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Abstract

Meat from lambs fattened on alfalfa is at risk of excessive pastoral flavours due to high levels of in-fat volatile indolic compounds (especially, skatole). Skatole has also been identified as a potential marker of interest for authenticating pasture-fed lamb meat. Here, we investigated the change in skatole and indole concentrations in kidney fat from lambs switched from an indoor-fed concentrate based diet to outdoor alfalfa grazing for various durations (0, 21, 42, 63 days) before slaughter. The study used a total of 219 lambs over 3 consecutive years. Kidney-fat skatole and indole concentrations increased from as early as 21 days on alfalfa, and then reached a plateau. Similarly, the proportion of lambs that had a kidney fat-skatole concentration above 0.15 µg/g liquid fat, a value that has been established as a sensory rejection threshold for pork, increased significantly from as early as 21 days on alfalfa and then reached a plateau. This value was reached or exceeded in a significant proportion of lambs fattened on alfalfa pastures (45.1%). However, skatole was not detected in kidney fat from 20 out of 164 alfalfa-fattened lambs (i.e., 12.2%) but was detected in 15 out of 55 concentrate-fed lambs (i.e., 27.3%). We thus conclude that while skatole content in kidney fat can inform on dietary changes made shortly before slaughter, it does not have the discrimination power needed to reliably authenticate pasture-fed lamb meat, let alone duration of finishing on pasture.

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

1. Introduction

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

Forage legumes offer many agro-environmental benefits and can play an important role in sustainable sheep production, notably by reducing the level of external inputs while improving animal productivity (Frank et al., 2016; Ponnampalam et al., 2022). These plants can improve soil fertility through their ability to fix atmospheric nitrogen. In addition, alfalfa (*Medicago sativa*) can improve soil stucture due to its deep root system compared to grass, which also makes it resistant to dry conditions. Grazing alfalfa had also been shown to improve the nutritional quality of lamb meat compared to grazing perennial ryegrass (*Lolium perenne*) or feeding feedlot pellets (Ponnampalam et al., 2019; Gruffat et al., 2020). Nevertheless, pastures including alfalfa or white clover (*Trifolium repens*) may result in increased fat concentration of volatile indolic compounds in lamb meat (Schreurs et al., 2007a and b; Devincenzi et al., 2014), and thus in increased meat flavour intensity (Schreurs et al., 2007a; Devincenzi et al., 2014 and 2019; Rivaroli et al., 2019) and increased risk of undesirable pastoral flavours (Park et al., 1972 and 1975), especially for consumers accustomed to meat from grain-based farming systems. However, this has not always been observed (Schreurs et al., 2007b; de Brito et al., 2016; Frank et al., 2016), sometimes due to the small number of animals under study (Schreurs et al., 2007b). Note that alfalfa can be consumed in different forms, but only fresh forage or silage can lead to high levels of skatole and indole in the rumen and fat, as drying and dehydration greatly reduce protein degradation in the rumen (INRA, 2018), and thus skatole and indole formation in the rumen.

Research has suggested that the detection threshold for skatole-related odour and flavour is about 0.05 µg/g fat in butter (Watkins et al., 2014) and up to 0.1 µg/g fat in pork (Banon et al., 2003; Lunde et al., 2010), and that the sensory rejection threshold for skatole is 0.15 µg/g fat in pork (Mörlein et al., 2012). This threshold can be considered as an indicator for the identification of carcasses whose meat is likely to be rejected by consumers. However, consumer acceptance of meat is highly dependent on how it is processed and consumed. As skatole and indole are lipophilic and volatile molecules, their concentration is much higher in fat than in lean tissue and their odour is more strongly perceived when the meat product has been heated. Thus, the risk of consumer rejection is higher for sausages or bacon than for rindless chops or ham (Parois et al., 2018). Furthermore, because lamb meat is less processed than pork and other volatile compounds may interfere with skatole and indole, such as androstenone in pork (Parois et al., 2018) or branched chain fatty acids in lamb meat (Young et al., 1997), generalization of this threshold established for pig carcasses to lamb carcasses must be done with caution. However, in the absence of comparable information for lamb carcasses, we used, in this study, that established for pig carcasses. Finally, it should be noted that the number of lambs used in previous studies was too small to enable proper assessment of the proportion of lambs that are above this latter threshold (0.15 µg/g fat) based on their diet.

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

Skatole in fat has also been investigated for its potential value in diet authentication, particularly for authenticating pasture-fed meat (Priolo et al., 2004; Vasta et al., 2006; Prache et al., 2020). However, the literature on this issue is so far inconclusive, probably due to both the high variability in animal response and the low number of animals under study (Vasta et al., 2006; Prache et al., 2020).

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

2. Material and methods

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

2.1. Experimental design, animal management and diet regimens

The experiment compared four durations of grazing alfalfa before slaughter: 0 day (L0), 21 days (L21), 42 days (L42), and 63 days (L63). The experiment was replicated three times during three consecutive years. Nineteen Romane weaned male lambs were used each year for each treatment group, giving 228 Romane lambs used in total. The lambs were born within a few weeks (between 8 and 30 April in year 1, between 6 and 30 April in year 2, and between 29 April and 1 May in year 3). Slaughter dates were scheduled each year so that the lambs would be slaughtered at approximately 5 months old, an age that yields carcasses of commercially acceptable weight and degree of fatness, as shown in previous research using Romane lambs fattened on alfalfa pastures after weaning (Devincenzi et al., 2019; Rivaroli et al., 2019; Eiras et al., 2022). Dates for turning out to pasture were then calculated based on the planned slaughter dates. Figure 1 gives a schematic diagram of the experimental layout.

