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26 reached a plateau. This value was reached or exceeded in a significant
27 proportion of lambs fattened on alfalfa pastures (45.1%). However, skatole was
28 not detected in kidney fat from 20 out of 164 alfalfa-fattened lambs (i.e., 12.2%)
29 but was detected in 15 out of 55 concentrate-fed lambs (i.e., 27.3%). We thus
30 conclude that while skatole content in kidney fat can inform on dietary changes
31 made shortly before slaughter, it does not have the discrimination power
32 needed to reliably authenticate pasture-fed lamb meat, let alone duration of
33 finishing on pasture.

34

35 **Keywords:** authentication, flavour, indole, lucerne, pasture-feeding, skatole

36

37 **1. Introduction**

38

39 Meat from pasture-fed lambs has better nutritional properties than meat from
40 lambs raised indoors with a concentrate diet (Aurousseau et al., 2007; Gruffat et
41 al., 2020). However, meat from pasture-fed lambs is darker (Priolo et al., 2002)
42 and may have a stronger flavour (Resconi et al., 2009) or a higher intensity of
43 skatole-related odour and flavour (Rivaroli et al., 2019; Devincenzi et al., 2019)
44 than meat from lambs fed indoors a concentrate diet, which may be seen as a
45 defect. The stronger flavour has in fact been partly associated with higher
46 concentrations of volatile indolic compounds, especially 3-methylindole (or
47 skatole), in the fat of pasture-fed lambs compared to concentrate-fed lambs
48 (Young et al., 1997 and 2003).

49 Forage legumes offer many agro-environmental benefits and can play an
50 important role in sustainable sheep production, notably by reducing the level of

51 external inputs while improving animal productivity (Frank et al., 2016;
52 Ponnampalam et al., 2022). These plants can improve soil fertility through their
53 ability to fix atmospheric nitrogen. In addition, alfalfa (*Medicago sativa*) can
54 improve soil structure due to its deep root system compared to grass, which also
55 makes it resistant to dry conditions. Grazing alfalfa had also been shown to
56 improve the nutritional quality of lamb meat compared to grazing perennial
57 ryegrass (*Lolium perenne*) or feeding feedlot pellets (Ponnampalam et al., 2019;
58 Gruffat et al., 2020). Nevertheless, pastures including alfalfa or white clover
59 (*Trifolium repens*) may result in increased fat concentration of volatile indolic
60 compounds in lamb meat (Schreurs et al., 2007a and b; Devincenzi et al.,
61 2014), and thus in increased meat flavour intensity (Schreurs et al., 2007a;
62 Devincenzi et al., 2014 and 2019; Rivaroli et al., 2019) and increased risk of
63 undesirable pastoral flavours (Park et al., 1972 and 1975), especially for
64 consumers accustomed to meat from grain-based farming systems. However,
65 this has not always been observed (Schreurs et al., 2007b; de Brito et al., 2016;
66 Frank et al., 2016), sometimes due to the small number of animals under study
67 (Schreurs et al., 2007b). Note that alfalfa can be consumed in different forms,
68 but only fresh forage or silage can lead to high levels of skatole and indole in
69 the rumen and fat, as drying and dehydration greatly reduce protein degradation
70 in the rumen (INRA, 2018), and thus skatole and indole formation in the rumen.
71 Research has suggested that the detection threshold for skatole-related odour
72 and flavour is about 0.05 µg/g fat in butter (Watkins et al., 2014) and up to 0.1
73 µg/g fat in pork (Banon et al., 2003; Lunde et al., 2010), and that the sensory
74 rejection threshold for skatole is 0.15 µg/g fat in pork (Mörlein et al., 2012). This
75 threshold can be considered as an indicator for the identification of carcasses

76 whose meat is likely to be rejected by consumers. However, consumer
77 acceptance of meat is highly dependent on how it is processed and consumed.
78 As skatole and indole are lipophilic and volatile molecules, their concentration is
79 much higher in fat than in lean tissue and their odour is more strongly perceived
80 when the meat product has been heated. Thus, the risk of consumer rejection is
81 higher for sausages or bacon than for rindless chops or ham (Parois et al.,
82 2018). Furthermore, because lamb meat is less processed than pork and other
83 volatile compounds may interfere with skatole and indole, such as
84 androstenone in pork (Parois et al., 2018) or branched chain fatty acids in lamb
85 meat (Young et al., 1997), generalization of this threshold established for pig
86 carcasses to lamb carcasses must be done with caution. However, in the
87 absence of comparable information for lamb carcasses, we used, in this study,
88 that established for pig carcasses. Finally, it should be noted that the number of
89 lambs used in previous studies was too small to enable proper assessment of
90 the proportion of lambs that are above this latter threshold (0.15 µg/g fat) based
91 on their diet.

92 Furthermore, recent studies in lambs switched to concentrate-feeding after a
93 period of alfalfa grazing demonstrated that the persistence of skatole and indole
94 in the fat was low (Gkarane et al., 2019; Eiras et al., 2022), but there is still a
95 lack of information on the time-related profile of these compounds in fat after a
96 switch to alfalfa grazing, including the time after which they become detectable
97 in fat after switching to alfalfa grazing.

98 Skatole in fat has also been investigated for its potential value in diet
99 authentication, particularly for authenticating pasture-fed meat (Priolo et al.,
100 2004; Vasta et al., 2006; Prache et al., 2020). However, the literature on this

101 issue is so far inconclusive, probably due to both the high variability in animal
102 response and the low number of animals under study (Vasta et al., 2006;
103 Prache et al., 2020).

104 The objective of the present study was to investigate the change in fat skatole
105 and indole concentrations in lambs switched from concentrate-feeding indoors
106 to alfalfa grazing for various durations before slaughter. We used a large
107 number of animals over three consecutive years in order to provide information
108 on the proportion of lambs at risk of developing excessive pastoral flavours, and
109 whether fat skatole levels can be used for authentication purposes.

