

## Review

# Use of rare earth elements as external markers for mean retention time measurements in ruminants

Laurence BERNARD\*, Michel DOREAU

Unité de Recherche sur les Herbivores, INRA de Clermont-Ferrand,  
63122 Saint-Genès-Champanelle, France

(Received 17 June 1999; accepted 2 March 2000)

**Abstract** — The present review deals with the utilisation of rare earth (RE) elements as particulate markers for ruminant nutrition studies. RE elements have similar chemical properties. They are attractive for use as a multiple marker system because they bind tightly to plant materials. RE binding of plant cell walls is cyclic throughout the RE series, which can be explained by the filling of the 4 f electron shell. RE markers may migrate from labelled feedstuffs under gastrointestinal conditions and particularly under acidic conditions, and they may decrease the digestibility of labelled feedstuffs. The various binding techniques used for labelling particulate matter with RE elements are evaluated in order to increase the stability of bound RE in different gastrointestinal conditions. The soaking procedure of plant cell walls to be labelled in an RE chelate solution, followed by washing to remove loosely bound RE, is recommended.

**rare earth / retention time / feedstuff / methodology / ruminant**

**Résumé** — Utilisation des terres rares comme marqueurs externes pour les mesures de temps de séjour moyen chez les ruminants. Cet article traite de l'utilisation des terres rares (TR) comme marqueurs des particules alimentaires pour la mesure du temps de séjour des aliments, dans les études de nutrition des ruminants. Ces différentes terres rares ont des propriétés chimiques proches, se fixent étroitement au matériel végétal et peuvent donc être utilisées de façon simultanée au cours d'un même essai. Les TR peuvent migrer des aliments sur lesquels elles ont été préalablement fixées du fait des conditions physico-chimiques rencontrées dans le tractus digestif, et aussi diminuer la digestibilité de ces aliments, probablement en réduisant la colonisation microbienne. Les différentes techniques employées pour marquer les aliments avec des TR ont été évaluées de façon à optimiser la stabilité du complexe TR-aliment. Le marquage par immersion de la fraction des constituants pariétaux de l'aliment dans une solution de TR, suivi d'un lavage de cette fraction afin d'éliminer la TR faiblement fixée, est recommandé.

**terre rare / temps de séjour / aliment / méthodologie / ruminant**

---

\* Correspondence and reprints  
E-mail: lbernard@clermont.inra.fr

## 1. INTRODUCTION

In ruminants, digestion is the result of different dynamic processes (reduction of the size of feed particles, fermentation, digesta flow) in which the ingested feedstuffs, the microbial population in the rumen, and the type of animal intervene. These processes are strongly inter-related. So, outflow rate of digesta from the rumen has an effect on rate and nature of rumen fermentation [51], on feed intake [6], and on efficiency of microbial synthesis [28]. Markers are used to estimate digesta flow and mean retention time by ruminants. Whereas a specific association of the marker with particles is not necessary for flow determination, the measurement of particle retention time requires an attachment of markers to the particles that are to be followed. The use of multiple markers to simultaneously tag different feedstuffs or feed particles allows the study of the dynamics of flow from the rumen. These systems of markers must meet certain criteria. General recommendations for gastrointestinal markers have been developed [32, 54, 57] and have been adapted to particulate markers, fixed on feedstuffs. Briefly, these markers must be intimately attached to the material to be marked and be unaffected by the digestive tract or its microbial population. They must not modify physical characteristics and fermentation kinetics of the feed fraction they mark. They must not affect the gastrointestinal tract microflora, nor modify gastrointestinal secretions, digestive and absorptive processes and gastrointestinal tract motility. They must be inert (without any toxic or physiological effects), and be easily determined without interfering with the determination of other substances. Moreover, they must be uniformly distributed throughout the feed fraction they mark. In a review [32] describing the markers used in nutrition studies, the interest in using RE elements as a multiple marker system was emphasised, since they are a group of elements with similar properties. RE elements

