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Authentication of meat and dairy products using rare earth element labeling and detection by solution based and laser ablation ICP-MS

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ABSTRACT

In order to meet the increasing customer demand for local food products, various methods for verification of food origin by means of region specific trace element fingerprinting have been developed. However, for products from conventional agriculture, without a close relationship to the local soil, other methods for food authentication are required. In an alternative approach, foodstuffs produced in a certain region, by a specific producer or under certain conditions can be safeguarded against imitation by chemical labeling.

The objective of the present study was to develop a method for labeling lamb meat and goat milk by selective enrichment of terbium and thulium in the feed for the animals. Therefore, a distinctive rare earth element (REE) pattern is artificially introduced which can be determined in labeled food products. Detection of REE labels was carried out using inductively coupled plasma mass spectrometry (ICP-MS) after acid digestion. Alternatively, laser ablation ICP-MS (LA-ICP-MS) was applied, allowing direct analysis of bone samples and analysis of meat and milk samples after dry ashing and pressing pellets.

After three weeks of administering 1000-fold terbium and thulium enriched feed to lambs, terbium and thulium enrichment was detected in all sample types except blood, following the trend bones > kidney > liver > heart > meat > kidney fat. Similarly, goat milk was successfully labeled after three weeks of feeding 500-fold terbium and thulium enriched feed.

Hence, the present method allows discrimination of labeled from unlabeled animal products, while REE contents in all labeled products remained low enough to avoid any health risk for the consumer.

1. Introduction

Recent years have seen a steadily increasing consumer demand for food products with a distinct regional identity. These products are often associated with assets such as environmentally friendly production, reduced carbon footprint, higher animal welfare standards, specific organoleptic qualities, or purported health benefits (Rees et al., 2016). Since many of these products are in the high price range, the possibility of adulteration by false declaration of cheaper products of an origin other than declared, must be taken into account, and therefore analytical methods for verification of food origin are required to avoid fraud.

Various analytical methods for origin determination of food products have already been developed, utilizing such diverse approaches as proteomics, trace element fingerprinting or isotope ratio measurement (Danezis, Tsagkaris, Camin, Brusic, & Georgiou, 2016). Food authentication methods based on trace element analysis, partly combined with stable isotope ratio measurement, have been proposed for a great variety of food products, including meat (Franke, Gremaud, Hadorn, & Kreuzer, 2005; Franke et al., 2008; Kim et al., 2017; Rees et al., 2016; Sun, Guo, Wei, & Fan, 2011; Zhao et al., 2013) as well as milk and dairy products (Benincasa, Lewis, Sindona, & Tagarelli, 2008; Magdas et al., 2016; Nečemer, Potočnik, & Ogrinc, 2016; Osorio, Koidis, & Papademas, 2015; Rodríguez-Bermúdez et al., 2018; Zain, Behkami, Bakirdere, & Koki, 2016). However, all these methods rely on a close relationship between animals, plants and the local geology, with trace elements being transferred from the soil into the plants on which the animals feed. For products from conventional agriculture, where animals are usually fed with commercially available complete feed of different or unspecified origin, this relationship is often not given and therefore innovative methods for food authentication are required.

In an alternative approach, food products produced in a certain region, by a specific producer or under certain conditions can be safeguarded against imitation by chemical labeling. The objective of the present study was to develop a method for labeling meat and milk by

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selective enrichment of two rare earth elements (REE), namely terbium and thulium, in the feed for lambs and dairy goats. Therefore, a distinctive i.e. non-natural REE pattern is artificially introduced which can be detected in food products such as meat and milk, and allows distinction from unlabeled products of other origin. In our laboratory, analogous methods for REE labeling of tomatoes (Bandoniene, Meisel, Rachetti, & Walkner, 2018) and of eggs and chicken meat (Bandoniene, Walkner, Zettl, & Meisel, 2018) have already been developed, and a similar method has been proposed for labeling farmed salmon in order to distinguish escaped animals from wild salmon (Pérez de Nanclares, Dessen, Rørvik, Thomassen, & Thomassen, 2016).

REE are particularly well suited for the purpose of chemical labeling, since natural REE levels in food products are generally very low, and therefore specific or characteristic labels can be introduced using very small amounts of REE. In China, REE have been used as growth promoting feed additives in animal husbandry for decades (Pagano et al., 2015; Redling, 2006), and the use of a feed additive containing mainly lanthanum and cerium citrates for weaned piglets has already been permitted in Switzerland (EFSA Panel on Additives and Products or Substances used in Animal Feed (FEEDAP), 2016). Therefore, some knowledge on the effects of REE enriched animal feed, including toxicity and potential adverse effects, already exists, although the mechanisms by which REE promote animal growth remain unclear (Abdelnour et al., 2019). REE are considered to be of low toxicity, especially when administered orally, mainly due to the poor absorption of REE from the gastrointestinal tract, with the bulk of ingested REE being quickly excreted via feces (Hirano & Suzuki, 1996; Wald, 1989). REE accumulation was found to occur mainly in liver, kidney, fat tissue and bones of REE supplemented animals, while little or no REE enrichment was detected in muscle tissue (Redling, 2006; Schwabe et al., 2012). Studies on lactating rats and mice showed that REE are also transferred into milk (Feige, Naharin, Lubin, & Sadeh, 1974; Marciniak, Chaś, & Baltrukiewicz, 1996).

Since food products usually contain only traces of REE besides large amounts of water, organic substances and elements such as potassium or calcium, REE contents in food are usually determined by means of inductively coupled plasma mass spectrometry (ICP-MS) after a sample preparation procedure involving acid digestion. However, ICP-MS is relatively prone to spectral and non-spectral interferences caused by matrix elements, which are usually counteracted by sample dilution, at the cost of higher detection limits. Analyte-matrix separation by means of ion exchange or chelation columns can help to avoid this dilemma, though it considerably increases sample preparation time and workload (Zawisza et al., 2011). In search for a simple and fit-for-purpose method for detection of REE labels in food samples, we developed a procedure consisting of dry ashing and laser ablation ICP-MS (LA-ICP-MS) analysis of pressed ash pellets, which was successfully applied to the analysis of chicken meat and bones (Bandoniene, Walkner, et al., 2018). Compared to conventional solution nebulization ICP-MS (SN-ICP-MS), LA-ICP-MS is more matrix tolerant and allows analysis of solid samples directly or with limited sample preparation. On the downside, detection limits in LA-ICP-MS are typically a few orders of magnitude higher, and accurate quantification of the results usually requires matrix-matched calibration standards and reference materials, which are often unavailable. However, in the present application higher detection limits can be at least partly compensated by preconcentration of inorganic sample components (including REE) during dry ashing. In addition, detection of REE enrichment relative to unlabeled samples (rather than absolute REE mass fractions) is sufficiently sensitive and accurate even without matrix-matched calibration standards. Therefore, LA-ICP-MS was applied as an alternative method for detecting REE labels in food products.

The present study aimed at developing a tool for assuring the origin of meat and milk products by labelling via REE spiking of the feed for lambs and goats, and detecting the labels using SN-ICP-MS and/or LA-ICP-MS. Since most studies on REE supplemented feed were focused on performance enhancement and used mixtures of REE containing mainly lanthanum and cerium, very few data on REE carry-over into meat and milk are available. It has to be noticed that La and Ce are the most abundant REE in nature and are thus the least expensive REE. Therefore, the primary goals of the present study were to assess the amounts of REE naturally present in various lamb tissues, and to test the REE concentrations and time of REE supplementation required to achieve significant REE labeling of food products whose origin needs to be guaranteed, while maintaining food safety for the customer.