110

111 **2. Material and methods**

112

113 The experiment was conducted at the INRAE's 'Herbipôle' experimental unit.
114 The protocol was approved by the ethical committee of the French Ministry of
115 Higher Education, Research and Innovation (committee approval 14289-
116 2017042411419479V4).

117

118 ***2.1. Experimental design, animal management and diet regimens***

119

120 The experiment compared four durations of grazing alfalfa before slaughter: 0
121 day (L0), 21 days (L21), 42 days (L42), and 63 days (L63). The experiment was
122 replicated three times during three consecutive years. Nineteen Romane
123 weaned male lambs were used each year for each treatment group, giving 228
124 Romane lambs used in total. The lambs were born within a few weeks (between
125 8 and 30 April in year 1, between 6 and 30 April in year 2, and between 29 April

126 and 1 May in year 3). Slaughter dates were scheduled each year so that the
127 lambs would be slaughtered at approximately 5 months old, an age that yields
128 carcasses of commercially acceptable weight and degree of fatness, as shown
129 in previous research using Romane lambs fattened on alfalfa pastures after
130 weaning (Devincenzi et al., 2019; Rivaroli et al., 2019; Eiras et al., 2022). Dates
131 for turning out to pasture were then calculated based on the planned slaughter
132 dates. Figure 1 gives a schematic diagram of the experimental layout.

133 Lambs were randomly selected from the existing flock in the experimental unit,
134 born during this 1-month period, and were randomly allocated to the treatment
135 groups on the basis of live-weight (LW) at birth, birth date, and growth rate
136 between birth and the beginning of the experiment. Mean lamb LW at birth was
137 4.0 kg (SD 0.84), and mean lamb age at slaughter was 143 days (SD 13).

138 Before grazing alfalfa, the L21, L42 and L63 lambs were kept indoors and-fed a
139 high-concentrate diet. During the stall-feeding period, concentrate was given at
140 a level adjusted to achieve similar mean patterns of growth between
141 concentrate-fed lambs and pasture-fed lambs, and straw was given *ad libitum*.

142 Pasture-fed lambs from the different treatment groups co-grazed in a single
143 group to limit potential confounding effects of sward characteristics, except
144 during the first 5 days following the turn-out to pasture to allow for a dietary
145 transition when switching from concentrate feeding to alfalfa grazing (Figure 1).

146 During this transition, lambs grazed a grass plot and were allowed access to an
147 adjacent alfalfa plot for increasing time duration over the course of the 5 days.

148 Two dry ewes were added to this group of pasture-finished lambs to train them
149 in pasture use. In each experimental year, lambs in the same treatment group
150 were slaughtered on the same day, so that all the pasture-fed lambs in a given

151 treatment group could be turned out from stall to pasture at the same date, thus
152 facilitating the 5-days diet transition. The plots were grazed rotationally, and
153 lambs were moved to a new grazing break as pasture conditions dictated so as
154 to ensure *ad libitum* availability of green alfalfa leaves. Pasture herbage was cut
155 and the trimmings removed, one month prior to commencement of the
156 experiment and after each grazing period, to ensure good-quality regrowths.
157 Pasture-fed lambs received drenches against digestive nematodes and
158 *Moniezia* if necessary, based on regular measurements of faecal egg counts
159 performed at monthly intervals after turn out to pasture.

160 When indoors, lambs were also managed in a single group. They were fed a
161 commercial concentrate, the composition of which is given in Table 1, along
162 with straw in collective feeding troughs and racks. The troughs and racks were
163 long enough for all animals to have access to the feed at the same time. The
164 concentrate was distributed every day at 9 a.m., after the previous concentrate
165 refusals had been discarded. All lambs had *ad libitum* access to water and salt
166 blocks.

167 The lambs were slaughtered in the INRAE's experimental slaughterhouse
168 located next to the experimental site. On arrival, the lambs were immediately
169 electrically stunned and slaughtered by throat cutting. Evisceration and skinning
170 were performed according to standard commercial practices. The carcasses
171 were then placed in a refrigerated room at 4°C until 24 h after slaughter.

172

173 **2.2. Measurements**

174

175 **2.2.1. Lamb live-weight**

176

177 Lambs were weighed at birth, then once weekly at the same time of the day
178 throughout the experiment, and on the day of slaughter (full weight).

179

180 *2.2.2. Carcass characteristics and fat sampling*

181

182 After chilling for 24 h at 4°C, the carcasses were weighed, graded for
183 conformation and assessed for fatness using a 15-point scale (Eiras et al.,
184 2022). Kidney fat weight and dorsal fat thickness over the last thoracic rib were
185 measured. A sample of kidney fat of approximately 30 g was then taken,
186 wrapped and vacuum-packed in a sealable polyamide bag, frozen, and stored
187 at -20° C until chemical analysis.

188

189 *2.2.3. Fat skatole and indole concentration analysis*

190

191 Kidney fat skatole and indole concentrations were determined by HPLC using
192 the procedure described by Batorek et al. (2012) as follows: fat samples were
193 liquefied in a microwave oven for 2 × 1 min at 350 W. The liquefied lipids were
194 centrifuged for 20 min at 11,200 g at 20°C. After centrifugation, the fat was
195 heated to 50°C and 0.5 ± 0.01 g of water-free liquid fat was transferred into 2.5
196 mL Eppendorf tubes with 1 mL methanol containing 0.050 mg/L 2 of 3-
197 methylindole (internal standard). After stirring for 30 s, the tubes were incubated
198 for 5 min at 30°C in an ultrasonic water bath, put on ice for 20 min, and
199 centrifuged for 20 min at 11,200 g at 4°C. For skatole and indole
200 determinations, 20 µL of supernatant was injected into an HPLC column, and

201 fluorescence was detected (excitation at 285 nm and emission at 340 nm) using
202 a HP1200 system (Agilent Technologies, Waldbronn, Germany).
203 Concentrations were expressed in μg per gram of lipid fraction from adipose
204 tissue. The limit of detection was $0.03 \mu\text{g/g}$ liquid fat.

