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Tissue turnover in ovine muscles and lipids as recorded by multiple (H, C, O, S) stable isotope ratios

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ABSTRACT

Multiple stable isotope ratios (δ^2H, δ^{13}C, δ^{18}O and δ^{34}S) were measured in muscle, muscle lipids and lipid fractions collected from 28 lambs, subjected to a diet-switch and raised on two energy allowances (EAs), to determine tissue turnover and diet-tissue fractionation. The diet-muscle fractionations prior to the diet-switch were estimated to be −44.0‰, +1.9‰ and 0‰ for H, C and S, respectively, while the drinking water was demonstrated to be the main source of muscle O and thus δ^{18}O variation. The diet-intra-muscular lipid fractionations prior to the diet-switch were estimated to be −172.7‰, −1.3‰ and −11.5‰ for H, C and O, respectively. The C half-lives of muscle were determined to be 75.7 and 91.6 days for animals receiving the high and low EA, respectively. Extracting temporally resolved pre-slaughter dietary information from meat by analysing bulk muscle, muscle lipids and muscle lipid fractions appeared to be not practicable due to possible incomplete turnover of lipids.

1. Introduction

The recent findings of elevated levels of dioxin in Irish pork and of formula milk contaminated with melamine in China were just the latest in a series of scares to beset the food sector following the previous outbreaks of bovine spongiform encephalopathy (BSE), foot-and-mouth disease (FMD) and avian influenza. These scares are largely responsible for raised consumer demands concerning clear origin labelling of food, especially meat (Food Standards Agency, 2001; Kelly, Heaton, & Hoogewerff, 2005). However, analytical tools are needed to enable confirmation of country of origin of animal products and to verify the authenticity of foods. The potential of stable isotope ratio analysis (SIRA) in this regard has been demonstrated (Camin et al., 2007; Franke et al., 2008; Heaton, Kelly, Hoogewerff, & Woolfe, 2008; Schmidt et al., 2005).

Measurements of multiple stable isotope ratios can be used to gain information about plant sources used as animal feed (Bahar et al., 2009; Boner & Förstel, 2004), the proximity to the sea of farms on which animals were raised (Bol, Marsh, & Heaton, 2007) and the latitude of the country of origin (Camin et al., 2007; Heaton et al., 2008). However, it is necessary to determine the diet-tissue fractionation for C (feed source), S (proximity to the sea) and H and O (latitude) as well as the half-lives of tissues that can be of use for meat authentication. Currently, this information is not available for livestock animals.

Recently, Phillips and Eldridge (2006) proposed a novel approach, the so-called "isotope clock", that potentially allows scientists to estimate the time of previous changes in the diet of animals. In order to use this isotope clock, it is necessary to analyse at least two tissues with distinctly different half-lives. For meat authentication, such tissues should be sourced from a single

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sample obtained from a single animal. Muscle and lipid were previously shown to fulfil this requirement in gerbils (Tieszen, Boutton, Tesdahl, & Slade, 1983). Furthermore, research on the half-lives of triacylglycerols in human skeletal muscle (Sacchetti, Saltin, Olsen, & van Hall, 2004) and polar lipids in rat brain tissue (Freysz, Bieth, & Mandel, 1969) revealed distinctively different half-lives for these two lipid fractions (29 h and 20–40 days, respectively). Thus, it may be possible to obtain more detailed pre-slaughter dietary information on meat animals by analysing different biochemical fractions of the same meat sample.

The objective of this research was to estimate half-lives of ovine muscle tissue and its associated lipids and lipid fractions by measuring multiple stable isotope ratios following a controlled diet-switch prior to slaughter. Furthermore, we estimated diet-tissue fractionation of various elements in several tissues. Finally, we focused on the possibility of extracting two tissues or tissue fractions with distinctive half-lives from meat that could be used as an isotope clock (Phillips & Eldridge, 2006).

2. Materials and methods

2.1. Animals and feeds

Between March and April 2006, 28 purebred Belclare lambs (14 males and 14 females) were born at the Teagasc Production Research Centre, Athenry, Co. Galway, Ireland and were taken from their mothers at pasture 2.5 ± 2.1 days (mean ± standard deviation (SD), $n = 28$) after birth and initially raised on artificial milk for 6 weeks. The lambs were slowly weaned from the milk substitute during this time and introduced to a commercial diet (control diet). The control diet (Thomas McDonagh & Sons Ltd., Dromod, Co. Leitrim, Ireland) consisted of a mixture of cooked and flaked C_3 and C_4 plant material including barley flakes, maize flakes, maize gluten, cane, molasses and oats. In June 2006, the animals were moved to the Teagasc Grange Beef Research Centre, Dunsany, Co. Meath, Ireland and maintained on the control diet.

Prior to the diet-switch, all lambs were statistically blocked according to sex and within treatment groups assigned at random to either a high energy allowance (HEA) or low energy allowance (LEA) of the experimental diet for 0 (control), 14, 28, 56, 98, 154 and 231 days (treatments T0, T14, T28, T56, T98, T154 or T231, respectively). The lambs were penned individually, weighed periodically and the feed allowances adjusted, based on body weight, to ensure a target weight gain of 50 g d^{-1} and 150 g d^{-1} for animals receiving the LEA and HEA, respectively.