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

Before grazing alfalfa, the L21, L42 and L63 lambs were kept indoors and-fed a high-concentrate diet. During the stall-feeding period, concentrate was given at a level adjusted to achieve similar mean patterns of growth between concentrate-fed lambs and pasture-fed lambs, and straw was given *ad libitum*. Pasture-fed lambs from the different treatment groups co-grazed in a single group to limit potential confounding effects of sward characteristics, except during the first 5 days following the turn-out to pasture to allow for a dietary transition when switching from concentrate feeding to alfalfa grazing (Figure 1). During this transition, lambs grazed a grass plot and were allowed access to an adjacent alfalfa plot for increasing time duration over the course of the 5 days. Two dry ewes were added to this group of pasture-finished lambs to train them in pasture use. In each experimental year, lambs in the same treatment group were slaughtered on the same day, so that all the pasture-fed lambs in a given treatment group could be turned out from stall to pasture at the same date, thus facilitating the 5-days diet transition. The plots were grazed rotationally, and lambs were moved to a new grazing break as pasture conditions dictated so as to ensure *ad libitum* availability of green alfalfa leaves. Pasture herbage was cut and the trimmings removed, one month prior to commencement of the experiment and after each grazing period, to ensure good-quality regrowths. Pasture-fed lambs received drenches against digestive nematodes and *Moniezia* if necessary, based on regular measurements of faecal egg counts performed at monthly intervals after turn out to pasture.

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

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

2.2. Measurements

2.2.1. Lamb live-weight

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

2.2.2. Carcass characteristics and fat sampling

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

2.2.3. Fat skatole and indole concentration analysis

Kidney fat skatole and indole concentrations were determined by HPLC using the procedure described by Batorek et al. (2012) as follows: fat samples were liquefied in a microwave oven for 2 × 1 min at 350 W. The liquefied lipids were centrifuged for 20 min at 11,200 g at 20°C. After centrifugation, the fat was heated to 50°C and 0.5 ± 0.01 g of water-free liquid fat was transferred into 2.5 mL Eppendorf tubes with 1 mL methanol containing 0.050 mg/L 2 of 3-methylindole (internal standard). After stirring for 30 s, the tubes were incubated for 5 min at 30°C in an ultrasonic water bath, put on ice for 20 min, and centrifuged for 20 min at 11,200 g at 4°C. For skatole and indole determinations, 20 µL of supernatant was injected into an HPLC column, and fluorescence was detected (excitation at 285 nm and emission at 340 nm) using a HP1200 system (Agilent Technologies, Waldbronn, Germany). Concentrations were expressed in µg per gram of lipid fraction from adipose tissue. The limit of detection was 0.03 µg/g liquid fat.

2.3. Data analyses

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

3. Results and discussion

3.1. Animal performance and carcass characteristics

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

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

The LW change during the 3 weeks after the switch from stall to pasture was in the order L63 (4.15 kg, range: -1.3 to 8.0 kg) > L42 (1.48 kg, range: -2.3 to 4.6 kg) > L21 lambs (0.01 kg, range: -6.0 to 6.0 kg), with a high variability between animals in a same feeding treatment. This abrupt change in diet and living conditions, which was dictated by the objectives of the study, caused some stress to some animals, particularly in the L21 and, to a lesser extent, L42 treatment groups, as had already been observed by Huang et al. (2015) and Eiras et al. (2022) for the reverse pattern of change in diet, i.e., switching from pasture-feeding to concentrate-feeding indoors. We chose to co-graze all pasture-fed lambs together in order to avoid confounding effects of sward characteristics (which could have induced undesirable differences in the quantity and quality of the herbage ingested and therefore, potentially, in the fat skatole and indole concentrations). However, co-grazing did not avoid unpredictable variability in animal performance, and resulted in lower carcass weight and degree of fatness in L21 and L42 lambs compared to L0 and L63 lambs. These differences were, however, relatively unsubstantial.

3.2. Fat skatole and indole concentrations

In this study, skatole and indole concentrations were measured in kidney fat to ensure that all lambs had sufficient fat for sampling. Previous studies in sheep have measured skatole and indole concentrations in kidney fat (Young et al., 2003; Girard et al., 2016), subcutaneous fat (Young et al., 1997; Priolo et al., 2004 and 2009), or both these fat depots (Devincenzi et al., 2014 and 2019; Rivaroli et al., 2019; Eiras et al., 2022). Devincenzi et al. (2014) observed a good correlation between these two fat depots for skatole concentration (r^2 = 0.78); such an analysis was not possible for indole concentration due to a large number of samples below the detection limit. Moreover, the effect of various treatments on fat skatole and indole concentrations showed high similarity between the two fat depots (Rivaroli et al., 1019; Devincenzi et al., 2019; Eiras et al., 2022). Skatole and indole, which are lipophilic compounds, are also expected to be present in intramuscular fat, and the corresponding analysis would be of great interest. However, this analysis poses technical difficulties because of the low level of intramuscular fat and thus the low concentrations of skatole and indole in the lean tissue. To our knowledge, no such data is available for lamb, but Devincenzi et al. (2014), in lambs fed various dietary proportions of fresh alfalfa forage showed that there was substantial similarity between the lean part of the chop and the fat part of the chop in the change in intensity of the skatole-related odour and flavour. Some studies on pork showed a significant correlation between subcutaneous fat and lean tissue for skatole and indole concentrations, and, as expected, lower concentrations in lean tissue than in subcutaneous fat (Rius and Garcia-Regueiro, 2001; Wauters et al., 2016; Meinert et al., 2017).

