ratios were set up and filtered by a one-way ANOVA (model: ratio of CCSC pretreated abundances of compound specific ion = type of feeding, $p < 0.05$) followed by a leave-one-out cross-validation procedure. Among the selected ratios, when several involved the same compound in the same tissue, whether in the numerator or the denominator, only the one with the best Fisher F was

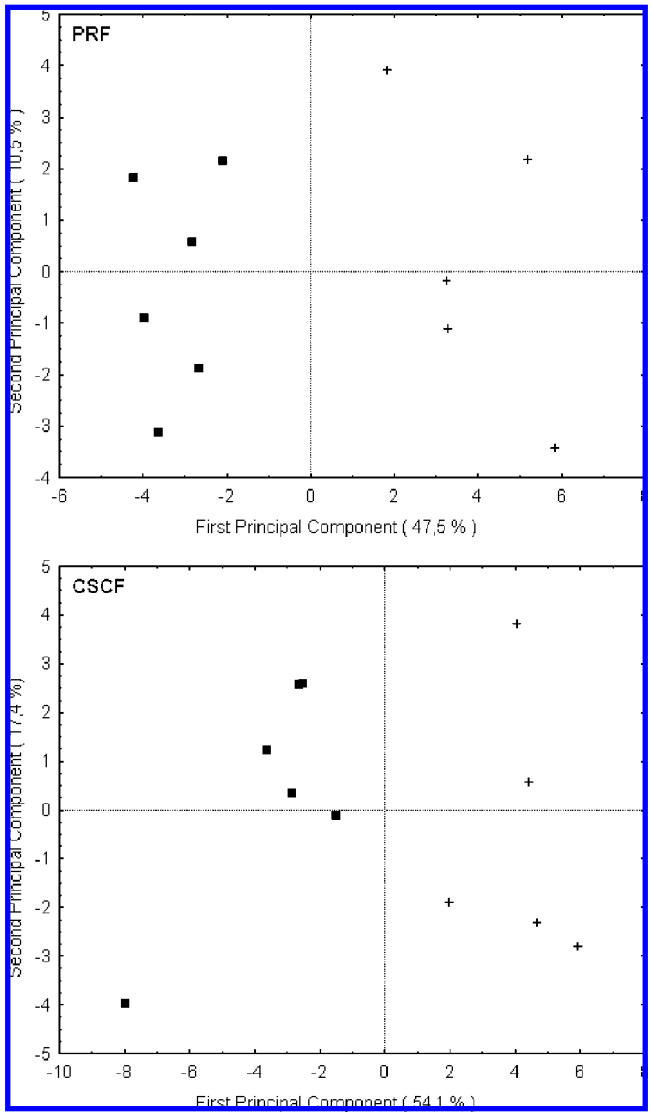

Figure 1. Differentiation of concentrate- and pasture-fed lambs based on GC-MS analysis of their PRF or CSCF: exclusive concentrate (■) and concentrate followed by a finishing period of 85 days at pasture (+). The first plan of normed PCAs was carried out from the CCSC pretreated abundances of the volatile compounds selected by one-way ANOVA ($p < 0.05$) in PRF ($n = 37$) and CSCF ($n = 28$).

selected. After the data were processing with a PCA, a Newman−Keuls mean comparison test was performed on the coordinates of the observations on the two principal components of the first plan to assess their significance in the differentiation of the four diets.

RESULTS AND DISCUSSION

Identification of the Lamb Feeding Tracers. A list of 122 compounds was first drawn up from the literature (*17*−*19*, *30*, *31*). These compounds were semiquantified in the PRF and CSCF of lambs fed either exclusive concentrate (C) or concentrate with a pasture-finishing period of 85 days (CP85). **Table 1** presents the compounds that distinguished between the two diets: 37 feeding tracers were found in PRF, 28 were found in CSCF, and 16 were common to the two tissues.