205

206 **2.3. Data analyses**

207

208 The data for lamb LW and LW gain, carcass weight, conformation, degree of
209 fatness, and dorsal fat thickness were analysed by ANOVA using a mixed
210 model, with treatment set as a fixed factor and year set as a random factor
211 (SAS Institute Inc, 2014), and using the Tukey test for pair-wise comparisons.
212 Differences between treatment groups in the distribution of lambs in the
213 different classes of fat skatole and indole concentrations (i) below or above the
214 limit of detection, i.e., $0.03 \mu\text{g/g}$ liquid fat, and (ii) below or above $0.15 \mu\text{g/g}$
215 liquid fat, the value for backfat skatole concentration identified as causing
216 sensory rejection in pork (Mörlein et al., 2012) were analysed using a Chi-
217 square test. As the variance for kidney fat weight and skatole and indole
218 concentrations differed between treatment groups and was not stabilized using
219 log-transformed data, these variables were analysed using non-parametric
220 statistics (Kruskal-Wallis test, pair-wise comparisons being performed with a
221 Bonferroni correction).

222

223 **3. Results and discussion**

224

225 **3.1. Animal performance and carcass characteristics**

226

227 Four lambs had to be removed from the experiment due to injury or sickness,
228 and six others died during the experiment. A final total of 55, 55, 56 and 52
229 lambs completed the experiment in the L0, L21, L42 and L63 treatment groups,
230 respectively. Lamb LW at the beginning of the grazing period and LW gain
231 during the grazing period are given in Table 2.

232

233 There were treatment effects on carcass weight, degree of carcass fatness,
234 dorsal fat thickness and kidney fat weight ($P < 0.001$ for all variables, Table 2
235 and Figure 2). Carcass weight and degree of fatness were lower in L21 and L42
236 lambs than in L0 and L63 lambs ($P < 0.05$ to $P < 0.001$), but not significantly
237 different between L21 and L42 lambs and between L0 and L63 lambs. Dorsal
238 fat thickness was higher in L0 lambs than in the other treatment groups ($P <$
239 0.001), which did not differ. Kidney fat weight was lower in L21 than in L0 ($P <$
240 0.01) and L63 ($P < 0.01$) lambs, with no other significant differences in pairwise
241 comparisons (Figure 2). Carcass conformation score averaged 6.72 (which
242 corresponds to R- in the EUROP classification) and showed no between-group
243 differences (Table 2).

244

245 The LW change during the 3 weeks after the switch from stall to pasture was in
246 the order L63 (4.15 kg, range: -1.3 to 8.0 kg) > L42 (1.48 kg, range: -2.3 to 4.6
247 kg) > L21 lambs (0.01 kg, range: -6.0 to 6.0 kg), with a high variability between
248 animals in a same feeding treatment. This abrupt change in diet and living
249 conditions, which was dictated by the objectives of the study, caused some
250 stress to some animals, particularly in the L21 and, to a lesser extent, L42

251 treatment groups, as had already been observed by Huang et al. (2015) and
252 Eiras et al. (2022) for the reverse pattern of change in diet, i.e., switching from
253 pasture-feeding to concentrate-feeding indoors. We chose to co-graze all
254 pasture-fed lambs together in order to avoid confounding effects of sward
255 characteristics (which could have induced undesirable differences in the
256 quantity and quality of the herbage ingested and therefore, potentially, in the fat
257 skatole and indole concentrations). However, co-grazing did not avoid
258 unpredictable variability in animal performance, and resulted in lower carcass
259 weight and degree of fatness in L21 and L42 lambs compared to L0 and L63
260 lambs. These differences were, however, relatively unsubstantial.

261

262 **3.2. Fat skatole and indole concentrations**

263

264 In this study, skatole and indole concentrations were measured in kidney fat to
265 ensure that all lambs had sufficient fat for sampling. Previous studies in sheep
266 have measured skatole and indole concentrations in kidney fat (Young et al.,
267 2003; Girard et al., 2016), subcutaneous fat (Young et al., 1997; Priolo et al.,
268 2004 and 2009), or both these fat depots (Devincenzi et al., 2014 and 2019;
269 Rivaroli et al., 2019; Eiras et al., 2022). Devincenzi et al. (2014) observed a
270 good correlation between these two fat depots for skatole concentration ($r^2 =$
271 0.78); such an analysis was not possible for indole concentration due to a large
272 number of samples below the detection limit. Moreover, the effect of various
273 treatments on fat skatole and indole concentrations showed high similarity
274 between the two fat depots (Rivaroli et al., 2019; Devincenzi et al., 2019; Eiras
275 et al., 2022). Skatole and indole, which are lipophilic compounds, are also

276 expected to be present in intramuscular fat, and the corresponding analysis
277 would be of great interest. However, this analysis poses technical difficulties
278 because of the low level of intramuscular fat and thus the low concentrations of
279 skatole and indole in the lean tissue. To our knowledge, no such data is
280 available for lamb, but Devincenzi et al. (2014), in lambs fed various dietary
281 proportions of fresh alfalfa forage showed that there was substantial similarity
282 between the lean part of the chop and the fat part of the chop in the change in
283 intensity of the skatole-related odour and flavour. Some studies on pork showed
284 a significant correlation between subcutaneous fat and lean tissue for skatole
285 and indole concentrations, and, as expected, lower concentrations in lean tissue
286 than in subcutaneous fat (Rius and Garcia-Regueiro, 2001; Wauters et al.,
287 2016; Meinert et al., 2017).