have been reported to bind tightly to plant materials [20, 33, 42], since they have low background levels in plant materials [47, 48], and they can easily be quantified by different techniques (atomic absorption and atomic or plasma emission procedures, neutron activation analysis...). A large number of markers have been used in nutrition studies: either internal markers when the marker is a natural and integral part of the plant material, or external markers when the marker is artificially integrated in the plant material. Methods and procedures for using various specific markers have been outlined in several reviews [21, 23, 24, 27, 45, 46], however without outlining any 'ideal' marker. The most widely used particle markers in nutrition studies are the RE elements and chromium. Chromium binds strongly to the cell wall and largely decreases feed digestibility [55], while RE elements are less tenaciously attached to feed leading to a smaller modification of feed digestibility.

*This paper will focus on the use of RE elements. Their chemical and labelling properties will be reported and their behaviour in the gastrointestinal tract (migration, modification of digestibility of plant material) in relation to used labelling technique, will be discussed.*

## 2. GENERAL PROPERTIES

Even if so-called, RE elements are neither rare nor earth. They are metallic elements which are more abundant than gold, silver, mercury or tungsten. RE are found in alkaline earth, lead minerals, rocks and in almost all ores, mainly at low concentrations. These elements have strikingly similar chemical properties, which make their separation difficult. Complex formation, fractional distillation and ion-exchange chromatographic methods have been successfully used to separate them. They are mainly used in the steel industry, metallurgy, ceramics, and as well as strong oxidizing agents in analytical chemistry. They are also used in making

optical products and sunglasses, and in orthodontics [43].

Rare-earth elements include the Lanthanide transition series (atomic number,  $Z = 57-71$ ) plus yttrium (Y) and scandium (Sc). All have similar chemical properties including trivalence. A total of sixteen elements are available for examination as particulate markers [4], excluding promethium (Pm) which has no stable isotope and does not exist naturally. The Lanthanide transition series starting with lanthanum (La,  $Z = 57$ ) includes fifteen elements with similar properties (including Pm). They form a sequence in which the 4 f electron shell (which is the antepenultimate level) is sequentially filled with up to 14 electrons. Consequently, a regular decrease in the ionic size from lanthanum (La,  $Z = 57$ ) through lutetium (Lu,  $Z = 71$ ) is observed. Ionic radii (valence 3) range from 1.14 Å (La) to 0.85 Å (Lu). As a result, throughout the Lanthanide series, a regular decrease in basic strengths, a regular increase in metallic properties, and an irregular increase in binding strength are observed. This irregularity is evident in the middle of the series where the 4 f electron shell is half filled. This periodicity within the rare-earth elements arises because the stability of electron spin configurations is maximum at 0, 7 or 14 electrons in the 4 f shell. It was demonstrated [50] that the irregularity of binding strength is cyclic and of similar pattern for gluconate, acetate and propionate. Later, workers [3] studying the binding strength of rare-earth elements on the cell wall (NDF, [56]) observed the same cyclic pattern with a peak of stability at samarium (Sm) and a trough at dysprosium (Dy) and holmium (Ho), and a maximum peak for thulium (Tm) [4].

Rare-earth elements easily complex because of their metallic properties; the RE element ionic complexes are soluble while RE non-ionic complexes (such as oxide) are insoluble. In the complexes, the electrons of the 4 f shell are not involved and the bonds are electrostatic: these are the same

type of bonds found with alkaline earth metal complexes in which the d shell electrons are not involved. In the lanthanide series, europium (Eu) and ytterbium (Yb) present properties closer to alkaline earth metals because they can achieve a stable conformation by reducing the valence to two.