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

Given that there was a treatment effect on kidney fat weight (Figure 2), it is questionable whether kidney fat weight may have been a confounding factor. There is no scientific literature on this kind of potential dilution effect. However, there was no significant correlation between kidney fat skatole content and kidney fat weight for either pasture-fed or concentrate-fed lambs. Moreover, the kidney fat skatole concentration found here was fairly similar to that found by Eiras et al. (2022), Rivaroli et al. (2019) and Devincenzi et al. (2019) in Romane lambs grazing alfalfa that had much higher kidney fat weights. This casts doubt on a possible dilution effect of kidney fat skatole content related to kidney fat weight. Here, kidney fat skatole concentration in the L63 lambs ranged from 0.01 to 0.54 µg/g liquid fat, whereas it ranged from 0.08 to 0.41 µg/g in Eiras et al. (2022), from 0.03 to 0.41 µg/g in Rivaroli et al. (2019), and from 0.09 to 0.56 µg/g in Devincenzi et al. (2019).

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

3.3. Proportion of lambs in which skatole concentration was above 0.15 µg/g liquid fat

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

The skatole concentration in kidney fat varied widely between lambs in a same treatment group, in line with previous studies (Young et al., 2003; Schreurs et al., 2008; Devincenzi et al., 2019; Rivaroli et al., 2019; Eiras et al., 2022). Six out of 52 concentrate-fed (L0) lambs had high levels of kidney fat skatole and were considered outliers. High skatole concentrations in kidney fat are rare in concentrate-fed lambs but have been observed in previous studies (Devincenzi et al., 2019; Eiras et al., 2022). Despite these empirical evidences, the underlying reasons remain poorly understood. Conversely, skatole and indole were not detected in a number of lambs fattened on alfalfa, as already observed in previous studies (Rivaroli et al., 2019; Eiras et al., 2022). This high variability between animals suggests that future research could be directed at assessing the content of these off-flavour-causing compounds in the live animal (e.g. in the blood), as proposed by Eiras et al. (2022), in order to furnish estimates of between-animal variation in fat skatole concentration and adapt the nutritional strategy to mitigate over-intense flavours if necessary.

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

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

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

Thus, another new finding from this study is that, even though skatole content in kidney fat can inform on dietary changes made shortly before slaughter (i.e., as early as 21 days of grazing), it does not have the discriminatory power to reliably authenticate pasture-fed lambs. Even though alfalfa is known to promote high levels of in-fat skatole, skatole was not detected in kidney fat from 12.2% of alfalfa-grazed lambs, but was detected in kidney fat from 27.3% of concentrate-fed lambs. Furthermore, the reliability of the discrimination between concentrate-fed and pasture-fed lambs based on kidney fat skatole content is likely lower when lambs graze grass pastures or legumes containing condensed tannins (such as sainfoin, sulla, or birdsfoot, for example), which are known to lead to lower fat skatole concentration (Schreurs et al., 2008; Girard et al., 2016; Rivaroli et al., 2019). This means that fat skatole concentration has to be combined with other tissue composition measures, preferably showing different pattern of change with duration of pasture-feeding before slaughter, to increase the reliability of diet authentication. A candidate compositional variable for this purpose could be meat n-3 PUFA content, as Noci et al. (2005) observed a linear increase in n-3 PUFA content in beef as a function of pasture-feeding duration before slaughter. Spectral methods that incorporate diet-related differences in tissue composition can take advantage of these different patterns of change with duration of pasture-finishing (Prache et al., 2020).

Conclusion

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

Ethics approval

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

Data and model availability statement

None of the data used were deposited in an official repository

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

Declaration of competing interest

None.

Data availability

Data will be made available on request

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References

Aurousseau, B., Bauchart, D., Faure, X., Galot, A.L., Prache, S., Micol, D., & Priolo, A. (2007). Indoor fattening of lambs raised on pasture: (1) Influence of stall finishing duration on lipid classes and fatty acids in the *longissimus thoracis* muscle. *Meat Science* 76, 241-252.

Banon, S., Costa, E., Gil, M.D., & Garrido, M.D. (2003). A comparative study of boar taint in cooked and dry-cured meat. *Meat Science*, 63, 381-388.

Batorek, N, Škrlep, M, Prunier, A, Louveau, I, Noblet, J, Bonneau, M, & Čandek-Potokar, M. (2012). Effect of feed restriction on hormones, performance, carcass traits, and meat quality in immunocastrated pigs. *Journal of Animal Science* 90, 4593–4603.