2. Materials and methods

2.1. Chemicals

REE chlorides for spiking the feed for lambs and goats ($TbCl_3 \cdot 6 H_2O$ and $TmCl_3 \cdot 6 H_2O$) were provided by Treibacher Industrie AG (Althofen, Austria). Nitric acid 65% m/m p.a. (Roth, Karlsruhe, Germany) was additionally purified by subboiling distillation. Hydrogen peroxide solution (30% m/m, suprapur, Merck KGaA, Darmstadt, Germany), ammonia solution (25% m/m, ROTIPURAN, Carl Roth GmbH, Karlsruhe, Germany) and acetic acid (96% m/m, ROTIPURAN, Carl Roth GmbH, Karlsruhe, Germany) were used as received. High purity water was prepared using the Siemens Ultra Clear system (18.2 MΩ cm resistivity, Siemens Water Technologies, Barsbüttel, Germany). 1% m/v HNO_3 was used for dilution of samples and calibration standards, and as carrier and rinsing solutions for SN-ICP-MS.

Because REE in foodstuff are not equally abundant but occur in patterns similar to Earth crust (lighter REE are more abundant than heavier REE, in addition to the Oddo-Harkins rule (Harkins, 1917; Migaszewski & Gałuszka, 2015)), calibration solutions containing REE in equal concentrations might impede accurate measurements due to memory effects, i.e. the persistence of an elemental signal after the analysis of a sample or standard solution with a high concentration and rinsing of the sample introduction system (McGinnis, Jain, & Neal, 1997; Pappas, 2012). This applies particularly to the analysis of samples with ultra-trace concentrations of e.g. REE after calibration with highly concentrated standard solutions. Therefore, a custom made REE multi-element standard (AHF-CAL-7, Inorganic Ventures, New Jersey, USA) was used, with a distribution pattern similar to continental crust (Rudnick & Gao, 2003). The concentrations were: 1000 μg mL^{-1} cerium, 500 μg mL^{-1} lanthanum, neodymium and yttrium, 100 μg mL^{-1} praseodymium, 150 μg mL^{-1} thorium, 50 μg mL^{-1} dysprosium, gadolinium, samarium and uranium, 20 μg mL^{-1} erbium, europium and ytterbium, 10 μg mL^{-1} holmium and terbium, and 5 μg μg mL^{-1} lutetium and thulium in 7% m/v HNO_3. A 50 ng mL^{-1} stock solution (calculated for lanthanum, neodymium and yttrium) was prepared and diluted accordingly with 1% m/v HNO_3 for a calibration range from 0 to 10 ng mL^{-1}. Internal standard solutions (100 ng mL^{-1} indium and rhenium for SN-ICP-MS, 10 μg mL^{-1} indium for LA-ICP-MS) were prepared from 1000 μg mL^{-1} single-element standard solutions (Merck KGaA, Darmstadt, Germany). Reference material MAPS-4 ("microanalytical phosphate standard", synthetic calcium phosphate pressed pellet in 19 mm ring (Jochum et al., 2014)) used as a calibration standard for LA-ICP-MS analysis of meat and bone samples was obtained from the United States Geological Survey, Denver, CO, USA. For LA-ICP-MS analysis of milk samples, an in-house standard for calibration was prepared by spiking five portions of commercial goat milk, each approximately 100 g, with each 1 mL of a 2 mg L^{-1} (calculated for lanthanum, neodymium, yttrium) AHF-CAL-7 REE multi-element standard solution. The portions were then subjected to the same preparation procedure as the unknown milk samples (see below), pooled, homogenized and pressed. In order to determine the actual REE contents, five 200 mg portions of the ash were subjected to a microwave-assisted acid digestion (see below), and analyzed using SN-ICP-MS.

Table 1
Natural REE contents in daily feed rations for lambs and goats, REE contents in spike solutions and spiked feed, and REE enrichment relative to natural REE content in spiked feed. Initial REE enrichment in feed for goats (experimental phase 1, *italic numbers*) were found to be insufficient for distinctive labelling and were increased two weeks after the beginning of the experiment (experimental phase 2, **bold numbers**).

	Element	Natural daily REE intake [μg]	Group	REE concentration in spike solution [mg/L]		Daily REE intake with spiked feed [μg]		REE enrichment in spiked feed	
Lambs	Tb	75	Low	750		7500		x 100	
			High	7500		75,000		x 1000	
	Tm	29.5	Low	295		2950		x 100	
			High	2950		29,500		x 1000	
Goats	Tb	30	Low	*6*	***30***	*60*	**300**	*x 2*	**x 10**
			Medium	*30*	***300***	*300*	**3000**	*x 10*	**x 100**
			High	*150*	***1500***	*1500*	**15,000**	*x 50*	**x 500**
	Tm	10.8	Low	*2.2*	***10.8***	*22*	**108**	*x 2*	**x 10**
			Medium	*10.8*	***108***	*108*	**1080**	*x 10*	**x 100**
			High	*54*	***540***	*540*	**5400**	*x 50*	**x 500**

2.2. Preparation of REE marker feed

For the present study, terbium and thulium were chosen as labeling elements because of their monoisotopic nature, low natural abundance and relatively low price. However, other REE can be expected to be equally applicable, and if the proposed method is to be implemented in food production, the sets of labeling elements applied would have to be changed regularly in order to avoid imitation.

Terbium and thulium contents of daily rations of unspiked feed for lambs and goats (hay, concentrated feed, pasture) were determined using SN-ICP-MS after acid digestion (Section 3.1). By multiplication of these numbers by enrichment factors ranging from 2 to 500 for goats and from 100 to 1000 for lambs, REE amounts needed for spiking of daily feed rations for the different feeding groups were calculated (Table 1). REE spike solutions for all feeding groups were prepared by dissolving appropriate amounts of $TbCl_3 \cdot 6\ H_2O$ and $TmCl_3 \cdot 6\ H_2O$ in deionized water. Feed spiking was carried out by mixing one 10 mL portion of the respective spike solution per day and animal with a ration of concentrated feed right before feeding. As initial analyses of milk samples did not show any significant REE enrichment (Section 3.4), the amounts of REE spike in feed for dairy goats were increased after two weeks (bold numbers in Table 1).

2.3. Labeling experiments

All animal experiments were reported to and approved by the Austrian Federal Office for Food Safety according to applicable Austrian law. Animals were kept in accordance with animal welfare standards at the Agricultural Research and Education Centre Raumberg-Gumpenstein, where the experiments were conducted in July – August 2013. For the sake of convenience, the first day the respective group of test animals (lambs or goats) received REE spiked feed will be referred to as day 1 in the following text, and subsequent experimental days will be counted accordingly. The REE labelling experiment with lambs was carried out between July 15th (day 1) and August 26th (day 43) with twelve male lambs of a crossbreed of Merinolandschaf and Berichon du Cher. At the age of 3–4 months, the animals weighed 40–50 kg. Prior to the experiment, they were allowed to acclimatize to the stable and the feed for one week. In order to be able to monitor feed uptake and excretion, each lamb was kept in a closed stall and furnished with a urinal connected to a collection vessel via a flexible tube, and a pouch for feces collection, both attached by means of straps. The lambs received two rations of feed per day, each of which consisted of 500 g hay, 250 g concentrated feed and 2 g iodinated salt. After feeding, residual hay was weighed, while concentrated feed was always completely consumed. Each four animals were assigned to control, “low” and “high” groups. From day 1 to day 21 (phase 1 of the experiment) REE spiked feed was administered to groups “low” and “high”, while the control group received unspiked feed. After 3 weeks (day 22), two animals of each group were slaughtered. All remaining lambs were fed untreated feed for another 3 weeks (phase 2 of the experiment), until they were also slaughtered.

The goat milk labelling experiment was carried out between July 7th (day 1) and August 29th (day 53). 24 goats of the Saanen breed were subdivided into four groups (control, “low”, “medium”, “high”). The animals were kept in a freestall barn and allowed to pasture for 10 h per day. Feed rations of 600 g hay and 600 g concentrated feed per day were provided. During feeding, the goats were tethered in order to make sure each animal was consuming its assigned ration. Groups “low”, “medium” and “high” received REE spiked feed from the beginning of the experiment (phase 1). After 2 weeks (day 15), spike concentrations were increased (Section 2.2), and kept at the higher level for another 3 weeks, until day 35 (phase 2). For the rest of the experimental period all animals received unspiked feed (phase 3).