288

289 In the present study, there were treatment effects on kidney-fat skatole ($P <$
290 0.001, Figure 3) and indole concentrations ($P <$ 0.001, Figure 4). Kidney-fat
291 skatole concentration was lower in L0 lambs than in L21, L42 and L63 lambs (P
292 $<$ 0.001 for all comparisons), but not significantly different between L21, L42
293 and L63 lambs. A new finding of this study was thus that skatole concentration
294 in kidney fat was significantly increased from as early as 21 days of alfalfa
295 grazing, and then stabilized for longer periods of fattening on alfalfa.

296 Given that there was a treatment effect on kidney fat weight (Figure 2), it is
297 questionable whether kidney fat weight may have been a confounding factor.
298 There is no scientific literature on this kind of potential dilution effect. However,
299 there was no significant correlation between kidney fat skatole content and
300 kidney fat weight for either pasture-fed or concentrate-fed lambs. Moreover, the

301 kidney fat skatole concentration found here was fairly similar to that found by
302 Eiras et al. (2022), Rivaroli et al. (2019) and Devincenzi et al. (2019) in Romane
303 lambs grazing alfalfa that had much higher kidney fat weights. This casts doubt
304 on a possible dilution effect of kidney fat skatole content related to kidney fat
305 weight. Here, kidney fat skatole concentration in the L63 lambs ranged from
306 0.01 to 0.54 $\mu\text{g/g}$ liquid fat, whereas it ranged from 0.08 to 0.41 $\mu\text{g/g}$ in Eiras et
307 al. (2022), from 0.03 to 0.41 $\mu\text{g/g}$ in Rivaroli et al. (2019), and from 0.09 to 0.56
308 $\mu\text{g/g}$ in Devincenzi et al. (2019).

309 Kidney-fat indole concentration was lower in L0 lambs than in L21, L42 and L63
310 lambs ($P < 0.001$ for all comparisons). Kidney-fat indole concentration was also
311 lower in L42 lambs than in L21 lambs ($P < 0.05$) (Figure 4). There were no other
312 significant differences in pairwise comparisons. Kidney fat indole concentration
313 was thus significantly increased from as early as 21 days of alfalfa grazing and
314 was similar between L21 and L63 lambs (0.12 and 0.11 $\mu\text{g/g}$, respectively), but
315 it was lower in L42 lambs than in L21 lambs (0.08 vs 0.11 $\mu\text{g/g}$). This latter
316 difference may be related to a slight change in grazing conditions at the end of
317 the fattening period. Actually, even though pasture-fed lambs co-grazed as a
318 single group, they were slaughtered on different days to accommodate a
319 feeding transition (when turned out to alfalfa pasture). This difference in indole
320 concentration is however of little importance for meat quality, as indole is much
321 less odorous than skatole.

322

323 **3.3. Proportion of lambs in which skatole concentration was above 0.15**
324 **$\mu\text{g/g}$ liquid fat**

325

326 There was a treatment effect on the proportion of lambs in which skatole
327 concentration was above 0.15 µg/g liquid fat ($P < 0.001$). This proportion was
328 lower in L0 lambs than in the other treatment groups (3.6% in L0 lambs vs.
329 50.0%, 46.4% and 38.5% in L21, L42 and L63 lambs, respectively; $P < 0.001$
330 for all pairwise comparisons), without significant differences between L 21, L42
331 and L63 lambs. The proportion of lambs for which skatole concentration in
332 kidney fat was above 0.15 µg/g liquid fat was thus significantly increased from
333 as early as 21 days of alfalfa grazing, and then stabilized for longer periods of
334 fattening on alfalfa. It should be noted, however, that this sensory rejection
335 threshold was established for pork and could be different for lamb meat, which
336 has a different composition of odorant molecules, resulting in a different
337 bouquet in which skatole could be less identifiable or a source of rejection or, on
338 the contrary, interact with other odorant molecules (Prache et al., 2022).

339 The skatole concentration in kidney fat varied widely between lambs in a same
340 treatment group, in line with previous studies (Young et al., 2003; Schreurs et
341 al., 2008; Devincenzi et al., 2019; Rivaroli et al., 2019; Eiras et al., 2022). Six
342 out of 52 concentrate-fed (L0) lambs had high levels of kidney fat skatole and
343 were considered outliers. High skatole concentrations in kidney fat are rare in
344 concentrate-fed lambs but have been observed in previous studies (Devincenzi
345 et al., 2019; Eiras et al., 2022). Despite these empirical evidences, the
346 underlying reasons remain poorly understood. Conversely, skatole and indole
347 were not detected in a number of lambs fattened on alfalfa, as already observed
348 in previous studies (Rivaroli et al., 2019; Eiras et al., 2022). This high variability
349 between animals suggests that future research could be directed at assessing
350 the content of these off-flavour-causing compounds in the live animal (e.g. in

351 the blood), as proposed by Eiras et al. (2022), in order to furnish estimates of
352 between-animal variation in fat skatole concentration and adapt the nutritional
353 strategy to mitigate over-intense flavours if necessary.