De Brito, G., McGrath, S.R., Holman, B.W.B., Friend, M.A., Fowler, S.M., van de Ven, R., & Hopkins, D.L. (2016). The effect of forage type on lamb carcass traits, meat quality and sensory traits. *Meat Science* 119, 95-101.

Devincenzi, T., Prunier, A., Méteau, K., Nabinger, C., & Prache, S. (2014). Influence of fresh alfalfa supplementation on fat skatole and indole concentration and chop odour and flavour in lambs grazing a cocksfoot pasture. *Meat Science* 98, 607-614.

Devincenzi, T., Prunier, A., Méteau, K., & Prache, S. (2019). How does barley supplementation in lambs grazing alfalfa affect meat sensory quality and authentication? *Animal* 13, 427-434.

Eiras, C.E., Prunier, A., Meteau, K., Nunes do Prado, I., & Prache, S. (2022). Is a short concentrate-finishing period in lambs raised on alfalfa pasture effective at reducing fat indoles contents and lightening meat colour? *Animal* 16, 100610.

Font-i-Furnols, M., Realini, C.E., Guerrero, L., Oliver, M.A., Sanudo, C., Campo, M.M., Nute, G.R., Caneque, V., Alvarez, I., San Julian, R., Luzardo, S., Brito, G., & Montossi, F. (2009). Acceptability of lamb fed on pasture, concentrate or combinations of both systems by European consumers. *Meat Science* 81, 196-202.

Frank, D., Watkins, P., Ball, A., Krishnamurthy, R., Piyasiri, U., Sewell, J., Ortuno, J., Stark, J., & Warner, R. (2016). Impact of brassica and lucerne finishing feeds and intramuscular fat on lamb eating quality and flavor. A cross-cultural study using chinese and non-chinese australian consumers. *Journal of Agricultural and Food Chemistry* 64, 6856-6868.

Girard, M., Dohme-Meier, F., Silacci, P., Kragten, S.A., Kreuzer, M., & Bee, G. (2016). Forage legumes rich in condensed tannins may increase n-3 fatty acid levels and sensory quality of lamb meat. *Journal of the Science of Food and Agriculture* 96, 1923-1933.

Gkarane, V., Brunton, N.P., Allen, P., Gravador, R.S., Claffey, N.A., Diskin, M.G., Fahey, A.G., Farmer, L.J., Moloney, A.P., Alcalde, M.J., Murphy, P., & Monahan, F.J. (2019). Effect of finishing diet and duration on the sensory quality and volatile profile of lamb meat. *Food Research International* 115, 54-64.

Gruffat, D., Durand, D., Rivaroli, D., do Prado, I.N., & Prache , S. (2020). Comparison of muscle fatty acid composition and lipid stability in lambs stall-fed or pasture-fed alfalfa with ou without sainfoin pellet supplementation. *Animal* 14, 1093-1101.

Huang, Y, Andueza, D, Oliveira, L, Zawadzki, F, & Prache, S. (2015). Visible spectroscopy on carcass fat combined with chemometrics to distinguish pasture-fed, concentrate-fed and concentrate-finished pasture-fed lambs. *Meat Science* 101, 5-12.

INRA (2018). INRA feeding system for ruminants. Wageningen Academic Publishers, Wageningen, the Netherlands, 640 pp.

Lunde, K., Skuterud, E., Hersleth, M., & Egelandsdal, B. (2010). Norwegian consumers' acceptability of boar tainted meat with different levels of androstenone or skatole as related to their androstenone sensitivity. *Meat Science* 86, 706-711.

Meinert, L., Lund, B., Bejerholm, C., & Aaslyng, M.D. (2017). Distribution of skatole and androstenone in the pig carcass correlated to sensory characteristics. *Meat Science* 127, 51-56.

Mörlein, D., Grave, A., Sharifi, A.R., Bücking, M., & Wicke, M. (2012). Different scalding techniques do not affect boar taint. *Meat Science*, 91, 435-440.

Noci, F., Monahan, F.J., French, P., & Moloney, A.P. (2005). The fatty acid composition of muscle fat and subcutaneous adipose tissue of pasture-fed beef heifers: Influence of the duration of grazing. *Journal of Animal Science* 83, 1167-1178.

Park, R.J., Corbett, J.L., & Furnival, E.P. (1972) Flavour differences in meat from lambs grazed on lucerne (*Medicago sativa*) or phlaris (*Phalaris tuberosa*) pastures. *Journal of Agricultural Science, Cambridge* 78, 47-52.

Park, R.J., Ford, A., Minson, D.J., & Baxter, R.I. (1975) Lucerne-derived flavour in sheep meat as affected by season and duration of grazing. *Journal of Agricultural Science, Cambridge* 78, 47-52.

Parois, S., Bonneau, M., Chevillon, P., Larzul, C., Quiniou, N., Robic, A., & Prunier, A. (2018) Odeurs indésirables de la viande de porcs mâles non castrés : problèmes et solutions potentielles. *INRA Productions Animales* 31(1), 23-36.

Ponnampalam, E.N., Vahedi, V., Giri, K., Lewandowski, P., Jacobs, J.L., & Dunshea, F.R. (2019) *Nutrients* 11, 723.