2.4. Sampling

Samples of hay, concentrated feed for lambs and goats, and pasture for goats were taken before starting the experiment. Feces and urine samples of lambs were taken on four days per week, by means of the sampling devices described in Section 2.3, weighed and pooled into weekly samples for each animal. Of each pooled urine sample, a subsample of approximately 50 mL was taken and stored at −20 °C. Pooled feces sample were air-dried and ground and subsamples of approximately 200 g were stored for analysis. During slaughtering of the lambs, samples of blood, last lumbar vertebra (bone and meat), shank (meat and bone), liver, heart, kidney and kidney fat were taken, vacuum-packaged and stored at −20 °C.

Goats were milked two times per day, and milk volumes were recorded. Milk samples for REE analysis of 100 mL volume were taken daily during the first milking and pooled into weekly samples, with the exception of days following changes in REE supplementation (days 1–5, days 16–20 and days 36–40), where separate samples for each day were kept. All samples were stored at −20 °C until sample preparation.

2.5. Sample preparation

For SN-ICP-MS analysis, samples were digested using a high pressure asher (HPA-S, Anton Paar, Graz, Austria). Subsamples of approximately 5 g of meat, liver, heart and kidney, 2 g of bone, 1 g of kidney fat and blood (each wet weight), and 0.5 g of feed and feces (air-dried) were weighed in quartz glass digestion vials, and 10 mL 65% m/m HNO_3 were added. Digestion was carried out at a temperature of 280 °C and a pressure of approximately 125 bar for 2.5 h. The resulting solutions were transferred into 15 mL round bottomed PFA vials, evaporated on a hot plate at 80 °C surface temperature and redissolved in

1% m/v HNO_3. Final sample volume was 5 mL, except for feed (20 mL) and feces samples (250 mL). Prior to SN-ICP-MS measurement, 100 µL portions of the indium and rhenium internal standard solution were added to 5 mL portions of the sample solutions. Two replicate subsamples were digested and analyzed for each sample.

Subsamples of approximately 10 g of goat milk were acidified with 1 mL 65% m/m HNO_3 in order to precipitate the proteins. After 5 min, when precipitation was complete, samples were centrifuged and the supernatant was separated using a paper filter and filtered through a syringe filter (0.45 µm pore size); the protein fraction was discarded. After addition of 5 mL 30% m/m H_2O_2, samples were evaporated to dryness on a hotplate (100 °C surface temperature). 5 mL 65% m/m HNO_3 were added to the residues, and digestion using a high pressure asher was carried out as described above.

Urine samples were only diluted 10-fold with 1% m/v HNO_3 and filtered through a syringe filter (0.45 µm pore size).

For LA-ICP-MS analysis, samples were prepared by dry ashing and pressing pellets (Bandoniene, Walkner, et al., 2018). Subsamples of meat (approximately 100 g wet weight) and bones (approximately 10 g wet weight) were dried at 120 °C over night and ashed in ceramic crucibles over a Bunsen burner until formation of smoke ceased. After cooling to room temperature, the residues were coarsely crushed using a glass rod, and 500 µL 10 µg mL^{-1} indium solution were added as an internal standard. The crucibles were then placed in a muffle furnace at a temperature of 550 °C for 5 h. Residues that appeared to contain residual carbon (i.e. dark gray to black in color) after this treatment were heated for another 5 h with addition of a few drops of 30% w/w H_2O_2 solution to facilitate complete ashing. This step was repeated if necessary. The remaining ash was homogenized in an agate mortar, and approximately 200 mg of each sample were pressed into a 13 mm diameter pellet using a hydraulic press, applying 10^5 N for 1 min.

Milk subsamples of approximately 50 g were weighed into ceramic crucibles, and 500 µL 10 µg mL^{-1} indium solution were added. Samples were evaporated on a hotplate (80 °C surface temperature). Complete ashing was achieved after 5 h in a muffle furnace at 550 °C. The ash was homogenized and pressed as described above, applying $5 \cdot 10^4$N for 1 min.

For direct LA-ICP-MS measurement of bone samples, pieces of approximately 30–50 mm^2 area were cut, dried at 120 °C over night and grinded using a glass file in order to remove the periosteum and create a flat surface.

2.6. ICP-MS measurement

SN-ICP-MS measurements were carried out using an Agilent 7500 ce (Agilent Technologies, Tokyo, Japan) equipped with a PFA nebulizer, a Peltier-cooled Scott-type spray chamber and nickel sampler and skimmer cones. Prior to each experiment, the instrument was tuned to maximum sensitivity while keeping oxide formation ratio (CeO^+/Ce^+) below 1% using a solution containing 1 µg L^{-1} lithium, cobalt, yttrium, cerium and thallium. Samples were taken up by an autosampler (SC-2 DX, Elemental Scientific, Omaha, NE, USA), loaded into a 2 mL sample loop via a six-port valve and conveyed to the nebulizer by means of a carrier solution (1% m/v HNO_3) by a peristaltic pump. Limits of quantitation (LOQ) were calculated as 10 times standard deviation of the calibration blank.

For the analysis of a part of the meat, liver, kidney and kidney fat samples and all of the milk samples analyzed after digestion, the ICP-MS was equipped with an automated on-line solid phase extraction system (sea*FAST*, Elemental Scientific, Omaha, Nebraska, USA) for preconcentration and matrix removal. A more detailed description of the system and its advantages and drawbacks regarding analysis of food samples has been published in a previous article (Bandoniene, Walkner, et al., 2018).

LA-ICP-MS analyses were carried out using a NWR 213 laser ablation system (Electro Scientific Industries, Portland, OR, USA) equipped with a TV 2 two-volume cell and coupled to an Agilent 8800 triple quadrupole ICP-MS (ICP-QQQ, Agilent Technologies, Tokyo, Japan). Sample aerosol was transported into the plasma by a He flow of 0.8 L/min, which was mixed with the carrier gas in a Liebig gas mixer. In order to increase the sensitivity, the ICP-QQQ was operated in single quadrupole mode and equipped with an additional foreline pump (McFarlane & Luo, 2012). For meat, bone and milk samples, five lines of 2 mm length per sample were performed, with 110 µm spot diameter, 20 Hz repetition rate, 50 µm s^{-1} scanning speed and approx. 2.5 J cm^{-2} fluence. For meat and bone samples, reference material MAPS-4 was used as a standard for element bias and drift correction, while an in-house standard (Section 2.1) was used for milk samples. Sets of 5 samples (i.e. 25 line scans) were bracketed by sets of 2 spot ablations of the respective calibration standard, with 80 µm spot diameter, 10 Hz repetition rate, 45 s dwell time and approx. 2 J cm^{-2} fluence. Time-resolved profiles for m/z = 43 ($^{43}Ca^+$), m/z = 89 ($^{89}Y^+$), m/z = 115 ($^{115}In^+$), m/z = 159 ($^{159}Tb^+$), m/z = 169 ($^{169}Tm^+$) were recorded and exported to a spreadsheet for data reduction. Calcium was used as an internal standard for quantitative analyses of bone samples, while indium was used for meat and milk samples. Analyte/internal standard count ratios as well as $^{159}Tb^+/^{89}Y^+$ and $^{169}Tm^+/^{89}Y^+$ count ratios were calculated for each data sweep, and from all data sweeps acquired during ablation after a 5 s stabilization period, 10% trimmed means were calculated in order to reduce spikes caused by incomplete atomization of large particles in the plasma. Results were then corrected for mass bias and drift by sample-standard-bracketing, assuming linear time-dependent drift between each 2 sets of standards. Element mass fractions were calculated by means of one point calibration, i.e. response factors (analyte/internal standard count ratio per mass fraction in ng/g) for the standards were calculated for each measurement session, and count ratios acquired for unknown samples were divided by the respective response factor. Limits of quantitation (LOQ) were calculated as 10 times standard deviation of 25 consecutive gas blank measurements.