A PCA was performed on the diet tracers of each adipose tissue. For both PRF and CSCF, the first plan (**Figure 1**) shows a clear segregation of the lamb tissue samples according to the type of feeding, confirming that the volatile fraction of each adipose tissue enables the authentication of these diets. Also, some tracers were found to be discriminant exclusively in PRF ($n = 21$) and CSCF ($n = 12$), showing that the two tissues contain different information on the lambs' diet.

Of the 49 compounds distinguishing between the C and CP85 diets, 46 had already been reported as diet tracers in previous studies. Thirteen compounds are stated as pasture diet tracers in the present work, following many other authors, and can be considered as universal. For some, the identification of a probable precursor in forage supports their reliability as pasture tracers. This is the case for 2,3-octanedione, which is derived from the oxidation of linoleic acid catalyzed by the action of the enzyme lipoxygenase that is present in leafy plants and not in seeds except for soybeans (*14*, *19*). Similarly, toluene may originate at least in part from the degradation of plant carotenoids (*19*, *32*). (*Z*)-4-Heptenal and (*E*)-2-pentenal are end products of the oxidation of n-3 polyunsaturated fatty acids, according to Elmore et al. (*15*), a lipid class found in greater amounts in pasture diet than in concentrate diet (*22*, *23*, *33*). Finally, three terpenes, α-pinene, *p*-cymene, and *trans*-cadina-1(6),4-diene, were also stated pasture tracers. Although terpenes, which originate from green herbages (*34*), are generally considered as too specific to the botanical composition of the pasture diet to be reliable (*35*−*37*), these three compounds have been recurrently identified as pasture diet tracers in previous studies (*17*−*19*).

Some compounds were determined as diet tracers in our previous study (*19*) and in the present one but not in the literature (**Table 1**). This can be explained by the use of both gentle/smooth analysis conditions, such as low temperatures, and of a procedure for instrument drift correction (*18*, *25*). Accordingly, these compounds may be considered as relevant for pasture diet authentication. According to **Table 1**, some compounds were shown to be significantly more abundant in fats of concentrate-fed lambs, whereas they were reported as potential pasture diet tracers by previous works (*18*, *19*, *31*). These discrepancies can be due to differences in the composition of the concentrate diet used in these studies, suggesting the poor robustness of these compounds as lamb diet tracers.

Determination of the Latency Rates of the Pasture Diet Tracers. The 49 feeding tracers that were identified above on the basis of their abundance in the fats of C and CP85 diet-fed lambs were also semiquantified in the fats of CP17- and CP51-fed lambs. A one-way ANOVA was processed to identify the tracers that discriminate ($p < 0.05$) the four diets. Twelve and ten pasture diet tracers were identified in PRF and in CSCF, respectively (**Table 2**), while seven and three concentrate diet tracers were found in the same tissues (**Table 3**). Some of the tracers listed in **Table 1** were not included in **Tables 2** and **3**. Even if these compounds were found distinctive of the diet in **Table 1**, their *p* value was lower but very close from 0.05 (data not shown). The second ANOVA processed for the construction of **Tables 2** and **3** showed that the discriminative power of these compounds was questionable. In contrast, some of these compounds like pyridine, (*E*)- and (*Z*)-2-octene, tridecane, or *trans*-cadina-1(6),4-diene were recurrently identified as pasture diet tracers by previous reports (*17*−*19*, *30*, *31*) and can be considered as relevant pasture diet tracers. These discrepancies with the literature may be explained by differences between the rearing protocol used by the previous and the present studies. The CP85 diet consisted in concentrate feeding for approximately 65 days after weaning, followed by pasture feeding for 85 days, while the minimum exclusive pasture feeding periods in the previous studies (*18*, *19*, *31*) was 120 days after weaning. Hence, we can assume that using the CP85 diet, the duration of the period where pasture is given, may be too short

Table 2. Latency of the Pasture Diet Tracers in Perirenal and Caudal Subcutaneous Lamb Fat Tissues