354 Figure 5 gives the lamb distribution stratified by kidney fat skatole concentration
355 classes, with data for the L0 and L63 lambs studied here pooled with data from
356 three previous experiments on lambs concentrate-fed indoors or fattened for at
357 least two months on alfalfa without any supplementation (Rivaroli et al., 2019;
358 Devincenzi et al., 2019; Eiras et al., 2022) (n = 94 and 93 for each type of
359 lambs, in 6 experimental years). Based on these data, 4.2% of concentrate-fed
360 lambs were above the target value of 0.15 µg/g liquid fat that Mörlein et al.
361 (2012) identified as causing sensory rejection in pork, whereas 45.9% of lambs
362 fattened for at least two months on alfalfa were above this target value. As there
363 is still insufficient knowledge (and therefore uncertainty) regarding the threshold
364 value for fat skatole concentration causing sensory rejection in lamb, we have
365 presented all the individual data in Figure 5. This could allow to better define in
366 the future the proportion of lambs finished on alfalfa that are at risk of
367 developing excessive pastoral flavours, if scientific studies better define this
368 threshold and complete this first database. It would actually be worthwhile to
369 study the relationship between fat skatole concentration and the intensity of
370 skatole-related odour and flavour on a large data set. The difficulty lies in the
371 fact that (i) odour and flavour appreciation and liking vary considerably across
372 consumer groups (Sanudo et al, 2007; Font-i-Furnols et al., 2009), (ii) there are
373 between-studies methodological differences in the qualifiers used in sensory
374 tests (Watkins et al.,2013) and in the method used for analysing skatole and

375 indole concentrations, (iii) as discussed above, other compounds affect meat
376 odour and flavour and may interact with skatole.

377

378 **3.4. Proportion of lambs in which skatole was detected (authentication**
379 **purpose)**

380

381 There was a treatment effect on proportion of lambs in which skatole was
382 detected in kidney fat ($P < 0.001$); this proportion was lower in L0 lambs than in
383 the other treatment groups (27.3 %, 96.4 %, 96.4 % and 69.2 % in L0, L21, L42
384 and L63 lambs, respectively; $P < 0.001$ for all pairwise comparisons). Analysis
385 with the alfalfa-finished lambs (L21, L42 and L63 lambs) pooled together
386 showed that the proportion of alfalfa-finished lambs in which skatole was
387 detected in kidney fat was higher than the proportion of L0 lambs (concentrate-
388 fed lambs indoors) in which skatole was detected in kidney fat (87.8% vs
389 27.3%, $P < 0.001$).

390 Thus, another new finding from this study is that, even though skatole content in
391 kidney fat can inform on dietary changes made shortly before slaughter (i.e., as
392 early as 21 days of grazing), it does not have the discriminatory power to
393 reliably authenticate pasture-fed lambs. Even though alfalfa is known to
394 promote high levels of in-fat skatole, skatole was not detected in kidney fat from
395 12.2% of alfalfa-grazed lambs, but was detected in kidney fat from 27.3% of
396 concentrate-fed lambs. Furthermore, the reliability of the discrimination between
397 concentrate-fed and pasture-fed lambs based on kidney fat skatole content is
398 likely lower when lambs graze grass pastures or legumes containing condensed
399 tannins (such as sainfoin, sulla, or birdsfoot, for example), which are known to

400 lead to lower fat skatole concentration (Schreurs et al., 2008; Girard et al.,
401 2016; Rivaroli et al., 2019). This means that fat skatole concentration has to be
402 combined with other tissue composition measures, preferably showing different
403 pattern of change with duration of pasture-feeding before slaughter, to increase
404 the reliability of diet authentication. A candidate compositional variable for this
405 purpose could be meat n-3 PUFA content, as Noci et al. (2005) observed a
406 linear increase in n-3 PUFA content in beef as a function of pasture-feeding
407 duration before slaughter. Spectral methods that incorporate diet-related
408 differences in tissue composition can take advantage of these different patterns
409 of change with duration of pasture-finishing (Prache et al., 2020).

410

411 **Conclusion**

412

413 The time to appearance of volatile indolic compounds in kidney fat after lambs
414 were switched from a concentrate-based diet indoors to alfalfa grazing was
415 short. Kidney fat skatole and indole concentrations were sharply increased from
416 as early as 21 days of alfalfa grazing, and their concentration then stabilized for
417 longer durations on alfalfa onwards. With respect to authentication, the results
418 of the present study show that, while skatole content in kidney fat can inform on
419 dietary changes made shortly before slaughter, it does not have the
420 discrimination power needed to reliably authenticate pasture-feeding in lambs,
421 let alone the duration of finishing at pasture.

422

423 **Ethics approval**

424

425 All procedures received ethical approval from Ethical Review Committee of the
426 French Ministry of Higher Education, Research and Innovation (committee
427 approval 14289-2017042411419479V4).

428

429 **Data and model availability statement**

430

431 None of the data used were deposited in an official repository

432

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438

439 **Author contributions**

440

441 **Sophie Prache:** conceptualization, investigation, writing, review and editing,
442 project administration, funding acquisition, supervision. **Lucille Rey-Cadilhac:**
443 investigation, statistical analysis, writing original draft. **Armelle Prunier:**
444 conceptualization, fat skatole and indole analysis, investigation, review. All
445 authors have read and agreed to the manuscript.