3. Results and discussion

3.1. Terbium and thulium contents in feed

REE contents of feed for lambs and goats, namely hay, concentrated feed and pasture, were determined using SN-ICP-MS after acid digestion (Table 2). From these data, natural terbium and thulium intake with daily feed rations were calculated. Feed intake during daily pasturing was assumed to be roughly 1 kg, based on preliminary measurements. In order to account for the uncertainty of this estimation, as well as factors such as varying water content of feed samples or incomplete consumption of feed rations, calculated values for daily terbium intake for lambs and goats (66.8 µg and 27.5 µg, respectively) were rounded up to 75 µg and 30 µg, respectively. Values for thulium intake for lambs and goats were increased accordingly (maintaining the measured terbium/thulium ratios) to 29.5 µg and 10.8 µg, respectively. Based on

Table 2
Tb and Tm contents determined in daily feed rations (hay, concentrated feed, pasture for goats) for lambs and goats, and calculated daily Tb and Tm uptake.

	Feed type	REE content [µg/kg]		Daily feed intake [kg]	daily REE intake [µg]	
		Tb	Tm		Tb	Tm
Lambs	Hay	61.6	23.3	1	61.6	23.3
	Conc. feed	10.5	5.6	0.5	5.3	2.8
	Total				**66.8**	**26.1**
Goats	Hay	35.3	12.6	0.6	21.2	7.6
	Conc. feed	1.9	0.9	0.6	1.2	0.5
	Pasture	5.1	2.2	1	5.1	2.2
	Total				**27.5**	**10.3**

Table 3
REE mass fraction range and median value determined in lamb samples using SN-ICP-MS after acid digestion. Measurands indicated as "control" only include control group samples, while other measurands are calculated from data for all samples.

		Y	La	Ce	Pr	Nd	Sm	Eu	Gd	Tb control	Tb	Dy	Ho	Er	Tm control	Tm	Yb	Lu	REE control	REE
		µg/kg																		
Meat (shank)	Minimum	0.03	0.05	0.14	0.01	0.07	0.01	0.00	0.01	0.02	0.02	0.01	0.001	0.000	0.003	0.003	0.003	0.001	0.77	0.64
	Maximum	0.46	0.48	2.08	0.22	0.21	0.04	0.04	0.04	0.12	0.23	0.03	0.007	0.142	0.045	0.118	0.018	0.025	3.38	3.38
	Median	0.06	0.08	0.26	0.08	0.08	0.02	0.03	0.02	0.05	0.08	0.02	0.003	0.013	0.010	0.029	0.012	0.008	1.30	0.86
Meat (loin)	Minimum	0.03	0.06	0.19	0.01	0.04	0.01	0.00	0.01	0.01	0.01	0.01	0.001	0.005	0.002	0.002	0.004	0.001	0.71	0.67
	Maximum	0.12	0.22	1.13	0.16	0.12	0.04	0.04	0.02	0.08	0.29	0.03	0.006	0.062	0.037	0.134	0.024	0.022	1.64	1.64
	Median	0.06	0.11	0.33	0.06	0.07	0.03	0.02	0.02	0.02	0.10	0.02	0.003	0.011	0.003	0.033	0.016	0.008	0.90	0.89
Kidney fat	Minimum	0.09	0.19	0.70	0.03	0.10	0.02	0.00	0.02	0.01	0.01	0.01	0.002	0.000	0.003	0.003	0.005	0.001	2.10	1.51
	Maximum	0.39	0.89	3.97	0.33	1.17	0.24	0.03	0.06	0.13	0.21	0.09	0.007	0.110	0.046	0.083	0.040	0.017	4.07	5.91
	Median	0.17	0.35	1.04	0.04	0.12	0.03	0.01	0.03	0.04	0.07	0.02	0.005	0.015	0.014	0.027	0.016	0.003	2.45	2.25
Kidney	Minimum	0.22	0.24	0.57	0.06	0.26	0.06	0.02	0.06	0.02	0.02	0.04	0.008	0.027	0.006	0.006	0.016	0.002	1.98	1.98
	Maximum	0.52	0.54	2.98	0.39	0.54	0.14	0.14	0.15	0.10	13.76	0.13	0.021	0.502	0.011	4.60	0.067	0.131	3.00	23.1
	Median	0.32	0.31	0.76	0.18	0.34	0.09	0.06	0.08	0.06	1.34	0.08	0.012	0.037	0.008	0.463	0.038	0.059	2.21	4.93
Liver	Minimum	0.13	1.09	1.33	0.47	0.90	0.14	0.07	0.08	0.05	0.05	0.05	0.007	0.019	0.005	0.005	0.021	0.036	5.55	5.55
	Maximum	0.43	2.51	4.53	1.20	1.93	0.33	0.20	0.18	0.17	10.31	0.09	0.014	0.184	0.010	1.84	0.040	0.157	8.51	23.0
	Median	0.16	1.64	2.48	0.58	1.20	0.19	0.09	0.11	0.08	0.94	0.06	0.008	0.023	0.007	0.177	0.027	0.070	6.59	7.90
Heart	Minimum	0.04	0.07	0.23	0.10	0.10	0.03	0.02	0.02	0.06	0.06	0.02	0.003	0.009	0.003	0.003	0.014	0.014	1.18	0.98
	Maximum	0.13	1.43	2.73	0.47	0.21	0.06	0.14	0.05	0.18	2.47	0.06	0.006	0.016	0.021	0.578	0.027	0.145	2.00	8.33
	Median	0.07	0.11	0.35	0.29	0.13	0.04	0.08	0.03	0.11	0.20	0.03	0.004	0.013	0.006	0.035	0.020	0.053	1.40	1.67
Blood	Minimum	0.04	0.10	0.23	0.01	0.03	0.01	0.00	0.01	0.01	0.01	0.01	0.003	0.009	0.003	0.003	0.009	0.001	0.80	0.80
	Maximum	0.29	0.38	0.99	0.03	0.14	0.04	0.02	0.04	0.03	0.04	0.04	0.008	0.056	0.018	0.018	0.020	0.005	1.02	1.89
	Median	0.09	0.16	0.37	0.02	0.07	0.03	0.01	0.02	0.02	0.03	0.02	0.006	0.012	0.004	0.007	0.014	0.004	0.97	0.95
Urine	Minimum	0.01	0.01	0.04	0.04	0.05	0.03	0.02	0.01	0.01	0.01	0.01	0.002	0.008	0.01	0.01	0.009	0.002	0.5	0.5
	Maximum	0.97	1.52	2.93	0.93	1.50	0.36	0.32	0.49	0.18	0.93	0.25	0.045	0.131	0.08	0.66	0.151	0.085	3.0	10.9
	Median	0.09	0.10	0.25	0.37	0.21	0.10	0.12	0.04	0.03	0.09	0.05	0.009	0.026	0.02	0.04	0.035	0.012	1.9	1.8
		mg/kg																		
Feces	Minimum	1.7	3.1	5.9	0.70	2.7	0.53	0.12	0.36	0.10	0.09	0.35	0.06	0.17	0.04	0.03	0.14	0.02	22	19
	Maximum	3.4	6.3	11.9	1.4	5.4	1.0	0.24	0.86	40.3	102	0.64	0.20	0.33	16	39	0.28	0.72	79	164
	Median	2.5	4.2	8.3	1.0	3.7	0.73	0.17	0.59	5.6	12	0.47	0.09	0.23	2.2	4.5	0.19	0.12	31	40

these numbers, terbium and thulium concentrations for spike solutions were calculated (Section 2.2).

It is worth noting that due to the low natural abundance of terbium and thulium, even in the spiked feed administered to the animals of the "high" groups during the present study, total REE content was only approximately 10% higher than in unspiked feed. Consequently, performance enhancing effects regarding weight gain or milk production, which were reported in literature after administration of more substantially REE enriched feed (Redling, 2006) were neither expected nor found.