		PRF[c] ($n = 12$)					CSCF[c] ($n = 10$)				
		diets[d]					diets[d]				
compounds[a] ($n = 16$)	ID[b]	C (%)	CP17 (%)	CP51 (%)	CP85 (%)	latency rate[e]	C (%)	CP17 (%)	CP51 (%)	CP85 (%)	latency rate[e]
alkanes											
dodecane	2	49 B	79 AB	115 A	100 A	medium					
1-ethyl-2-methyl-cyclododecane	1	52 B	63 B	86 AB	100 A	long	37 B	52 AB	67 AB	100 A	long
alkenes											
(*Z*)-2-octene	1	69 B	151 AB	212 A	100 B	other*					
pentadec-1-ene	1	49 C	61 BC	85 AB	100 A	medium	37 B	52 AB	68 AB	100 A	long
alcohols											
1-penten-3-ol	1	54 B	48 B	71 AB	100 A	long					
aldehydes											
butanal	1						58 B	123 A	88 B	100 B	other*
3-methyl-butanal	1	65 A	51 A	82 A	100 A	very long					
(*Z*)-4-heptenal	1	56 B	88 A	96 A	100 A	short	52 B	147 A	77 B	100 B	other*
benzene compounds											
toluene	2	2 B	72 A	86 A	100 A	short	9 B	93 A	102 A	100 A	short
ketones											
hexan-2-one	2						77 B	149 A	105 AB	100 AB	other*
3-cyclohepten-1-one	1	44 B	134 AB	193 A	100 AB	other*					
2,3-octanedione	1	18 B	22 B	40 B	100 A	long	7 B	19 B	60 AB	100 A	long
3,5-octadien-2-one	1	68 A	71 A	105 A	100 A	very long					
sulfur compounds											
dimethyl-sulfone	1	9 B	60 AB	106 A	100 A	medium	10 B	80 A	107 A	100 A	short
terpenes											
α-pinene	3						11 B	55 AB	58 AB	100 A	long
p-cymene	3						7 B	59 A	69 A	100 A	short

[a] Compounds found to distinguish between the four types of diet. The amounts of the compounds in the volatile fraction of the tissues are expressed in percentages of their amount in the CP85 diet. [b] Method used for the identification of the volatile compounds: 1 = tentative mass spectrum + LRI from the literature (*17*−*19, 31*), 2 = 1 + LRI of purchased commercial compounds, and 3 = 1 + LRI of laboratory database (*27*). [c] Adipose tissues: PRF and CSCF. [d] Type of lamb diet related to the compound considered in this study: C = exclusive concentrate; CP17, CP51, and CP85 = alternating concentrate and finishing for 17, 51, and 85 days, respectively, with concentrate. For each tissue and each compound, values with different letters (A−C) within the same row were found to be significantly different ($p < 0.05$) according to a Newman−Keuls mean comparison test. [e] Latency degree resulting from the Newman−Keuls mean comparison test; * compounds with a persistency or latency rate undefined because their maximum amounts were significantly reached in CP17 or CP51 diets.

Table 3. Persistence of the Concentrate Diet Tracers in Perirenal and Caudal Subcutaneous Lamb Fat Tissues

		PRF[c] ($n = 7$)					CSCF[c] ($n = 3$)				
		diets[d]					diets[d]				
compounds[a] ($n = 10$)	ID[b]	C (%)	CP17 (%)	CP51 (%)	CP85 (%)	persistence rate[e]	C (%)	CP17 (%)	CP51 (%)	CP85 (%)	persistence rate[e]
alkanes											
hexane	2	147 A	102 B	103 B	100 B	short					
2,6,10-trimethyl-dodecane	1	275 A	166 AB	154 AB	100 B	long					
2,3,7-trimethyl-decane	1	189 A	125 AB	142 AB	100 B	long					
pentadecane	2	216 A	155 AB	117 B	100 B	medium					
alkenes											
pent-1-ene	1	156 A	152 A	121 AB	100 B	long					
alcohols											
2,3-butanediol	2	147 AB	184 A	193 A	100 B	very long					
1-octen-3-ol	2						290 B	495 A	213 B	100 B	other*
ketones											
1-octen-3-one	2						149 B	557 A	233 B	100 B	other*
tridecan-2-one	2	188 A	150 AB	117 AB	100 B	long					
nitrogen compounds											
pyrazine	2						228 A	127 B	117 B	100 B	short