446

447 **Declaration of competing interest**

448

449 None.

450

451 **Data availability**

452

453 Data will be made available on request

454

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456

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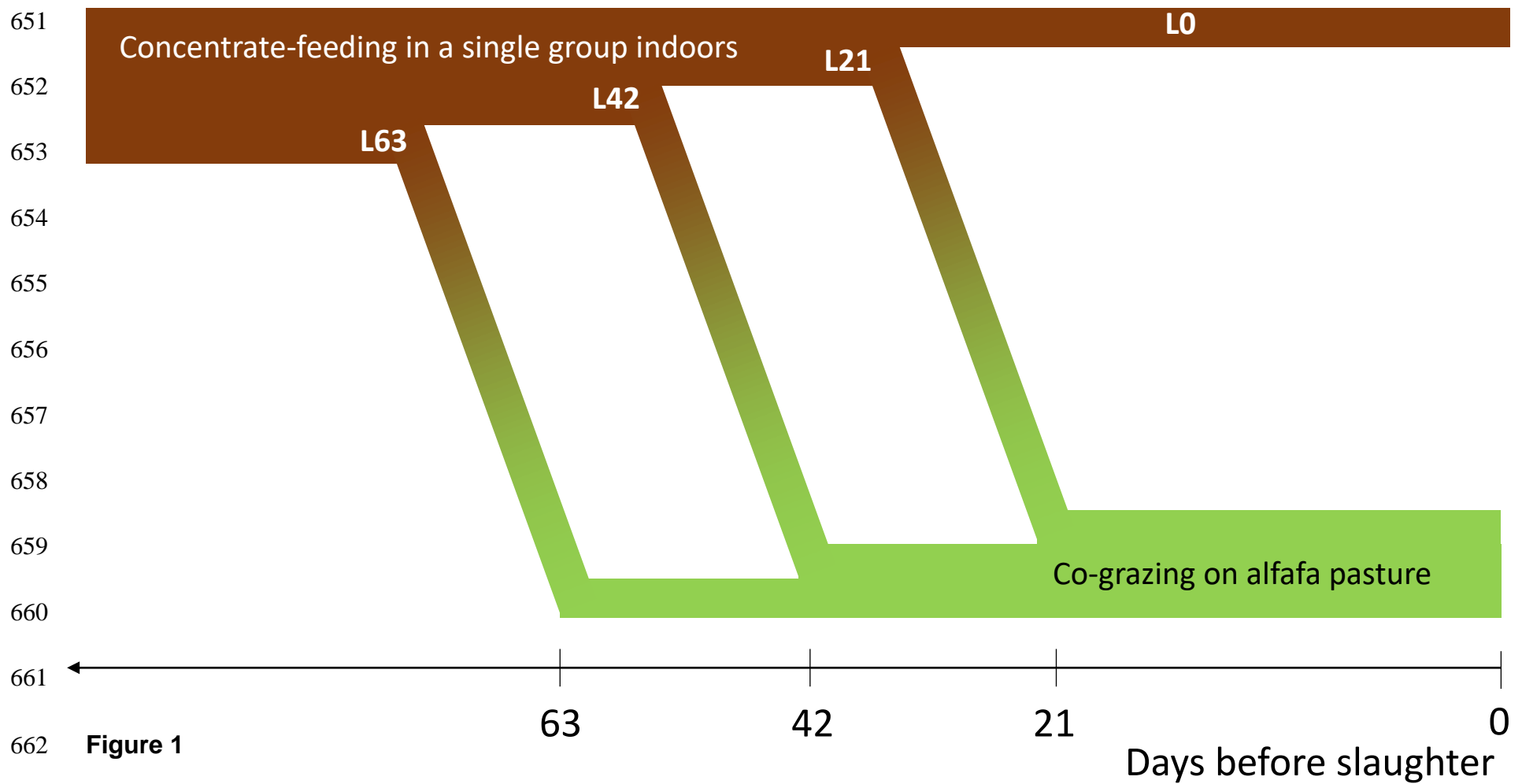
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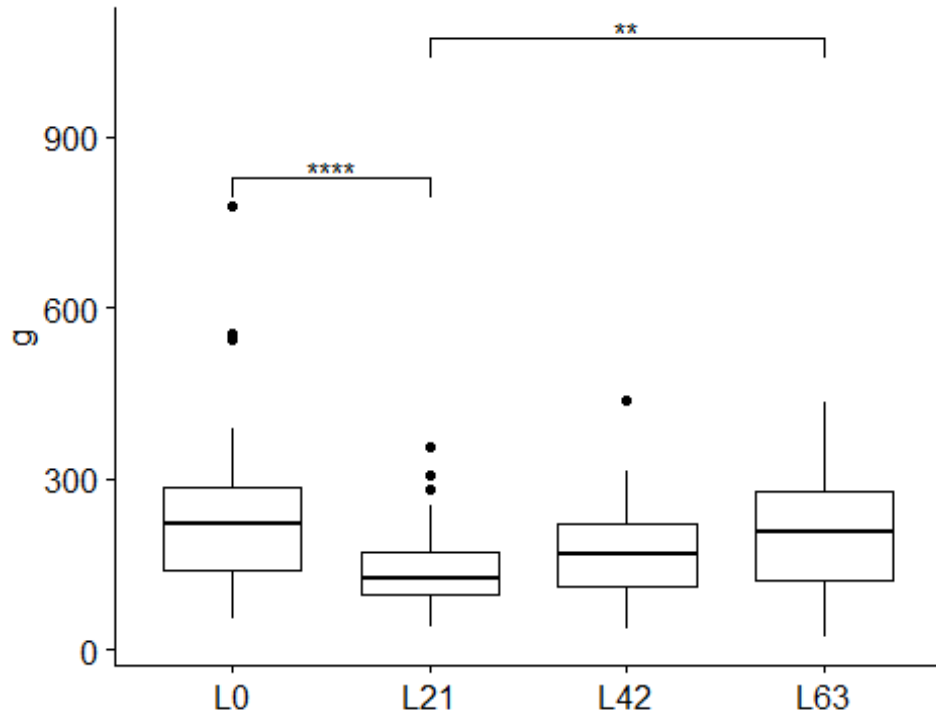
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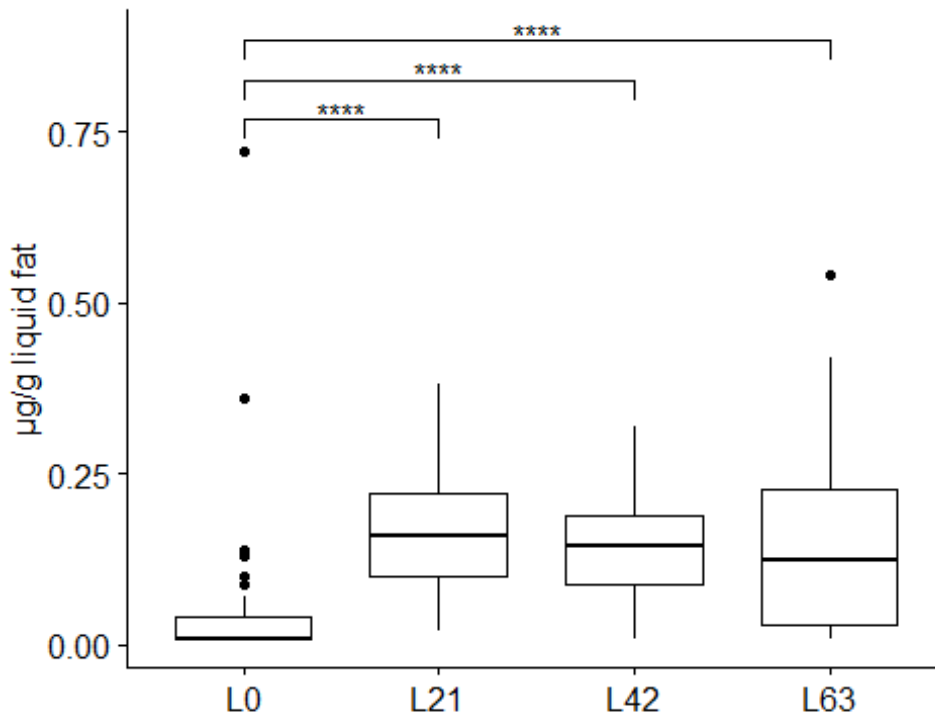
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666 **Figure 2**