3.2. Calculation of terbium and thulium anomaly

Since REE are not equally abundant in natural samples, but vary according to the Oddo-Harkins rule (Harkins, 1917; Oddo, 1914) it is customary to normalize REE mass fractions by division by a suitable reference data set in order to facilitate identification of anomalies in the REE profiles (Bandoniene, Zettl, Meisel, & Maneiko, 2013; Bandoniene, Walkner, et al., 2018). In the present work, data acquired for unlabeled samples were used as reference data sets (one phase 2 control group sample each for urine and feces, and one phase 1 control group loin meat sample for all meat, blood, heart, kidney, kidney fat and liver samples).

While normalized values for all elements in unlabeled samples approximate 1, normalized terbium and thulium values for labeled samples deviate significantly from the reference value. The magnitude of this deviation is referred to as REE anomaly and is calculated as REE_n/REE_n^*, where REE_n is the normalized mass fraction of the respective element, i.e. terbium or thulium, and REE_n^* is the expected normalized value calculated by interpolation between the two respective "neighboring" elements, i.e. gadolinium and dysprosium for terbium and erbium and ytterbium for thulium (Bandoniene, Walkner, et al., 2018; Lawrence, Greig, Collerson, & Kamber, 2006). REE anomalies constitute a demonstrative measure of REE enrichment and are also relatively robust towards varying absolute REE levels caused by tissue heterogeneity, unequal water content or even contamination (with material of natural REE composition) or sample loss during sample preparation.

Acquisition of complete REE profiles in food samples using LA-ICP-MS was not feasible due to its generally lower sensitivity. This also applies for SN-ICP-MS analysis of milk samples because of their exceptionally low natural REE levels. Alternatively, normalized terbium/yttrium and thulium/yttrium ratios (REE_n/Y_n) were used as a measure of REE enrichment. Yttrium is particularly well suited as a reference element, since it resembles terbium and thulium in ionic radius and chemical behavior, while being far more abundant in nature. Values for REE_n/Y_n were calculated as $REE/Y/REE_{ref}/Y_{ref}$, where REE/Y is the ratio of terbium or thulium and yttrium intensity ratios, and REE_{ref}/Y_{ref} is the respective reference value calculated as the mean of all measured REE/Y ratios for control group samples of the same type (Bandoniene, Walkner, et al., 2018).

Terbium and thulium levels in unlabeled bone samples were below the respective limits of quantitation (Section 3.3), and calculating REE_{ref}/Y_{ref} and, consequently, REE_n/Y_n ratios based on these data would result in high uncertainty or even erroneous values. Therefore, Tb/Y and Tm/Y ratios of calcium phosphate reference material Durango apatite (Yang et al., 2014), 0.031 and 0.011, respectively, were used as REE_{ref}/Y_{ref} instead. Similarly, Tm_{ref}/Y_{ref} for milk samples was extrapolated from the respective Tb_{ref}/Y_{ref} ratio using the Tb/Tm ratio of the reference material, 2.8. Since the differences in ionic radii and hence chemical behavior between yttrium, terbium and thulium are small, and the reference material can be considered matrix matched at least for bone samples, the extrapolated values can be assumed to reasonably approximate the true values. It should also be noted that the ability of the present method to discriminate between labeled and unlabeled samples does not depend on their trueness. Of the samples analyzed using LA-ICP-MS, all meat samples of group "high" and all meat

Table 4
Tb and Tm contents and Tb and Tm anomalies (Tb_n/Tb_n^*, Tm_n/Tm_n^*) determined in lamb samples using SN-ICP-MS after acid digestion: Mean values (n = 2) for each experimental group and phase. Phase1: Spiked feed; Phase 2: Unspiked feed.

	Group	Phase	Tb µg/kg	Tm µg/kg	$\frac{Tb_n}{Tb_n^*}$	$\frac{Tm_n}{Tm_n^*}$
Meat (shank)	Control	1	0.06	0.031	0.8	6.4
		2	0.02	0.003	0.4	0.1
	Low	1	0.09	0.041	1.6	1.7
		2	0.02	0.007	0.4	0.6
	High	1	0.23	0.104	4.9	2.9
		2	0.12	0.041	1.5	2.7
Meat (loin)	Control	1	0.01	0.020	1.1	3.6
		2	0.02	0.002	0.3	0.2
	Low	1	0.15	0.101	3.8	10.7
		2	0.04	0.008	0.6	0.5
	High	1	0.18	0.081	6.0	4.0
		2	0.13	0.033	1.8	2.4
Kidney fat	Control	1	0.07	0.035	0.6	1.1
		2	0.01	0.003	0.1	0.2
	Low	1	0.12	0.051	1.2	2.2
		2	0.04	0.006	0.3	0.4
	High	1	0.15	0.072	2.8	8.5
		2	0.13	0.023	1.1	1.0
Kidney	Control	1	0.02	0.009	0.1	0.3
		2	0.09	0.008	0.3	0.2
	Low	1	2.45	0.705	7.2	23.6
		2	1.37	0.361	2.4	6.4
	High	1	13.76	3.850	38.3	90.4
		2	6.01	1.764	19.6	52.8
Liver	Control	1	0.05	0.009	0.2	0.4
		2	0.17	0.005	0.4	0.2
	Low	1	1.47	0.224	3.8	9.3
		2	0.96	0.138	2.2	4.4
	High	1	9.31	1.772	27.8	49.7
		2	10.31	1.272	19.0	40.3
Heart	Control	1	0.06	0.013	0.7	0.9
		2	0.16	0.004	1.0	0.2
	Low	1	0.22	0.044	2.0	2.4
		2	0.15	0.024	1.6	1.6
	High	1	1.39	0.469	12.5	23.5
		2	1.76	0.244	11.6	14.9
Blood	Control	1	0.01	0.011	0.3	0.8
		2	0.02	0.003	0.4	0.3
	Low	1	0.03	0.011	0.3	0.9
		2	0.03	0.006	0.3	0.3
	High	1	0.04	0.016	0.9	1.0
		2	0.04	0.009	0.4	0.5

samples of group “low” taken after phase 1, all bone samples of groups “high” and “low”, and all milk samples taken from days 18 to 37 showed Tb/Y and Tm/Y ratios significantly higher than the respective control group samples, confirmed by one-tailed t-tests (P = 0.05, data not shown).

3.3. Lamb meat labeling experiment

Minimum, maximum and median values for all REE mass fractions determined using SN-ICP-MS after high pressure acid digestion are summarized in Table 3. Table 4 contains mean terbium and thulium mass fractions and anomalies determined in samples obtained from the two animals per group sacrificed after the first three weeks of the experiment (phase 1), and from the remaining two animals per group sacrificed after another three weeks (phase 2). A graphical representation of terbium anomalies determined in lamb tissues and blood is given in Figs. 1 and 2. In general, thulium anomalies follow the same trends, and therefore only terbium will be discussed in the rest of the text.

No significant terbium enrichment could be detected in blood samples. In all other sample types (in samples from groups “low” and “high”) a small but significant terbium anomaly is visible after phase 1 and persists during phase 2 in group “high” samples. Amongst the tissue types analyzed, terbium anomalies as well as terbium mass fractions follow the trend kidney > liver > heart > meat > kidney fat > blood, largely in agreement with previously published studies (Redling, 2006; Schwabe et al., 2012). However, the results differ from those of a previous study on terbium and thulium labeling of chicken meat conducted in our laboratory, where terbium and thulium anomalies found in meat samples were not only higher than in the present study (about 50 to 100-fold), but also higher compared to liver samples (no heart or kidney samples were analyzed) (Bandoniene, Walkner, et al., 2018).

Table 5 shows terbium and thulium mass fractions determined in urine and feces samples. While values determined for urine compare to those for tissue samples, extremely high amounts of terbium and thulium were found in feces samples. This finding is not surprising considering the reportedly poor absorption of orally administered REE, with the bulk of ingested REE passing through the gastrointestinal tract in unaltered form (Hirano & Suzuki, 1996; Wald, 1989). It should, however, be noted that relatively high terbium and thulium enrichment was detected even in control group feces samples. Although the actual cause of these anomalies could not be located, contamination during sampling or sample preparation is the most likely explanation. Therefore, the numbers given for control and “low” groups can be regarded as erroneous, but are given in Table 5 for the sake of completeness.