[a] Compounds found to distinguish between the four types of diet. The amounts of the compounds in the volatile fraction of the tissues are expressed in percentages of their amount in the CP85 diet. [b] Method used for the identification of the volatile compounds: 1 = tentative, mass spectrum + LRI from the literature (*17*−*19, 31*), 2 = 1 + LRI of purchased commercial compounds, and 3 = 1 + LRI of laboratory database (*27*). [c] Adipose tissues: PRF and CSCF. [d] Type of lamb diet related to the compound considered in this study: C = exclusive concentrate; CP17, CP51, and CP85 = alternating concentrate and finishing for 17, 51, and 85 days, respectively, with concentrate. For each tissue and each compound, values with different letters (A−C) within the same row were found to be significantly different ($p < 0.05$) according to a Newman−Keuls mean comparison test. [e] Persistence degree resulting from the Newman−Keuls mean comparison test; * compounds with a persistency or latency rate undefined because their maximum amounts were significantly reached in CP17 or CP51 diets.

to significantly increase the level of these pasture diet tracers. Accordingly, we can assume that these compounds have "very long" latencies.

To characterize the latency and the persistence of the diet tracers in the fat tissues of concentrate -fed lambs finished with increasing grazing periods, a Newman−Keuls mean comparison

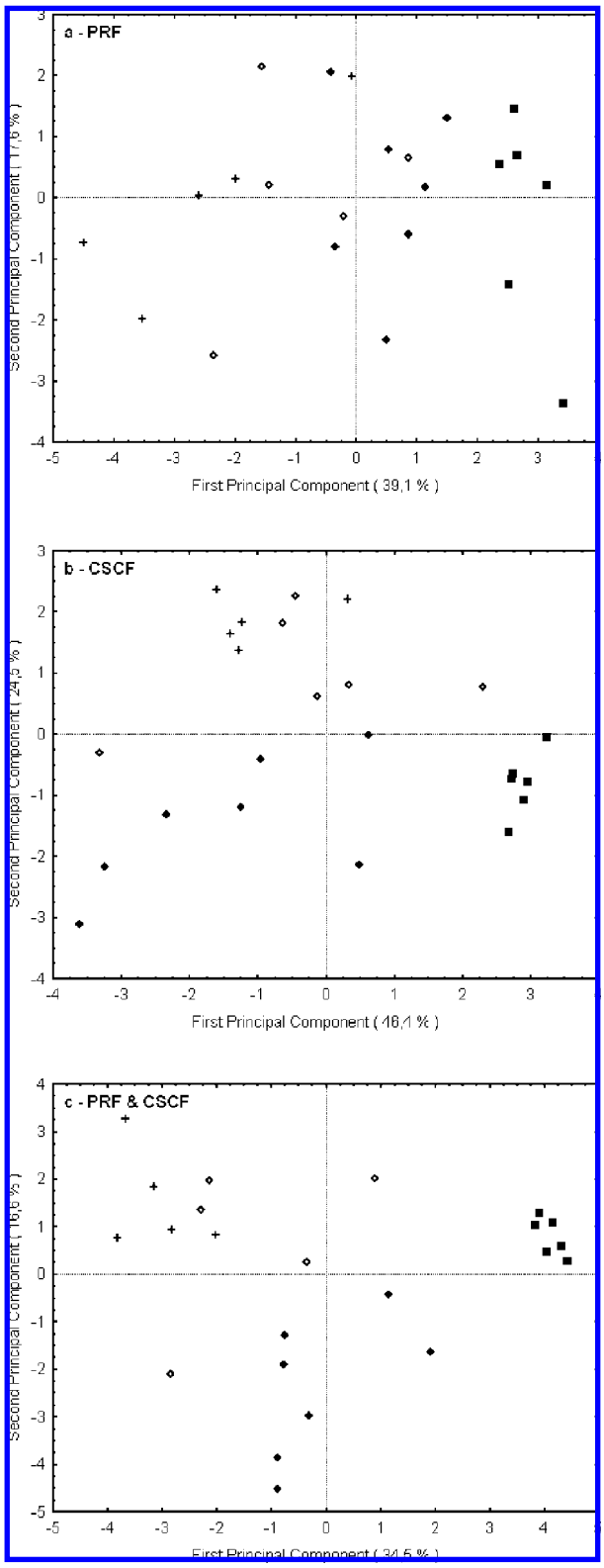

Figure 2. Differentiation of concentrate-fed lambs finished at pasture for various durations based on GC-MS analysis of their PRF or CSCF: exclusive concentrate (■) and concentrate followed by finishing periods of 17 (♦), 51 (◊), and 85 days (+) at pasture. The first plan of normed PCAs was carried out from the CCSC pretreated abundances of the volatile compounds differentiating the four diets selected by one-way ANOVA ($p < 0.05$) followed by a leave-one-out cross-validation procedure. (a) PRF ($n = 13$), (b) CSCF ($n = 10$), and (c) PRF and CSCF = data set incorporating the tracers from the two tissues ($n = 23$).

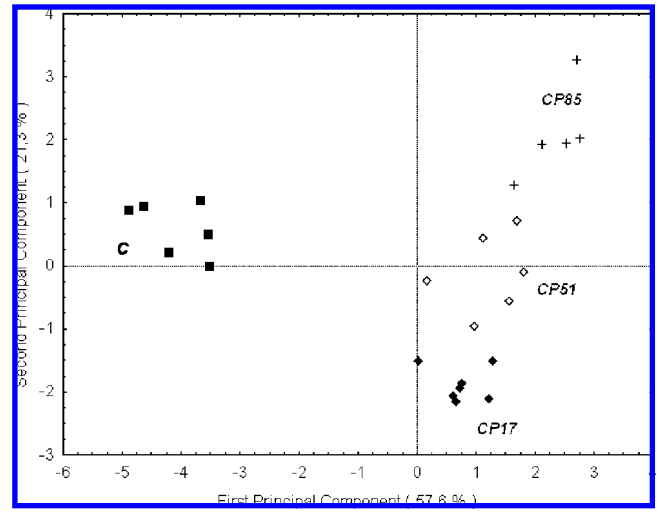

