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Nitrogen stable isotope and carotenoid pigments signatures in the meat as tools to trace back the diet: Comparison between two sheep breeds

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ABSTRACT

We compared the response of two dietary biomarkers (nitrogen stable isotope and carotenoid pigments signatures in lamb meat) in two sheep breeds when fed two dietary lucerne levels, and we deduct subsequent implications for diet authentication issues. Two breeds (Limousine and Romane) and two daily dietary lucerne levels (H: High, 500 g; L: Low, 0 g) were used for 60 days pre-slaughter. Treatment H comprised 35 lambs of each breed, individually penned and fed. Treatment L comprised 8 lambs of each breed, fed in groups. The $\delta^{15}N$ value of the muscle did not differ between breeds and gave a correct classification score of 92.9% comparing lambs from both feeding treatments. The absolute value of the mean integral of perirenal fat reflectance spectrum, which quantifies light absorption by carotenoid pigments present in perirenal fat, and plasma carotenoid content were similar in L Romane and L Limousine lambs, but were higher in H Romane lambs than in H Limousine lambs As the carotenoid pigments are highly involved in the discrimination process between pasture-fed lambs and concentrate-fed lambs using visible spectroscopy on the fat, further work is required to test the extent to which this between-breed variability may modulate the reliability of pasture-feeding authentication using visible spectroscopy of the fat over a range of breeds.

1. Introduction

Several factors have contributed to research interest in the reconstruction of animal diet from the meat, such as (i) the consumer demand for guarantee about the animal diet, because of food crises such as the Bovine Spongiform Encephalopathy, which arose because of the use of feeds of animal origin, (ii) the evidence that the animal diet strongly influences the nutritional (Aurousseau et al., 2007a,b) and sensory (Priolo et al., 2002a) quality of the meat, and (iii) the interest for producers to obtain market recognition and to avoid piracy of their brands.

Carotenoid pigments and nitrogen (N) stable isotope signatures in the meat have already been used successfully to trace back beef and lamb diet. Actually, as the principal source of carotenoid and N in herbivorous animals is plant feed, the carotenoid content and isotopic composition of plant feed are the most important factor in carotenoid content and N isotopic composition of animal tissues (Camin et al., 2016). Nitrogen stable isotope ratio is measured using isotope ratio mass spectrometry (IRMS). Regarding carotenoid pigments present in the fat, Prache and Theriez (1999) proposed a mathematical analysis of the fat reflectance spectrum as a method to quantify their light absorption. The last fifteen years has witnessed important diffusion of this method for meat authentication (see reviews by Monahan et al., 2013 and Alvarez et al., 2015), because it is rapid, non-invasive and can be easily implemented in the abattoir with a portable spectrocolorimeter.

Carotenoid pigments signature in the perirenal fat has thus shown high potential for authenticating meat produced from herbivores fed green forage-based diets and particularly from pasture-fed herbivores, in sheep (Prache and Theriez, 1999; Priolo et al., 2002b; Ripoll et al., 2008; Huang et al., 2015a) and cattle (Serrano et al., 2006; Röhrle et al., 2011; Ripoll et al., 2015). The N stable isotope signature has also been proposed as a valuable tool to authenticate meat produced from pasture-fed animals (Monahan et al., 2012) and more specifically from organic beef (Schmidt et al., 2005; Bahar et al., 2008). Also, N stable isotope signature in the *longissimus thoracis et lumborum* muscle has been shown to be related to the dietary proportion of legume plants and has been successfully used to discriminate pasture-fed lambs that ate 50% of legumes in their diet from those that ate only graminae species (Devincenzi et al., 2014).

However, the extent to which carotenoid and N isotope signatures in the meat may be subjected to variations across breeds has not been investigated. These variations may yet modulate the reliability of diet authentication, which may therefore require breed-specific databases. Schwertl et al. (2003) found no difference between breeds in the N

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isotopic signatures in cattle hair, but they did not analysed the meat. Regarding carotenoid pigments, it has been suggested that the tendency to accumulate them has a genetic component: Morris et al. (2002) for example, reported, in large cow populations, that the carotenoid contents in both plasma and milk were positively correlated to the Jersey-to-Friesian gene ratio, the effect of the breed being maximal under high dietary carotenoid level. The colour of carcass fat also differs between breeds in steers (Dunne et al., 2009) and lambs (Baker et al., 1985) raised on pasture. However, despite empirical evidence, there are practically no experimental studies comparing carotenoid accumulation in different breeds fed similar dietary carotenoid levels.

The aim of this study was therefore to compare the response of both dietary biomarkers in animal tissues in two sheep breeds when fed two dietary lucerne pellets levels and deduct subsequent implications for diet authentication issues. Dehydrated lucerne pellets were of experimental interest for this purpose as (i) they contain high levels of carotenoid pigments, (ii) they are composed of a legume plant species and (iii) they are easy to use as a dietary component. We compared the Romane and the Limousine breeds, because previous studies suggested potential differences between both breeds in plasma and fat carotenoid concentrations (Dian et al., 2007a and Prache et al., 2009 in Romane pasture-fed lambs; Dian et al., 2007b in Limousine pasture-fed lambs).

2. Materials and methods

The experiment was conducted at the experimental farm of the Herbivores Research Unit run by the Clermont-Ferrand/Theix INRA Centre in France. The animals were handled by specialized personnel who ensured their welfare in accordance with the European Union Directive 2010/63/EU for animal experiments.