Ponnampalam, E.N., & Holman, B.W.B. (2022) Sustainable animal production and meat processing. In Toldra, F. (Ed.) 'Lawrie's Meat Science Textbook-Edition 9'.

Prache, S., Martin, B., & Coppa, M. (2020). Review: Authentication of grass-fed meat and dairy products from cattle and sheep. *Animal* 14, 854-863.

Prache, S., Schreurs, N., & Guillier, L. (2022). Review: Factors affecting sheep carcass and meat quality attributes. *Animal* 16, 100330.

Priolo, A., Micol, D., Agabriel, J., Prache, S., & Dransfield, E. (2002). Effect of grass or concentrate feeding systems on lamb carcass and meat quality. *Meat Science* 62, 179-185.

Priolo, A., Cornu, A., Prache, S., Krogmann, M., Kondjoyan, N., Micol, D., & Berdagué, J.L. (2004). Fat volatiles tracers of grass feeding in sheep. *Meat Science* 66, 475-481.

Priolo, A., Vasta, V., Fasone, V., Lanza, C.M., Scerra, M., Biondi, L., Bella, M., & Whittington, F.M. (2009). Meat odour and flavour and indoles concentration in ruminal fluid and adipose tissue of lambs fed green herbage or concentrates with or without tannins. *Animal* 3, 454-460.

Resconi, V.C., Campo, M.M., Font-i-Furnols, M., Montossi, F., & Sanudo, C. (2009). Sensory evaluation of castrated lambs finished on different proportions of pasture and concentrate feeding systems. *Meat Science* 83, 31-37.

Rius, M.A., & Garcia-Regueiro, J.A. (2001). Skatole and indole concentrations in *Longissimus dors*i and fat samples of pigs. *Meat Science* 59, 285-291.

Rivaroli, D., Prunier, A., Méteau, K., do Prado, I.N., & Prache, S. (2019). Tannin-rich sainfoin pellet supplementation reduces fat volatile indoles content and delays digestive parasitism in lambs grazing alalfa. *Animal* 13, 1883-1890.

Sanudo, C., Alfonso, M., San Julian, R., Thorkelsson, G., Valdimarsdottir, T., Zygoyiannis, D., Stamataris, C., Piasentier, E., Mills, C., Berge, P., Dransfield, E., Nute, G.R., Enser, M., & Fisher, A.V. (2007). Regional variation in the hedonic evaluation of lamb meat from diverse production systems by consumers in six European countries. *Meat Science* 75, 610-621.

SAS Institute Inc 2014. SAS 9.4 Language Reference: Concepts, 3rd edition. SAS Institute Inc., Cary, NC, USA.

Schreurs, N.M., Tavendale, M.H., Lane, G.A., Barry, T.N., McNabb, W.C., Cummings, T, Fraser, K., & Lopez-Villalobos, N. (2007a). The effect of supplementation of a white clover or perennial ryegrass diet with grape seed extract on indole and skatole metabolism and the sensory characteristics of lamb. *Journal of the Science of Food and Agriculture* 87, 1030-1041.

Schreurs, N.M., McNabb, W.C, Tavendale, M.H., Lane, G.A., Barry, T.N., Cummings, T., Fraser, K., Lopez-Villalobos, N., & Ramirez-Restrepo, C.A. (2007b). Skatole and indole concentration and the odour of fat from lambs that had grazed perennial ryegrass/white clover pasture or *Lotus corniculatus*. *Animal Feed Science and Technology* 138, 254-271.

Schreurs N.M., Lane, G.A., Tavendale, M.H., Barry, T.N., & McNabb, W.C. (2008). Pastoral flavour in meat products from ruminants fed fresh forages and its amélioration by forage condensed tannins. *Animal Feed Science and Technology* 146, 193-221.

Vasta, V., & Priolo, A. (2006). Ruminants fat volatiles as affected by diet. A review. *Meat Science* 73, 218-228.

Watkins, P.J., Frank, D., Singh, T.K., Young, O.A., & Warner, R.D. (2013). Sheepmeat flavor and the effect of different feeding systems: a review. *Journal of Agricultural and Food Chemistry* 61, 3561-3579.

Watkins, P.J., Kearney, G., Rose, G., Allen, D., Ball, A.J., Pethick, D.W., & Warner, R.D. (2014). Effect of branched-chain fatty acids, “-methylindole and 4-

methylphenol on consumer sensory scores of grilled lamb meat. *Meat Science* 96, 1088-1094.

Wauters, J., Vercruysse, V., Aluwé, M., Verplanken, K., & Vanhaecke, L. (2016). Boar taint compound levels in back fat versus meat products: Do they correlate? *Food Chemistry* 206, 30-36.

Young, O.A., Berdagué, J.L., Viallon, C., Rousset-Akrim, S., & Thériez, M. (1997). Fat-borne volatiles and sheepmeat odour. *Meat Science* 45, 183-200.

Young, O.A., Lane, G.A., Priolo, A., & Fraser, K. (2003). Pastoral and species flavour in lambs raised on pasture, lucerne or maize. *Journal of the Science of Food and Agriculture* 83, 93-104.