Figure 3. Differentiation of concentrate-fed lambs finished at pasture for various durations based on GC-MS analysis of their PRF or CSCF: exclusive concentrate (■) and concentrate followed by finishing periods of 17 (♦), 51 (◊), and 85 days (+) at pasture. The first plan of normed PCA was carried out from 11 selected ratios of diet tracers.

test was performed on the abundances of the compounds that are listed in **Tables 2** and **3**. Considering the two tissues, five pasture diet tracers were found to distinguish between C and each of the other diets and so were considered as exhibiting "short" latency (**Table 2**). Three compounds, only in PRF, were found to distinguish between C and concentrate diets followed by a period longer than or equal to 51 days at pasture (CP51 and CP85) and so were considered as having "medium" latency. Three tracers in PRF and four in CSCF distinguished only between C and CP85 and so were considered as exhibiting "long" latency. 3-Methyl-butanal and 3,5-octadien-2-one, which were not found to distinguish between C and any of the other diets, were considered as diet tracers with "very long" latency. Finally, the fat content in most of the pasture diet tracers exhibits a more or less progressive increase, which is consistent with the pasture ingestion process of lambs (**Table 2**). Similarly, regarding the concentrate diet tracers (**Table 3**), hexane in PRF and pyrazine in CSCF were found to exhibit "short" latency rates, while pentadecane, four other tracers, and 2,3-butanedione, all from PRF, had "medium", "long", and "very long" latencies, respectively. The decreases in amounts of these concentrate diet tracers are consistent with the switching from concentrate to pasture diet. By contrast, five compounds in CSCF [1-octen-3-ol, butanal, (*Z*)-4-heptenal, hexan-2-one, and 1-octen-3-one] peaked in fats of lambs fed the CP17 diet, while (*Z*)-2-octene and 3-cyclohepten-1-one strongly decreased in PRF of lambs allowed to graze 85 days at pasture (CP85). These "other" patterns are unexpected and require further investigation in order, for instance, to explain how 1-octen-3-ol and 1-octen-3-one can be categorized either as concentrate diet tracers or as pasture diet tracers, depending on the duration of the grazing finishing period.