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671 **Figure 3**

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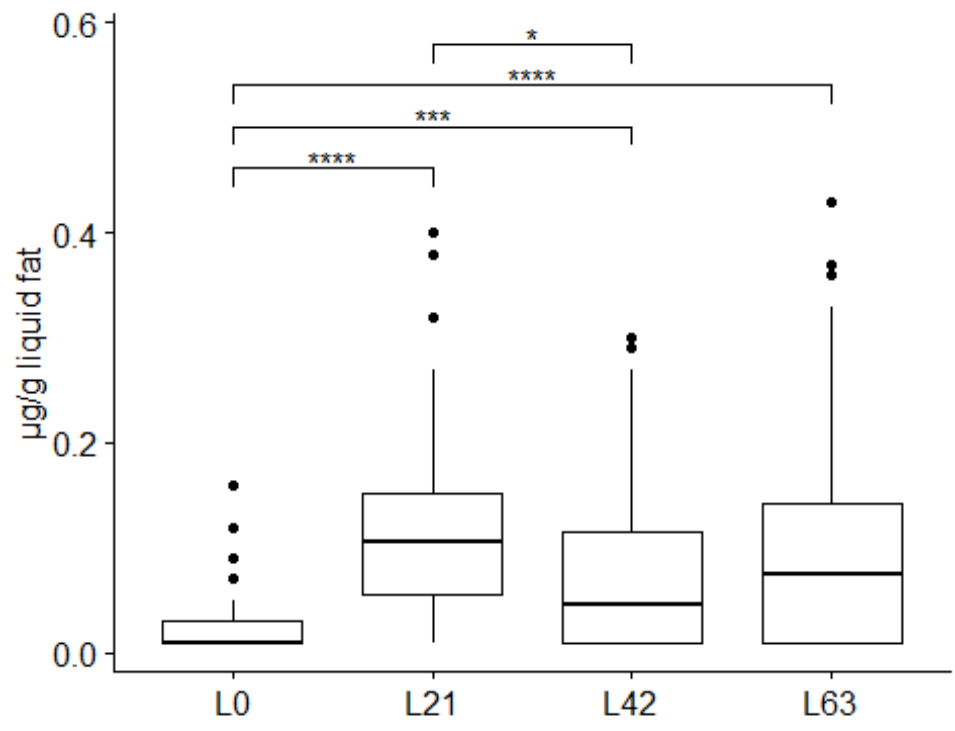
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679 **Figure 4**

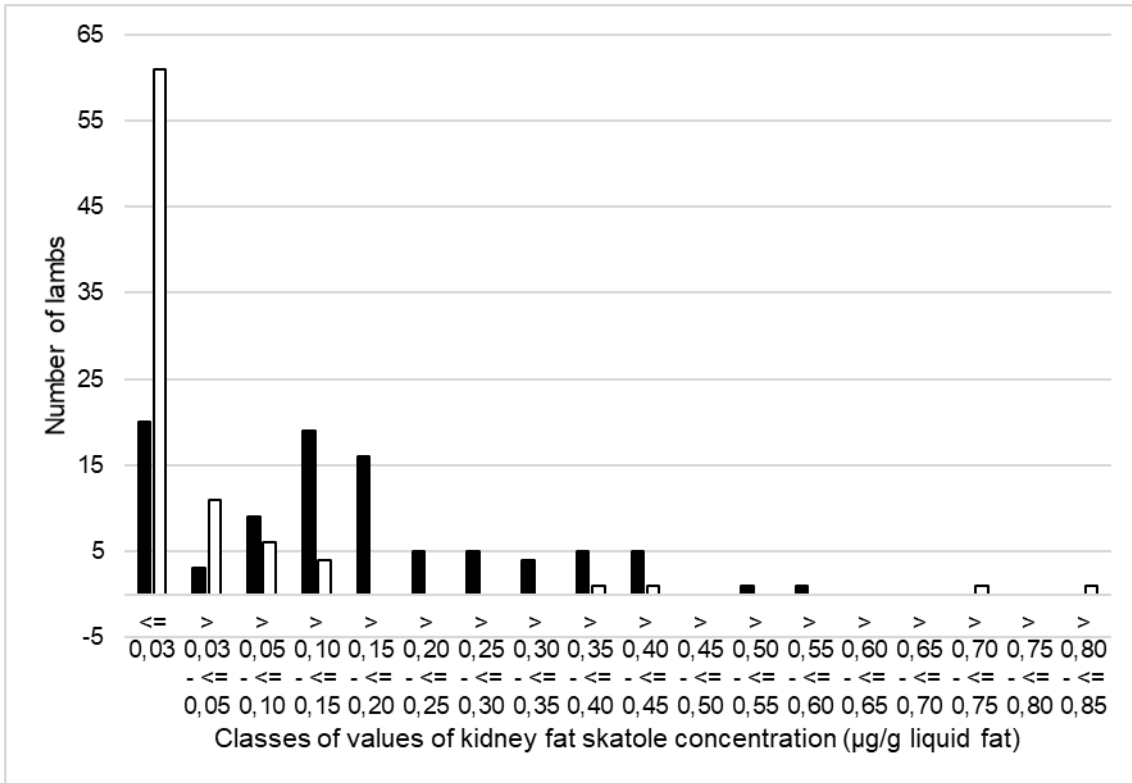
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686 **Figure 5**

