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Influence of fresh alfalfa supplementation on fat skatole and indole concentration and chop odour and flavour in lambs grazing a cocksfoot pasture

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ABSTRACT

We investigated the influence of the level of fresh alfalfa supplementation on fat skatole and indole concentration and chop sensory attributes in grazing lambs. Four groups of nine male Romane lambs grazing a cocksfoot pasture were supplemented with various levels of alfalfa for at least 60 days before slaughter. Perirenal fat skatole concentration was higher for lambs that consumed alfalfa than for those that consumed only cocksfoot. The intensity of 'animal' odour in the lean part of the chop and of 'animal' flavour in both the lean and fat parts of the chop, evaluated by a trained sensory panel, increased from the lowest level of alfalfa supplementation onwards and did not increase further with increasing levels of alfalfa supplementation. The outcome of this study therefore suggests that these sensory attributes may reach a plateau when perirenal fat skatole concentration is in the range 0.16–0.24 μg/g of liquid fat.

1. Introduction

Low-input and organic farming livestock systems embody features that consumers value, such as animal welfare, food healthiness and environmental acceptability (Montossi, Font-i-Furnols, del Campo, San Julián, Brito & Sañudo, 2013). The presence of forage legumes in these systems is of major importance, because nitrogen-fixing plants improve pasture quality and reduce dependency on external inputs. However, the occurrence of off-flavours and off-odours in the meat has been shown to increase in lambs grazing legume-rich pastures (Prache, Gatellier, Thomas, Picard, & Bauchart, 2011; Schreurs, McNabb, Tavendale, Lane, Barry, Cummings, Fraser, Lopez-Villalobos & Ramirez-Restrepo, 2007; Young, Lane, Priolo, & Fraser, 2003). Some legume species, such as white clover (*Trifolium repens*) or alfalfa (*Medicago sativa*), play a prominent role in the ruminal synthesis of indole and skatole, which are smelling volatile components (Schreurs, Lane, Tavendale, Barry, & McNabb, 2008; Watkins, Frank, Singh, Young, & Warner, 2013; Young et al., 2003). Flavours described as animal, pastoral and faecal have been related to the presence of indole and skatole in lamb meat (Watkins et al., 2014; Young, Berdagué, Viallon, Rousset-Akrim, & Theriez, 1997; Young et al., 2003). In ruminants, indole and skatole are formed in the rumen from the microbial deamination and decarboxylation of tryptophan (Deslandes, Gariépy, & Houde, 2001; Schreurs et al., 2008). Their ruminal synthesis has been found to depend on diet (Carlson, Hammond, Breeze, Potchoiba, & Heinemann, 1983), and to increase when the pasture is rich in legume species with high degradable protein content (Schreurs, McNabb, et al., 2007; Schreurs et al., 2007). However, although the proportion of legume in the animal's diet can vary widely according to its proportion in the pasture and sward availability, there has not yet been any published information on the effect of the dietary legume level on the chop's sensory attributes. This study was therefore undertaken to evaluate the influence of different levels of alfalfa in the diet on fat indole and skatole concentrations and chop odour and flavour attributes.

2. Materials and methods

The study was conducted at the Herbivore Research Unit at the INRA Clermont-Ferrand/Theix Research Centre, France. The animals were handled by specialized staff who ensured their welfare in accordance with European Union Directive No. 609/1986.

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2.1. Experimental design, animals and diets

We compared four levels of fresh alfalfa (*M. sativa)* forage supplementation in lambs grazing a cocksfoot (*Dactylis glomerata*) pasture for at least 60 days before slaughter: no supplementation (U), low (L), medium (M) and high (H) level of supplementation. We used 36 non-castrated male Romane lambs from 9 rams and 28 dams. The supplementation level was calculated so that the alfalfa should represent 0%, 25%, 50% and 75% of the voluntary dry matter (DM) intake in U, L, M and H lambs respectively.

Two conterminal monocultures of alfalfa (1.0 ha) and cocksfoot (1.6 ha) were sown in October 2010. From 30 April 2012 onwards, both pastures were divided into four plots of similar area. In March 2012, both pastures received 67 kg P/ha and 100 kg K/ha. In April 2012, the cocksfoot pasture received 48 kg N/ha. Each week, one alfalfa plot was mown to ensure the provision of young forage throughout the experiment. The cocksfoot pasture was mown on 29 May to ensure a vegetative regrowth.

Lambs were born within 8 days (18 March–26 March 2012). Before weaning, the animals were managed uniformly and received no legumes in their diet (i.e. from the beginning of the gestation period for the dams). For approximately 50 days after lambing, the lambs and their dams were housed in a sheepfold, and the lambs were offered a commercial concentrate. From 04 May to 10 May, the lambs were offered freshly cut cocksfoot grass distributed indoors. Lambs and their dams were then turned out to the cocksfoot pasture for 18 days until weaning on 29 May (day 0, d0), at 69 days on average.

On d1, the 36 lambs were assigned to nine blocks of similar animals according to birth weight and average daily gain (ADG) between birth and d0. They were then randomly assigned from within the blocks to one of the four treatments. The treatments were thus applied to one lamb of each block. Mean lamb birth weight and ADG between birth and weaning were 4.2 (SD 0.71) kg and 301 (SD 49.6) g/d; lambs weighed 24.9 (SD 3.49) kg at weaning.