2.1. Experimental design, animals and diets

Two dietary levels of dehydrated lucerne (*Medicago sativa* L.) pellets (H: 500 g lucerne offered per lamb per day vs. L: no lucerne) and two breeds (Limousine vs. Romane) were compared in a 2 × 2 factorial design. All the animals were offered the feeding treatments for 60 d before slaughter.

We used 86 male lambs. In treatment H, we used 35 Limousine lambs from 7 rams and 32 dams, and 35 Romane lambs from 14 rams and 29 dams, fed indoors in individual pens in order to control the diet at the individual level. In treatment L, we used 8 Limousine lambs from 5 rams and 8 dams, and 8 Romane lambs from 6 rams and 8 dams, penned in two groups (one for each breed). There was no need to feed L lambs individually, as the L diet did not contain green vegetative matter or legumes. The large number of rams used ensured that there was no risk of a ram effect.

The rationale for choosing the number of lambs under study was as follows. Previous studies showed that the variability in the plasma and fat carotenoid concentrations between individual lambs on the same feeding treatment increased with the dietary carotenoid intake level (Dian et al., 2007a). It is also well documented that this inter-individual variability is much higher in pasture-fed lambs that in concentrate-fed lambs (Dian et al., 2007a,b). For lambs receiving the high dietary lucerne level, we calculated the sample size which was necessary to reach a probability of finding a significant difference between Limousine and Romane lambs in both plasma and fat carotenoid concentrations by using the mean and variance values we previously observed in pasture-fed lambs (Dian et al., 2007a for Romane lambs; Dian et al., 2007b for Limousine lambs). The output of this calculation gave a minimum sample size of 35 lambs per breed. For lambs receiving no lucerne in their diet, as the variability between individual lambs in carotenoid accumulation was known to be much lower, we chose a sample size of 8 lambs per breed.

Mean lamb live weight at birth, and mean age and live weight at the beginning of the experiment were 4.2 ± 0.9 kg, 75 ± 6.5 days and 25.4 ± 3.6 kg, respectively; these animal characteristics were similar for L and H feeding treatments in both breeds.

The animals were housed indoors from birth to slaughter and were managed uniformly before the experiment. They received *ad libitum* a commercial concentrate which did not contain green vegetative matter or legumes from three weeks of age until the beginning of the experiment. The commercial concentrate comprised 300 g/kg barley, 230 g/kg wheat, 140 g/kg sugar beet pulp, 110 g/kg colza cake, 90 g/kg wheat bran, and 130 g/kg corn, sugar beet molasses, calcium carbonate, sodium chloride, ammonium chloride and vitamins (5.040 IU/kg of vitamin A, 1.501 IU/kg of vitamin D3, 20 IU/kg of vitamin E). The dams were also kept indoors and received a commercial concentrate which did not contain green vegetative matter or legumes and *ad libitum* access to hay.

For 60 days prior to slaughter, each H lamb received daily 500 g of dehydrated lucerne pellets and 200 g of wheat straw (as-fed); they were also offered 100 g barley grain per day (as-fed) for two weeks, then 300 g barley grain per day (as-fed) from the day 15 of the experiment onwards. The level of dehydrated lucerne pellets offered to H lambs was chosen to mimick the dietary carotenoid level we previously observed in lambs fed green vegetative pastures (Prache et al., 2009; Dian et al., 2007b), and the level of barley offered was chosen to obtain animal performances close to those observed in pasture fed lambs. All the H lambs were fed at 08:30 with the different feeds offered in separate feed tubs. The L lambs were fed the commercial concentrate and 200 g wheat straw per lamb per day (as-fed). The concentrate level offered to L lambs was adjusted every week to achieve a mean ADG similar to that of the H lambs. Thus we expected similar live weight (LW) and degree of fatness at slaughter in all the groups, thereby avoiding the confounding effect of degree of fatness on carotenoid concentration in fat (Prache et al., 2003; Huang et al., 2015a). The concentrate offered to L lambs was the same as that fed previously from three weeks of age.

Feed was offered in the morning. All feed tubs were emptied every morning and each refusal was weighed, recorded and discarded daily. Representative samples of offered and refused feed were collected daily for dry matter (DM) measurements by drying in a force-air oven for 48 h at 60 °C. Representative samples of the feed offered were collected twice weekly and bulked for analysis of carotenoid content and N stable isotope ratio. Water and salt blocks were made constantly available for all animals. The salt blocks contained (g/kg, on an as-fed basis) 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.

2.2. Slaughter procedures

There were five slaughter sessions with lambs from each treatment slaughtered on each session. Animals were slaughtered in the morning and not fed on the day of slaughter. They were transported by truck to the abattoir, which was located within 500 m of the experimental farm. Immediately on arrival, the animals were electrically stunned and slaughtered by throat cutting. The carcasses were placed in a chiller set at 4 °C until 24 h post mortem and were kept in the dark throughout.

2.3. Measurements

2.3.1. Animal live weight and carcass characteristics at slaughter

Lambs were weighed at 09:30 at the beginning of the experiment, then weekly and finally the day before slaughter. Carcass and perirenal fat weights were measured after 24 h shrinkage.

2.3.2. Carotenoid concentration in the feed and the animal tissues

Determination of carotenoid concentration in lucerne pellets, barley, straw and concentrate was carried out according to the method described by Chauveau-Duriot et al. (2010). Briefly, carotenoids were extracted from freeze-dried, ground forages three times with acetone until depletion. Dry matter (DM) was determined simultaneously on