687

688 **Table 1** Composition of the concentrate given to lambs when raised indoors (g/kg). The
689 net energy for meat production and the crude protein content were 0.93 Unités
690 Fourragères Viande/kg DM and 133 g/kg DM, respectively.

Barley	302
Wheat	228
Sugar beet pulp	142
Rapeseed meal	110
Sunflower meal	76
Wheat red shorts	37
Wheat bran	30
Corn grain	20
Sugarcane molasses	15
Calcium carbonate	10
Palm oil	10
Vegetable extracts to prevent renal calculus	6
Trace elements and vitamins	10.5
Salt	3

691

692

693 **Table 2** Carcass characteristics of lambs fattened on alfalfa pasture for various
694 durations before slaughter

Treatment group ⁽¹⁾	L0	L21	L42	L63	SEM ⁽²⁾	<i>P</i> -value
Number of lambs	52	56	55	55		
LW ⁽³⁾ at the beginning of the grazing period (kg)	-	37.3a	31.9b	27.0c	2.82	<i>P</i> < 0.001
LW ⁽³⁾ gain during the grazing period	-	0.01a	7.71b	15.26c	1.048	<i>P</i> < 0.001
Carcass weight (kg)	18.75a	17.29b	17.60b	19.41a	1.518	<i>P</i> < 0.001
Carcass conformation	6.83	6.54	6.60	6.89	0.292	<i>P</i> = 0.41
Carcass fatness	7.44a	6.14b	6.33b	6.99a	0.515	<i>P</i> < 0.001
Dorsal fat thickness (mm)	3.2b	2.6a	2.6a	2.9a	0.291	<i>P</i> < 0.001

695 Means with unlike letters are significantly different.

696 ⁽¹⁾ L0, L21, L42 and L63: lambs fattened on alfalfa pasture for 0 day, 21 days, 42
697 days and 63 days before slaughter, respectively.

698 ⁽²⁾ SEM: standard error of the mean.

699 ⁽³⁾ Live-weight

700

701

702 **Figure captions**

703

704 **Figure 1** Overview of the course of the experiment. Red and green refer to
705 indoor feeding with a concentrate-based diet and alfalfa grazing periods,
706 respectively.

707

708 **Figure 2** Box-plot representation of kidney-fat weight in lambs that have grazed
709 lucerne for 0, 21, 42 or 63 days before slaughter (n = 55, 55, 56 and 52 lambs,
710 respectively). The upper edge of the box indicates the 75th percentile of the
711 data-set, the bold line in the box indicates the median value, and the lower edge
712 indicates the 25th percentile of the data-set. The black circles represent the
713 outliers. *: $P < 0.05$; **: $P < 0.01$; ***: $P < 0.001$; ****: $P < 0.0001$.

714

715 **Figure 3** Box plot representation of kidney fat skatole concentration in lambs
716 that have grazed lucerne for 0, 21, 42 or 63 days before slaughter (n = 55, 55,
717 56 and 52 lambs, respectively). The upper edge of the box indicates the 75th
718 percentile of the data-set, the bold line in the box indicates the median value,
719 and the lower edge indicates the 25th percentile of the data-set. The black
720 circles represent the outliers. *: $P < 0.05$; **: $P < 0.01$; ***: $P < 0.001$; ****: $P <$
721 0.0001 .

722

723 **Figure 4** Box plot representation of the kidney fat indole concentration in lambs
724 that have grazed lucerne for 0, 21, 42 or 63 days before slaughter (n = 55, 55,
725 56 and 52 lambs, respectively). The upper edge of the box indicates the 75th

726 percentile of the data-set, the bold line in the box indicates the median value,
727 and the lower edge indicates the 25th percentile of the data-set. The black
728 circles represent the outliers. *: $P < 0.05$; **: $P < 0.01$; ***: $P < 0.001$; ****: $P <$
729 0.0001.

730

731 **Figure 5** Distribution of lambs fattened for at least two months on alfalfa
732 pastures without any supplementation (solid symbols) and lambs fed indoors a
733 concentrate-based diet (open symbols), stratified into classes of kidney fat
734 skatole concentration. The data ($n = 93$ and 94 for lambs fattened for at least
735 two months on alfalfa pastures and lambs fed indoors a concentrate-based diet,
736 respectively) come from four experiments (this study, plus Rivaroli et al., 2019,
737 Devincenzi et al., 2019 and Eiras et al., 2022) conducted during 6 consecutive
738 years.

739