2.1.1. Pre-experimental adaptation period

During this 35-d pre-experimental period, the lambs were individually fed ad libitum indoors. From d1 to d8, all lambs were fed freshly cut cocksfoot. From d9 to d35, U, L, M and H lambs were fed a diet containing 0%:100%, 25%:75%, 50%:50% and 75%:25% alfalfa forage:cocksfoot forage (on a DM basis). The levels of fresh alfalfa and fresh cocksfoot offered were calculated on the basis of their assigned proportion in the diet and the estimation of their DM content. The two forages were offered in separate tubs.

2.1.2. Experimental period

From d36 until slaughter, each group of lambs was assigned to one of the four cocksfoot pasture plots and supplemented with freshly cut alfalfa using racks. Both cocksfoot pasture quality and availability were assumed not to limit lamb voluntary DM intake. The voluntary DM intake level of cocksfoot forage was assumed to be 78.1 g DM/LW$^{0.75}$, where LW was the average live weight of the group (Dulphy, Faverdin, & Jarrige, 1989). The amount of fresh alfalfa offered to each group was then based on the assigned proportion of alfalfa in the diet and the estimation of its DM content. Care was taken to use sufficiently large racks to avoid between-animal competition for alfalfa.

Fresh forages were cut at 6 cm above the ground every morning at 8 a.m. and offered half in the morning at 9 a.m., and half in the afternoon at 4 p.m. after storage at 4 °C. Feed tubs and racks were emptied every morning before the distribution of the fresh forage, and the refusals were weighed, recorded and discarded. The estimation of the forage DM content was made daily (except on weekends) in duplicate using a microwave oven. Representative samples of offered and refused forages were collected daily for final DM measurements (made in duplicate). Water and salt blocks were always available. The salt blocks contained (g/kg, as-fed) 60 Ca, 20 P, 10 Mg, 280 Na, 17.5 Zn, 1.5 Fe, 5.5 Mn, 0.03 Co, 0.03 I, and 0.01 Se.

The cocksfoot pasture was grazed continuously during the experimental period, but the groups of lambs were changed weekly from one cocksfoot plot to another to avoid confounding effects of sward characteristics for cocksfoot forage intake level. All lambs received anthelmintic drenches monthly.

2.2. Slaughter procedures

The lambs were slaughtered at the INRA Clermont-Ferrand Centre experimental slaughterhouse, according to European Union welfare guidelines. Three, 2 and 4 blocks of lambs were slaughtered on d97, d100 and d121. Blocks were selected for slaughter on the basis of mean LW, with the priority given to the heaviest blocks. Lambs were thus slaughtered at mean age 177 (SD 12) days, after an experimental period ranging from 61 to 85 days according to block. The lambs had access to food and water until approximately 30 min before slaughter, and were transported by truck to the slaughterhouse located close to the experimental pastures (<500 m). Immediately on arrival, the lambs were electrically stunned and slaughtered by throat cutting. The carcasses were placed in a refrigerated room at 4 °C until 24 h post mortem.

2.3. Measurements

2.3.1. Animal body weight

Lambs were weighed once weekly before the alfalfa distribution in the morning using an electronic scales.

2.3.2. Pasture availability

Fifty measurements of the cocksfoot sward surface height per plot were made weekly using a sward stick. Herbage mass was estimated weekly with a rising-plate metre (RPM, weight 430 g, 0.30 m × 0.30 m) using a double-sampling technique (Prache, Duby, & Froment, 1989). For each measurement date, a regression of herbage mass on RPM height was established on 12 quadrats (0.30 m × 0.30 m, three randomized quadrats per plot). At each location, the RPM height was measured, and the herbage in the quadrat was cut 1.5 cm above ground level with a "mini mower", and oven-dried at 60 °C for 72 h. Fifty measurements of the RPM height were then made on each cocksfoot plot. Herbage mass was then estimated for each plot using the mean RPM height together with the regression of herbage mass on RPM height.

2.3.3. Carcass characteristics and meat and fat sampling

Carcass weight, perirenal fat weight and subcutaneous fat thickness over the last thoracic rib were measured after 24 h cooling. A sample of approximately 25 g of perirenal fat was taken for all lambs. The subcutaneous fat was taken from the posterior end of the loin when it was available in sufficient amount (at least 10 g), which was the case for 18 lambs (8 H, 7 M, 2 L and 1 U lambs). Fat samples were wrapped, vacuum-packed in sealable polyamide bags and frozen at −20 °C until indole and skatole concentration analysis.

After chilling of the carcass for 24 h, a sample of the left *longissimus thoracis et lumborum* (LTL) muscle was taken from the last thoracic rib, vacuum packed and frozen at −20 °C until N isotope ratio analysis. The value of the nitrogen stable isotope ratio ($^{15}N/^{14}N$) in LTL muscle largely depends on the dietary $^{15}N/^{14}N$ ratio which is lower in legume than in grass plant species due to the capacity of leguminous plants to fix atmospheric nitrogen (DeNiro & Epstein, 1981; Devincenzi, Delfosse, Andueza, Nabinger, & Prache, 2014). Here, we used the $\delta^{15}N$ value of the LTL muscle as an indicator of the dietary proportion of alfalfa at the individual lamb level. The $\delta^{15}N$ value of the LTL muscle was calculated as $([(^{15}N/^{14}N_{muscle}) - (^{15}N/^{14}N_{air})] / (^{15}N/^{14}N_{air})) \times 1000)$.

The saddles (muscle, fat and bones) were removed from the posterior end of the loin from the right side of the carcass, wrapped and vacuum-