Different latency or persistence values suggest different accumulation and clearance mechanisms that do not appear to be related to particular chemical families or types of tissue in this work. Also, in comparison with previous studies on the persistence of pasture diet tracers in grazing animals finished with concentrate, it is clear that the persistence and latency values for a given compound are not necessarily similar. For example, 2,3-octanedione was identified as exhibiting a "short" persistence (*13*), while here, it exhibited a "long" latency. Hence,

it is necessary to determine both the persistence and the latency of diet tracers to segregate different feeding situations where diets alternate.

Authentication of Four Different Types of Diet. A PCA was performed on the tracers listed for each tissue in **Tables 2** and **3**. The first plan of the corresponding PCA (**Figure 2**) confirms the differentiation of C and CP85 for both tissues. As shown in Figure **2a**,**b**, the PCA performed on CSCF data also shows a distinction between C, CP85, and a group formed by CP17 and CP51. The Newman−Keuls mean comparison test performed on the coordinates of the individuals in the first two principal components confirmed this segregation in three groups for both CSCF and PRF. In contrast, neither of the two tissues enabled us to distinguish between the CP51 and the CP85 groups. Following a previous study (*19*), data of the two tissues were pooled, and a PCA was run on the new data set (the same compound in two different tissues being considered as two distinct variables). As shown in **Figure 2c**, merging the data set enhanced the discrimination even though the distinction between CP17 and CP51 remained imprecise.

For confirmation, a DA was performed on the data set ($n = 23$) including tracers from both PRF and CSCF. According to Sivadier et al. (*19*), these tracers were combined into quartets, and the Wilks' λ values of the corresponding models were calculated, enabling an assessment of the model's ability to differentiate lambs' diets: The lower the λ value, the better the discriminative power (data not shown) was. Comparison of λ values corresponding to the 100 most discriminative quartets of compounds from PRF, CSCF, or the two shows that the combinations involving tracers from the two adipose tissues give the model with the best discriminative power.

According to a previous study (*11*), tracers of the two tissues were combined in ratios to improve the discrimination. A one-way ANOVA enabled us to filter 310 significant ratios according to the type of lamb diet (*11*). Of those involving the same compound from the same tissue (numerator or denominator), the one that had the best Fisher *F* was selected. The final data set thus comprised 11 ratios, five of them including tracers from both tissues. The first plan of the PCA performed on these ratios (**Figure 3**) exhibited a clear segregation of the four groups, which was confirmed by a Newman−Keuls test ($p < 0.05$) performed on the principal component. While the first principal component clearly segregates the lambs fed with the exclusive concentrate diet (C) against an alternating diet (CP17, CP51, and CP85), the second axis differentiates the different alternating diets. These results confirm the interest of combining data in ratios, to enhance their informative content (*11*).

In the present work, we show that the parallel DH-GC-MS analysis of two adipose tissues of lambs enables us to differentiate diets varying in pasture-finishing duration. Combined with the results of another current study designed to distinguish between tissues of lambs fed on pasture with ranging concentrate-fattening levels, these data will help us to construct models to authenticate meat products from grazing lambs.

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