The experimental diet was formulated to have a similar metabolisable energy (Agricultural and Food Research Council, 1993) as the commercially available control diet. The control diet had a metabolisable energy of 11.4 ± 0.3 MJ kg^{-1} dry matter (DM) (mean ± SD, $n = 8$) while the pelleted maize concentrate and maize silage had metabolisable energies of 12.6 ± 0.1 MJ kg^{-1} DM (mean ± SD, $n = 9$) and 10.1 ± 0.3 MJ kg^{-1} DM (mean ± SD, $n = 9$), respectively (see Table 1). Thus, the experimental diet had a theoretical metabolisable energy of 12.4 MJ kg^{-1} DM after consideration of DM and DM digestibility of each diet component. The experimental diet consisted of 76% (wet weight basis) of pelleted maize concentrate containing seaweed (48 kg t^{-1}concentrate; Arramara Teoranta, Kilkieran, Co. Galway, Ireland), produced in a single batch at the Teagasc Moorepark Dairy Production Research Centre, Fermoy, Co. Cork, Ireland, and 24% (wet weight basis) of maize silage. The feed was offered to the animals in a single meal each morning while the lambs had *ad libitum* access to water at all times.

This study was carried out under licence from the Irish Government Department of Health and Children and with the approval of Teagasc, the Irish Agricultural and Food Development Authority. All procedures used complied with national regulations concerning experimentation on farm animals.

2.2. Feed and water samples

Weekly feed samples were collected and stored at −20 °C until analysis. For SIRA, monthly samples of the three feed stuffs (control diet, pelleted maize concentrate and maize silage) were oven dried at 40 °C for 48 h. The dried samples were then first milled to a particle size of <1 mm using a commercial feed mill (Christy & Norris Ltd., Chelmsford, UK) before being powdered using a ball mill (Glen Creston Ltd., Stanmore, UK). For H and O analysis, feed was defatted according to Radin using a mixture of *n*-hexane and 2-propanol (3:2, v/v) (Radin, 1981). Powdered (C and S) or powdered, lipid-free (H and O) feed were finally weighed into tin capsules for SIRA.

Water samples were collected regularly throughout the experiment. In total, 19 samples were collected; four samples at the research station where the animals were born (Athenry, Co. Galway) and a further 15 samples at the research station where the diet-switch experiment was carried out (Dunsany, Co. Meath). All water samples were kept frozen at −20 °C until analysis.

2.3. Preparation of muscle and lipid samples

The muscle *Longissimus dorsi* (LD) was collected from all animals 24 h *post-mortem*, trimmed of superficial adipose tissue, vacuum packed and stored frozen at −20 °C until analysis. Furthermore, a sample from the subcutaneous adipose tissue (SAT) located close to the LD was collected 24 h *post-mortem*. For SIRA, a sample from the geometrical centre of the muscle was collected and freeze dried (Edwards Pirani 501 Freeze Dryer, Edwards Ltd., Crawley, UK) for 168 h to ensure absolute dryness of the samples. Prior to analysis, 300 mg of muscle were sub-sampled from every collected sample and defatted according to Radin (1981) and the extracted lipid was collected. Dry, lipid-free muscle samples were stored in Eppendorf vials in a desiccator prior to weighing into tin capsules for SIRA.

The intra-muscular lipid (IML) fraction obtained following defatting was retained and stored in $CHCl_3$. The amount of IML was estimated following evaporation of the solvent under a stream of N_2 and determined as % of muscle dry matter (% DM). The SAT col-

Table 1
Metabolisable energy, δ^2H, $\delta^{13}C$, $\delta^{18}O$ and $\delta^{34}S$ of all feed stuffs (dried) and δ^2H and $\delta^{18}O$ of drinking water at both research stations (means ± SD).

Feed component	Energy [MJ kg^{-1} DM]	δ^2H [‰]	$\delta^{13}C$ [‰]	$\delta^{18}O$ [‰]	$\delta^{34}S$ [‰]
Control diet ($n = 8$)	11.4 ± 0.3	−53.6 ± 3.9	−22.6 ± 1.4	23.8 ± 1.2	4.1 ± 1.0
Experimental diet					
Pelleted concentrate ($n = 9$)	12.6 ± 0.1	−21.4 ± 2.6	−12.5 ± 0.5	25.9 ± 0.5	8.3 ± 0.8
Maize silage ($n = 9$)	10.1 ± 0.3	−45.9 ± 7.1	−12.1 ± 0.3	26.1 ± 1.3	5.1 ± 0.6
Composite (theoretical)	12.4	−23.6	−12.5	25.9	8.2
Water					
Water (Athenry; $n = 4$)		−30.7 ± 0.3		−5.0 ± 0.0	
Water (Dunsany; $n = 15$)		−44.6 ± 0.7		−6.7 ± 0.2	