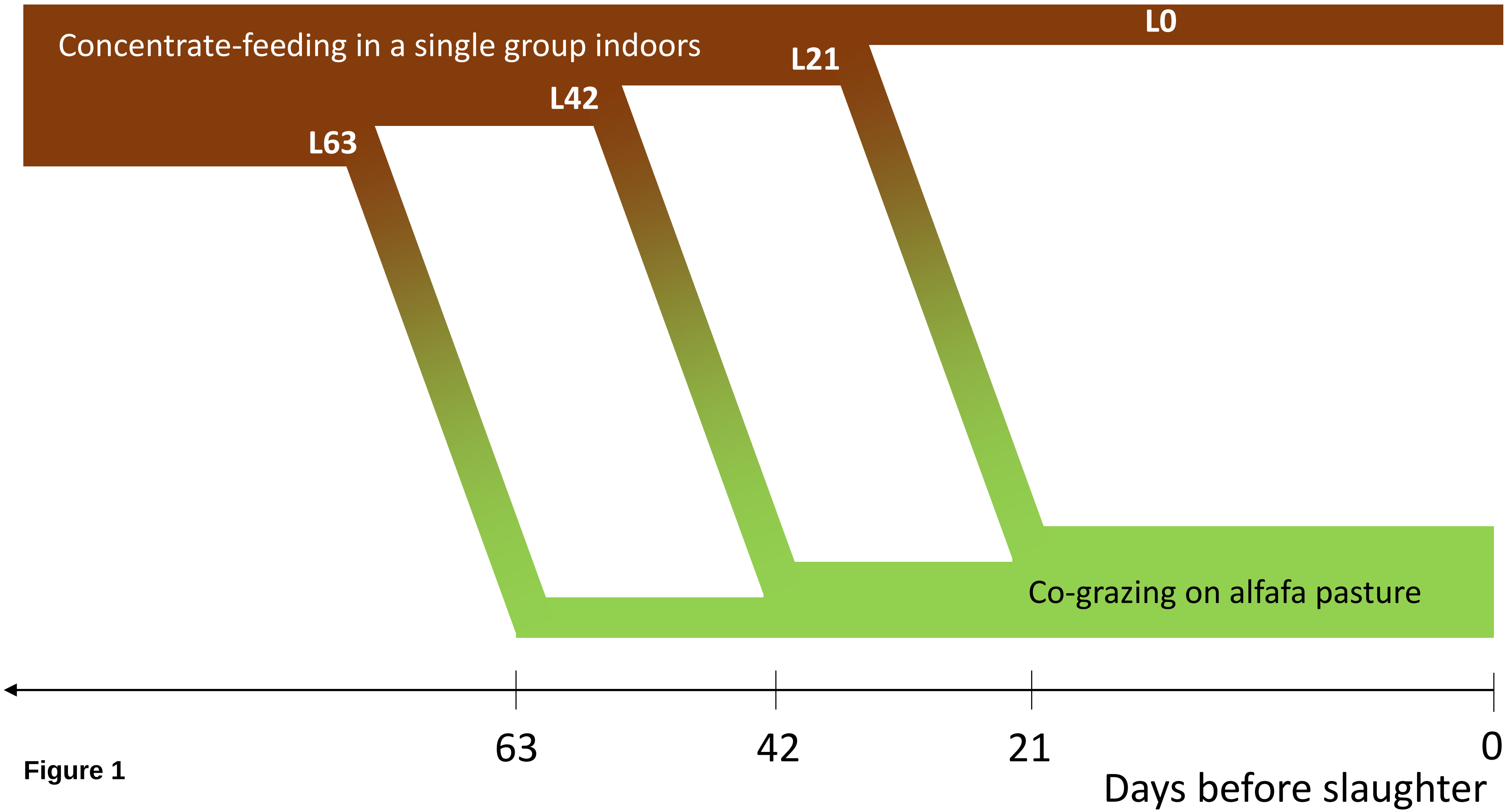
Concentrate-feeding in a single group indoors
L0
L21
L42
L63
Co-grazing on alfafa pasture
63
42
21
0
Days before slaughter