REE contents of animal tissues are usually very low, while matrix elements such as potassium, calcium or magnesium are abundant, especially in bones. Therefore, LA-ICP-MS was tested as an alternative method for detecting terbium and thulium labels in meat samples, while bone samples were exclusively analyzed by LA-ICP-MS. The proposed sample preparation method, consisting of dry ashing and pressing pellets for LA-ICP-MA analysis, allows processing and homogenization of relatively large meat and bone samples (approximately 100 g and 10 g, respectively), making it more robust towards sample heterogeneity and superficial contamination. On the downside, in the absence of matrix matched calibration standards, quantification of LA-ICP-MS results has to rely on one-point calibration using reference material MAPS-4.

Table 6 contains yttrium, terbium and thulium mass fractions and terbium and thulium anomalies determined in meat and bone samples using LA-ICP-MS. Terbium anomalies obtained for meat and bone samples are summarized in Figs. 3 and 4, respectively. Due to the large enrichment factors achieved during ashing of meat samples, LA-ICP-MS proved sufficiently sensitive for quantification of yttrium, terbium and thulium in labeled and unlabeled samples. Terbium and thulium anomalies thus determined are comparable to those measured using SN-ICP-MS.

Although terbium and thulium contents in unlabeled bone samples were below the limits of quantitation for LA-ICP-MS, the method was sensitive enough for detecting terbium and thulium in labeled samples from both “low” and “high” groups. They actually contained surprisingly high amounts of the labeling elements, with anomalies (calculated relative to reference material Durango apatite) of approximately 170 for thighbone and approximately 400 for lumbar vertebra samples of the “high” group. These numbers are distinctly higher than those determined in previous studies on chickens (Bandoniene, Walkner, et al., 2018) and bulls (Schwabe et al., 2012), where only REE anomalies of approximately 10 were registered. The particularly high terbium and thulium anomalies determined in the present study can possibly explained by the fact that the lambs were in a phase of growth at the beginning of the labeling experiment, when REE were incorporated during the generation of new bone tissue.

The relative ease with which the REE labels could be determined in bone samples by LA-ICP-MS hinted at the possibility of direct analysis of bone samples, without the sample preparation steps of ashing and pressing. Fragments of appropriate size were cut from thighbone and lumbar vertebra samples from phase 1, dried and polished and LA-ICP-MS analysis was performed without any further preparation steps. As

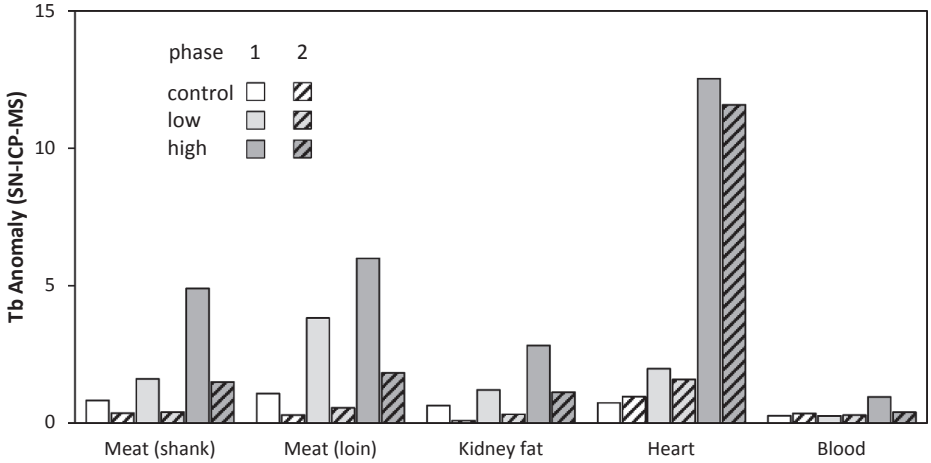

Fig. 1. Mean terbium anomaly (Tb_n/Tb_n*) detected in lamb tissue samples obtained after experimental phase 1 (during which the animals received spiked feed; plain bars) and phase 2 (during which the animals received unspiked feed; hatched bars) using SN-ICP-MS.

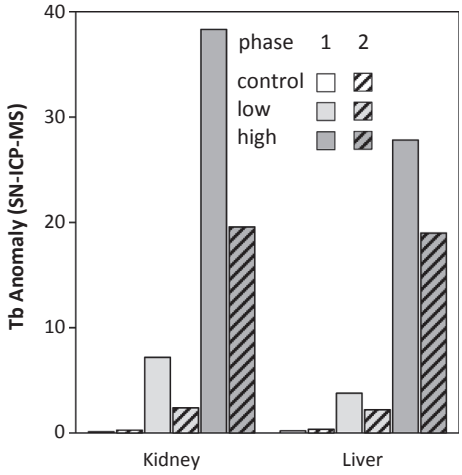

Fig. 2. Mean terbium anomaly (Tb_n/Tb_n*) detected in lamb kidney and liver samples obtained after experimental phase 1 (during which the animals received spiked feed; plain bars) and phase 2 (during which the animals received unspiked feed; hatched bars) using SN-ICP-MS.

Table 5
Tb and Tm contents and Tb and Tm anomalies (Tb_n/Tb_n*, Tm_n/Tm_n*) determined in lamb urine and feces samples (each one weekly pooled sample per animal) using SN-ICP-MS after acid digestion: Median values for each experimental group for phase 1 (n = 12) and phase 2 (n = 6). Phase1: Spiked feed; Phase 2: Unspiked feed.

	Group	Phase	Tb µg/kg	Tm µg/kg	$\frac{Tb_n}{Tb_n^*}$	$\frac{Tm_n}{Tm_n^*}$
Urine	Control	1	0.01	0.01	0.41	0.61
		2	0.05	0.02	0.73	1.32
	Low	1	0.02	0.02	0.74	1.03
		2	0.05	0.02	0.73	1.32
	High	1	0.12	0.11	3.12	4.42
		2	0.10	0.04	3.78	5.36
			mg/kg	mg/kg		
Feces	Control	1	10	3.8	91	91
		2	2.7	1.1	23	25
	Low	1	14	5.3	141	140
		2	1.5	0.6	12	13
	High	1	72	28	750	773
		2	1.8	0.7	15	16

Table 6
Y, Tb and Tm contents and Tb and Tm anomalies (Tb_n/Y_n, Tm_n/Y_n) determined in lamb samples using LA-ICP-MS: Mean values (n = 2) for each experimental group and phase. Phase1: Spiked feed; Phase 2: Unspiked feed.

	Group	Phase	Y µg/kg	Tb µg/kg	Tm µg/kg	$\frac{Tb_n}{Y_n}$	$\frac{Tm_n}{Y_n}$
Meat (shank)	Control	1	0.055	0.029	0.012	1.5	1.4
		2	0.071	0.014	0.007	0.5	0.6
	Low	1	0.063	0.057	0.028	2.8	3.1
		2	0.043	0.018	0.011	1.1	1.7
	High	1	0.036	0.085	0.041	8.1	8.6
		2	0.024	0.050	0.022	6.7	6.6
	LOQ		0.009	0.003	0.002		
Meat (loin)	Control	1	0.120	0.031	0.017	1.4	1.4
		2	0.138	0.016	0.008	0.6	0.6
	Low	1	0.118	0.061	0.036	2.7	2.8
		2	0.065	0.014	0.007	1.0	0.9
	High	1	0.059	0.104	0.047	9.1	7.4
		2	0.040	0.057	0.020	7.9	5.1
	LOQ		0.010	0.004	0.003		
Thighbone (pellets)	Control	1	1.4	< LOQ	< LOQ	n/a	n/a
		2	1.5	< LOQ	< LOQ	n/a	n/a
	Low	1	1.5	0.7	0.2	16 [a]	15 [a]
		2	1.7	0.9	0.3	18 [a]	18 [a]
	High	1	1.5	7.3	2.7	167 [a]	174 [a]
		2	2.1	9.9	3.5	169 [a]	162 [a]
	LOQ		0.4	0.1	0.1		
Lumbar vertebra (pellets)	Control	1	1.4	< LOQ	< LOQ	n/a	n/a
		2	1.3	< LOQ	< LOQ	n/a	n/a
	Low	1	1.4	1.7	0.7	42[a]	47[a]
		2	1.7	1.8	0.7	34[a]	38[a]
	High	1	1.6	16.6	6.6	358[a]	397[a]
		2	1.3	16.0	5.9	408[a]	422[a]
	LOQ		0.17	0.06	0.04		

[a] Ratios calculated based on reference values published for calcium phosphate reference material Durango apatite.

can be seen in Fig. 4b, terbium enrichment is clearly visible in samples from both "low" and "high" group. Terbium and thulium contents in unlabeled samples were, again, below the respective limits of quantitation and hence anomalies were calculated relative to reference material Durango Apatite. Due to the heterogeneity of bones, data obtained for ashed and homogenized and for native bone samples are not directly comparable. However, both methods have shown to be capable of detecting the REE labels introduced. With regard to practical application in assuring the origin of lamb meat, direct LA-ICP-MS analysis of bones would be the most convenient method, since most lamb meat cuts on the market include bones.

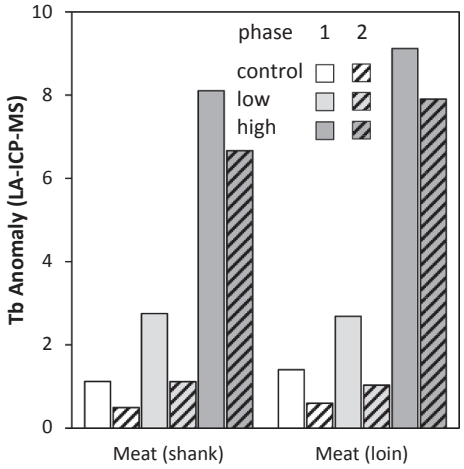

Fig. 3. Mean terbium anomaly (Tb_n/Y_n) detected in lamb meat samples obtained after experimental phase 1 (during which the animals received spiked feed; plain bars) and phase 2 (during which the animals received unspiked feed; hatched bars) using LA-ICP-MS after dry ashing and pressing.

3.4. Milk labeling experiment

Amongst the sample types analyzed within the present study, goat milk samples contained the lowest amount of REE. In addition, acid digestion of milk samples is complicated by their high water, protein and lipid content; therefore we devised a modified sample preparation procedure. Since REE were assumed to be primarily present in whey, the milk protein fraction was removed beforehand by precipitation in order to facilitate digestion. Water was then evaporated from the supernatant, adding H_2O_2 in order to start digestion of organic constituents and prevent excessive foaming on addition of acid. The residue was then subjected to the usual digestion procedure. Therefore, clear and colorless solutions were achieved, and REE from 10 g of milk could be concentrated into 5 mL of sample solution. Using this procedure, samples from experimental days 20, 25 and 32 of experimental phase 2 (when up to 500-fold terbium and thulium enriched feed was fed) and from days 37, 38, 39, 40 and 46 of experimental phase 3 (when unspiked feed was fed again) were analyzed. Table 7 shows results for yttrium, terbium and thulium mass fractions and terbium and thulium anomalies thus acquired. However, for analysis of the complete set of goat milk samples available, the LA-ICP-MS method was preferred due to the considerably lower time and workload required. Median values for yttrium, terbium and thulium contents and terbium and thulium anomalies determined using LA-ICP-MS are summarized in Table 8. Mass fractions obtained using both methods are comparable considering the limited number of samples analyzed by SN-ICP-MS, the generally low REE levels in milk, and the possibility of a REE losses during protein separation.

Fig. 5 shows the evolution of terbium anomalies determined using LA-ICP-MS for all groups, i.e. control, "low", "medium" and "high", throughout the experiment. Thulium contents in most samples from control, "low" and "medium" groups and in phase 1 samples from the "high" group were below the limit of quantitation for the LA-ICP-MS

Table 7
Median values for each experimental group for Y, Tb and Tm contents and Tb and Tm anomalies (Tb_n/Y_n, Tm_n/Y_n) determined in goat milk samples using SN-ICP-MS.

Group	Y µg/kg	Tb µg/kg	Tm µg/kg	$\frac{Tb_n}{Y_n}$	$\frac{Tm_n}{Y_n}$
Control (n = 45)	0.023	0.003	0.0007	0.8	0.8
Low (n = 44)	0.022	0.003	0.0006	0.6	0.7
Medium (n = 42)	0.018	0.003	0.0008	0.8	1.2
High (n = 47)	0.020	0.008	0.0037	2.4	4.8
LOQ	0.002	0.002	0.0005		

Table 8
Y, Tb and Tm contents and Tb and Tm anomalies (Tb_n/Y_n, Tm_n/Y_n) determined in goat milk samples using LA-ICP-MS: Median values for each experimental group for phase 1 (n = 36), phase 2 (n = 42) and phase 3 (n = 42). Phase 1: Up to 50-fold terbium and thulium enriched feed; Phase2: Up to 500-fold terbium and thulium enriched feed; Phase 3: Unspiked feed.

Group	Phase	Y µg/kg	Tb µg/kg	Tm µg/kg	$\frac{Tb_n}{Y_n}$	$\frac{Tm_n}{Y_n}$
Control	1	0.017	0.005	< LOQ	1.0	n/a
	2	0.019	0.004	< LOQ	0.9	n/a
	3	0.022	0.005	< LOQ	0.8	n/a
Low	1	0.014	0.005	< LOQ	1.3	n/a
	2	0.016	0.004	< LOQ	1.3	n/a
	3	0.019	0.005	< LOQ	1.0	n/a
Medium	1	0.015	0.004	< LOQ	1.2	n/a
	2	0.015	0.006	< LOQ	1.7	n/a
	3	0.017	0.006	< LOQ	1.4	n/a
High	1	0.013	0.006	< LOQ	1.5	n/a
	2	0.012	0.016	0.005	4.8	4.8 [a]
	3	0.015	0.017	0.008	4.3	5.3 [a]
LOQ		0.006	0.002	0.002		

[a] Ratios calculated based on reference values published for calcium phosphate reference material Durango apatite.

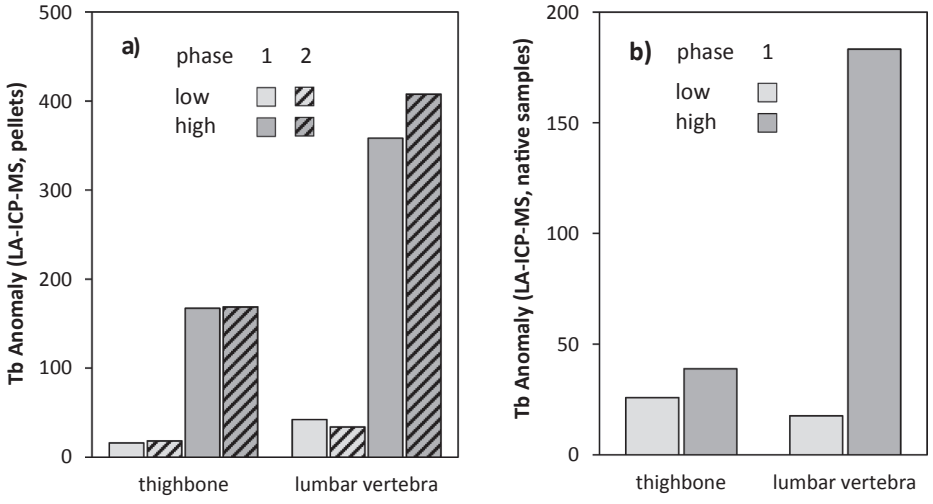

Fig. 4. Mean terbium anomaly (Tb_n/Y_n) detected in lamb bone samples using LA-ICP-MS. (a) Values obtained after dry ashing and pressing. Plain bars: Experimental phase 1 (during which the animals received spiked feed); hatched bars: Phase 2 (during which the animals received unspiked feed). (b) Values obtained by direct LA-ICP-MS measurement of native bone samples of experimental phase 1.