Figure 1

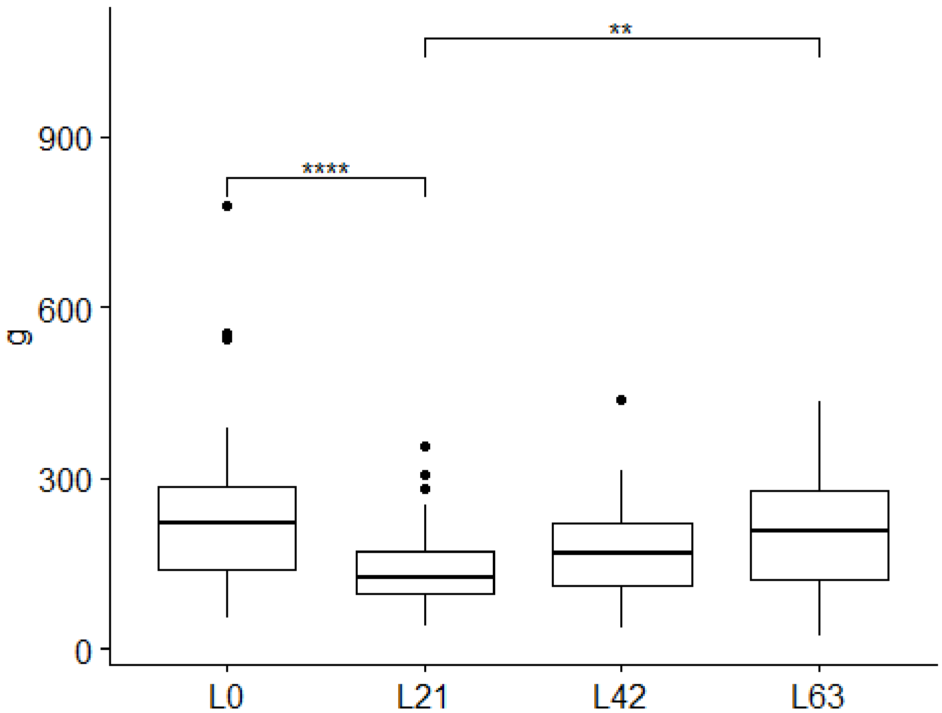

Figure 2

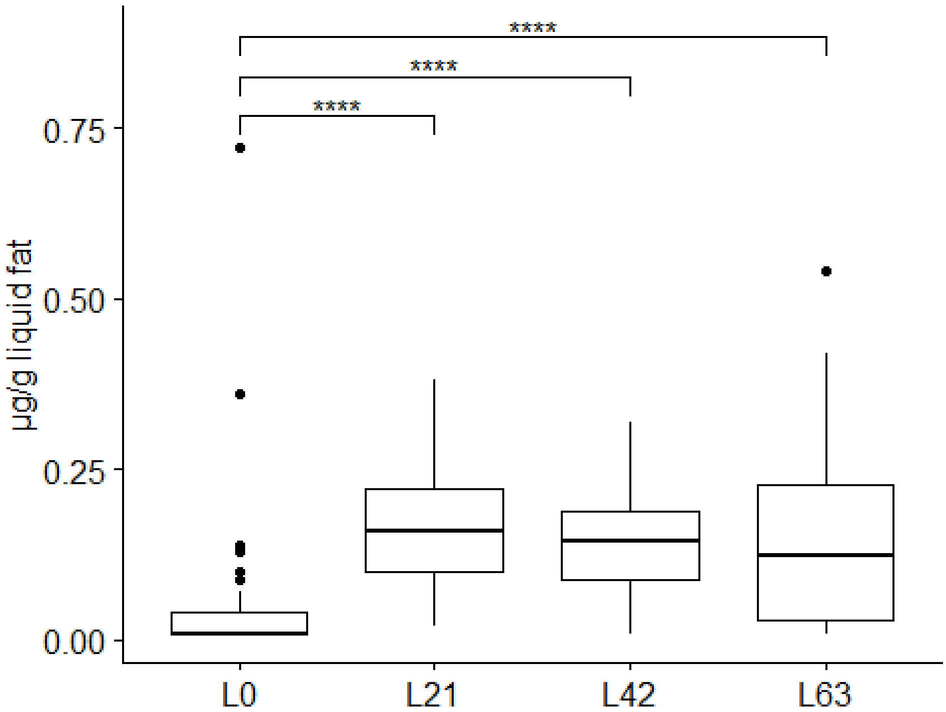

Figure 3

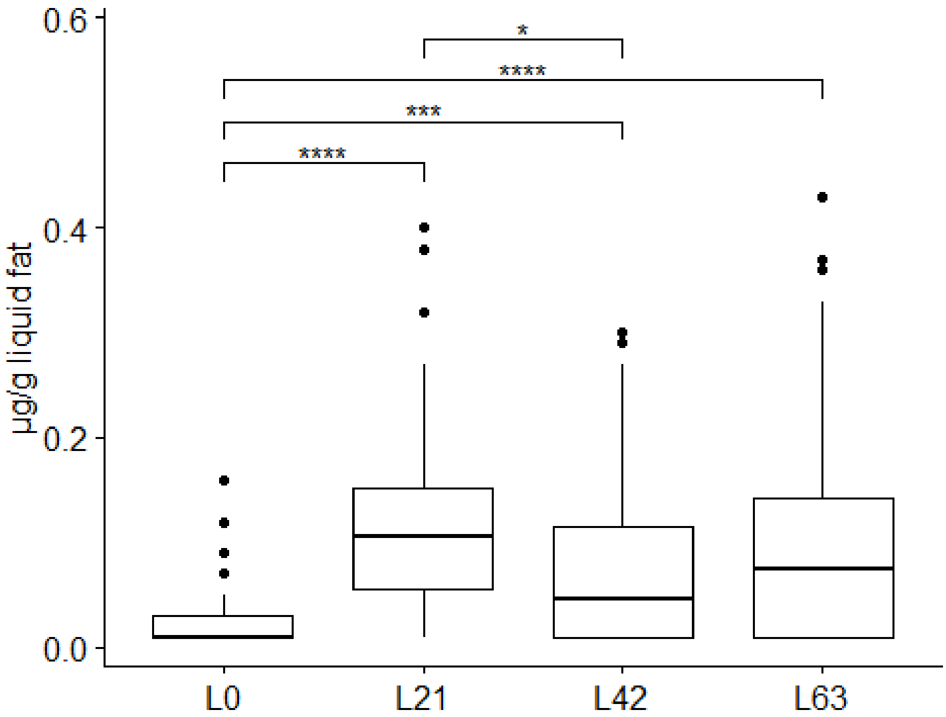

Figure 4

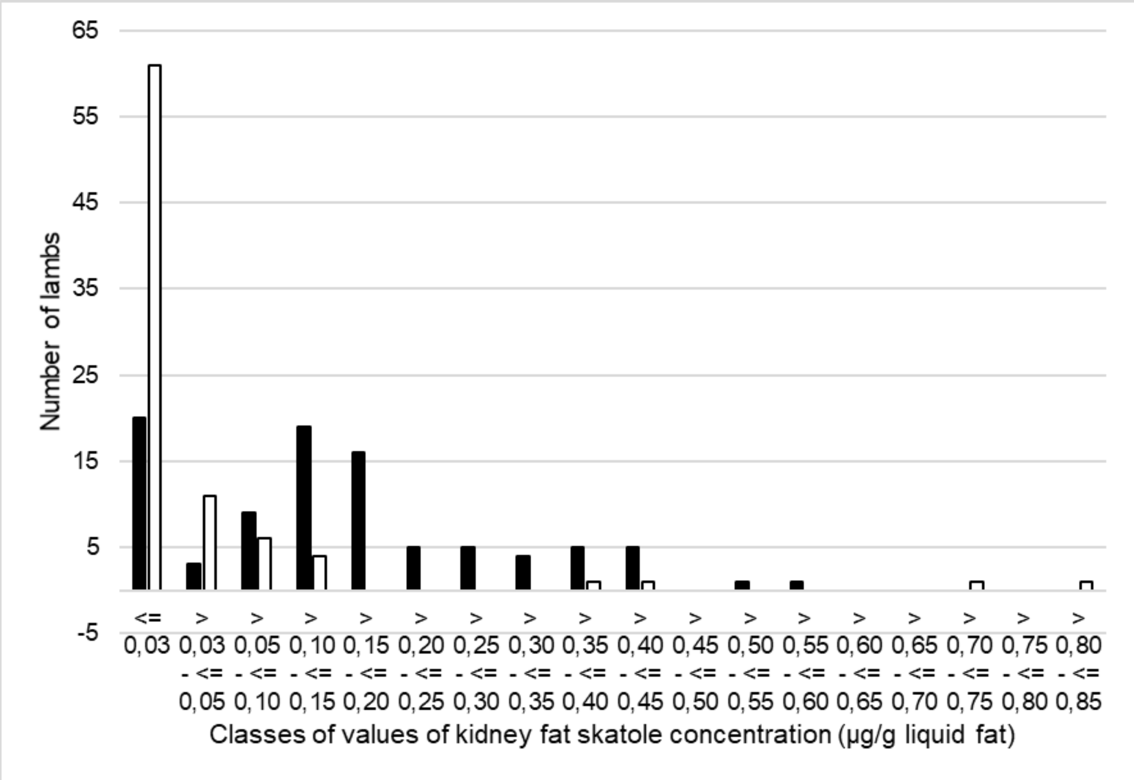
Number of lambs
65
55
45
35
25
15
5
-5
<= 0,03
> 0,03 - <= 0,05
> 0,05 - <= 0,10
> 0,10 - <= 0,15
> 0,15 - <= 0,20
> 0,20 - <= 0,25
> 0,25 - <= 0,30
> 0,30 - <= 0,35
> 0,35 - <= 0,40
> 0,40 - <= 0,45
> 0,45 - <= 0,50
> 0,50 - <= 0,55
> 0,55 - <= 0,60
> 0,60 - <= 0,65
> 0,65 - <= 0,70
> 0,70 - <= 0,75
> 0,75 - <= 0,80
> 0,80 - <= 0,85
Classes of values of kidney fat skatole concentration (µg/g liquid fat)

Figure 5

Table 1 Composition of the concentrate given to lambs when raised indoors (g/kg). The net energy for meat production and the crude protein content were 0.93 Unités 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

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

Treatment group[1]	L0	L21	L42	L63	SEM[2]	*P*-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	$P < 0.001$
LW[3] gain during the grazing period	-	0.01a	7.71b	15.26c	1.048	$P < 0.001$
Carcass weight (kg)	18.75a	17.29b	17.60b	19.41a	1.518	$P < 0.001$
Carcass conformation	6.83	6.54	6.60	6.89	0.292	$P = 0.41$
Carcass fatness	7.44a	6.14b	6.33b	6.99a	0.515	$P < 0.001$
Dorsal fat thickness (mm)	3.2b	2.6a	2.6a	2.9a	0.291	$P < 0.001$

Means with unlike letters are significantly different.

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

[2] SEM: standard error of the mean.

[3] Live-weight

Figure captions

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

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

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

Figure 4 Box plot representation of the kidney fat indole concentration in lambs that have grazed lucerne for 0, 21, 42 or 63 days before slaughter (n = 55, 55, 56 and 52 lambs, respectively). The upper edge of the box indicates the 75th percentile of the data-set, the bold line in the box indicates the median value, and the lower edge indicates the 25th percentile of the data-set. The black circles represent the outliers. *: $P < 0.05$; **: $P < 0.01$; ***: $P < 0.001$; ****: $P < 0.0001$.

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