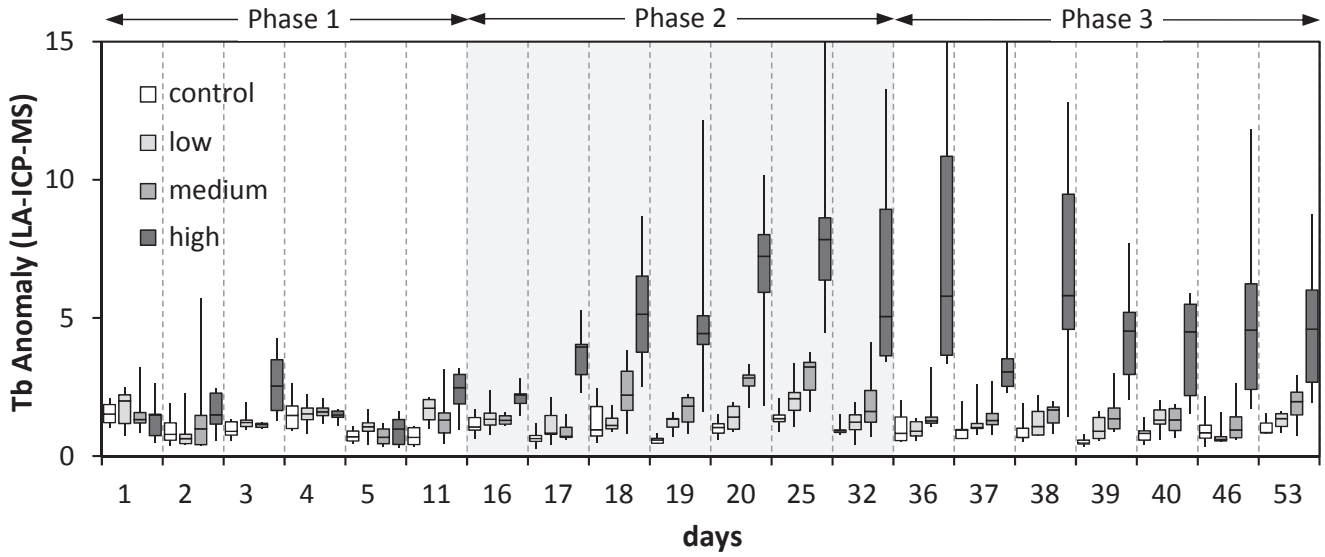

Fig. 5. Evolution of terbium anomaly (Tb_n/Y_n) detected in goat milk samples throughout the labeling experiment using LA-ICP-MS after dry ashing and pressing. Phase 1: Up to 50-fold terbium and thulium enriched feed; Phase2: Up to 500-fold terbium and thulium enriched feed; Phase 3: Unspiked feed.

method applied, and therefore no thulium anomalies could be calculated for these samples. As can be seen from Fig. 5, no significant terbium anomaly developed during phase 1, when the animals received feed enriched up to 50-fold in terbium (Table 1). Therefore, terbium end thulium enrichment in feed was increased, resulting in a 500-fold terbium enrichment for the "high" group. This resulted in the development of a small but significant terbium anomaly during phase 2, which decreased gradually during phase 3. Therefore, at least in the "high" group, milk was successfully labeled. The low terbium and thulium contents, roughly 0.02 μg kg^{-1} and 0.01 μg kg^{-1}, respectively, determined even in milk samples taken from the "high" group during phase 2 and phase 3 are somewhat surprising given the high calcium content in milk (De la Guardia & Garrigues, 2015) and the reported partial replacement of Ca^{2+} ions by lanthanide ions in biological systems (Evans, 2013). However, the data available are certainly not sufficient for hypothesizing on the mechanisms involved. For comparison, approximately 0.04 μg kg^{-1} terbium and 0.02 μg kg^{-1} thulium were measured in blood samples taken from lambs of the "high" group after phase 1 of the meat labelling experiment (Section 3.3), after receiving 1000-fold enriched feed. It appears that these numbers more or less represent the overall terbium and thulium levels in the body fluids of animals receiving a diet enriched in these elements, and that no further enrichment takes place during milk generation. In summary, labeling of milk has been shown to be feasible using 500-fold terbium and thulium enriched feed. It is possible that after a prolonged period of administering REE supplemented feed, lower REE enrichment in the feed would be sufficient for significant REE labeling of milk. However, the constraints of the present study did not allow for such an experiment.

3.5. Food safety

As the target of the present study is labeling of food products, the safety of these products for the customer is of course a crucial point. At present, no standards to limit the concentration of REE in meat and dairy products have been established yet (Jiang, Yang, Zhang, & Yang, 2012). The Chinese Ministry of Health has issued maximum limited levels for REE in vegetables and cereals ranging between 0.5 and 2.0 mg/kg (Chinese Ministry of Health, 2005). In literature, acceptable daily intake values for total REE were proposed ranging between 0.1 and 2 mg/kg body weight (He & Rambeck, 2000; Li, Chen, Chen, & Zhang, 2013). Since the elements used for labeling in this study, terbium and thulium, are amongst the least abundant REE in nature, the impact of their enrichment on the total REE content of food products is low. As can be seen from Table 3, no increase in total REE content could be detected in lamb meat (both shank and loin), i.e. the amount of terbium and thulium introduced by labeling is small compared to the natural variation in REE content. In contrast, a significant increase in total REE content occurred in lamb liver and kidney samples. However, even these numbers are well (by a factor of 20–100) below the limits issued in China, and for a person of 70 kg, a daily intake of 0.1 mg/kg body weight would still correspond to approximately 300 kg of labeled kidney or liver per day (calculated using the maximum values for total REE content in Table 3). Therefore no adverse effects are to be expected from the consumption of usual amounts of these products.

Since only yttrium, terbium and thulium were measured in goat milk samples, their total REE content cannot be quantified. However, based on the values determined for yttrium (Table 8), which are about one order of magnitude smaller than in meat samples, and assuming natural REE distribution, the total REE content in goat milk can be estimated to be correspondingly low. Therefore, considering also the minor enrichment of terbium and thulium achieved, no effects on consumer safety are to be expected from labeled milk products either.

4. Conclusions

The presented experiments have shown that food products, namely lamb meat and goat milk, can be distinctively labeled by spiking the feed for the animals with selected rare earth elements, such as terbium and thulium in this work. These labels can be detected in food products using ICP-MS after sample preparation involving complete acid digestion under high pressure and temperature. Therefore, products of a certain origin can be distinguished from unlabeled products of other origin and thus be safeguarded against imitation and fraud. In practice, the choice of elements and element ratios used for labeling can of course be varied, from season to season.

It has also been shown, that in milk, meat and bone samples, terbium and thulium enrichment can also be detected using laser ablation ICP-MS after a sample preparation procedure consisting of dry ashing and pressing. In bone samples, rare earth element labels could even be detected directly without any sample preparation apart from drying and polishing. Therefore, the developed LA-ICP-MS method which is fit for this purpose has been demonstrated to be simpler, faster, less matrix sensitive and which consumes lower amounts of chemicals and time than classical ICP-MS analysis.

Due to the extremely low levels of rare earth elements naturally

present in food products, distinctive labels can be introduced with absolute amounts of rare earth elements low enough to virtually exclude any detrimental effects on the potential customer.

CRediT authorship contribution statement

Donata Bandoniene: Conceptualization, Investigation, Supervision, Project administration, Writing - review & editing. **Christoph Walkner:** Investigation, Writing - original draft. **Ferdinand Ringdorfer:** Investigation, Resources. **Thomas Meisel:** Conceptualization, Writing - review & editing.

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