The sensitivity of analysis by neutron activation analysis ranges from 0.00003 mg for Dy to 0.2 µg for Cerium (Ce) and Tm (Catalogue of the General Atomic Co., San Diego, CA). Analysis by plasma emission spectroscopy may allow an even greater sensitivity [12]. The sensitivity of atomic absorption spectrophotometry analysis ranges from 0.12 to 0.62 mg·L<sup>-1</sup>, respectively for Yb and Eu [Manual of analytical methods for atomic absorption spectrophotometry, Perkin Elmer, Norwalk, Connecticut, USA], because of variable interference conditions among RE. The utilisation of RE radioisotopes allows the detection of a multi-labelled sample with a sensitivity of about 0.02 Bq. In addition, radio-activation analysis can be used [15, 18, 29].

*Rare earth ( $z = 57-71$ ) are a group of elements with similar properties allowing their utilisation as a multiple marker system. Through the series, the 4 f electron shell is sequentially filled up to 14 electrons, leading to a cyclic binding strength for diverse substrates. The RE have a strong ability to form RE-complexes because of their metallic properties.*

### 3. RARE-EARTH BINDING SITES IN FEEDSTUFFS

The fiber matrix of plants consists of botanical structures such as xylem, phloem or sclerenchyma, which comprise the components of the plant cell wall, hemicellulose, cellulose, lignin and a small amount of protein. These entities have functional groups, such as carboxyl and amino groups with known ionisation potentials which

determine the cation exchange capacity of the plant. Depending upon rumen pH, these may become charged. For example, as pH increases, a greater proportion of cell wall carboxyl groups becomes negatively charged. This phenomenon leads to a difference in the cation exchange capacity of the fiber which measures the ability of the fiber matrix to bind and hold metal ions on its surface. The fiber serves as a reservoir, exchanging sodium, potassium, as well as calcium, magnesium and other multivalent cations for hydrogen when the pH drops. Consequently, the cation exchange capacity may affect the extent of microbial attachment, (with the cation being involved in this attachment mechanism as a link between the bacterial glycocalyx and cellulose [34]) and thus the rate of digestion [37]. Moreover, the cation exchange capacity of the fiber may also provide significant buffering capacity in the gastrointestinal tract [38]. The cation exchange capacities vary widely among feedstuffs and depend on their nature. Nevertheless, the cation exchange capacity of forages presents little variation. Furthermore, the cation exchange capacity of feeds in the rumen is largely dependent upon their NDF (Neutral Detergent Fiber) content. The cation exchange capacity ranges from low to high for maize silage, straws, brans, grass hays, and legume hays [5]. This is the property which is used to label feedstuffs with RE elements.

The study of RE binding sites will allow the characterisation of such sites both in terms of their abundance, determining the RE quantity fixed, and in terms of their affinity for RE, which will determine both labelling conditions and ability for migration. The RE binding capacity is related to the cation exchange capacity of the feedstuffs to which labelling takes place.

It was demonstrated that feedstuffs exhibit a continuous distribution of affinities for RE [19]. Teeter et al. [52] measured the binding strength and capacities of different feedstuffs (mainly concentrates; 7 cereal grains,

1 roughage and 1 cottonseed hull) for Yb at pH 3.8, and reported total binding capacities ranging from 15.9 to 210.4  $\mu\text{mol Yb}\cdot\text{g}^{-1}$  feed for whole corn and cottonseed hulls, respectively. From binding affinity studies, partition between Yb concentrations in feed and in the saturated solution allowed to calculate the number of heterogeneous binding sites and their binding capacity and affinity for Yb following the Scatchard curve procedure [25]. The authors simply estimated, at least, 2 groups of binding sites: weaker sites with molar association constants ( $K_a$ ) of 4.5 to  $9.2 \times 10^5$  and stronger sites with  $K_{as}$  of 1.2 to  $3.8 \times 10^7$ .

McBurney et al. [39] measured the cation exchange capacity of neutral detergent fiber residues from 12 different feedstuffs (forages and concentrates) with a method utilising praseodymium (Pr) [5]. They reported binding capacities ranging from 104.0 to 490.9  $\mu\text{mol}\cdot\text{g}^{-1}$  feed for oat and rapeseed NDF, respectively. These binding capacities were correlated to lignin ( $r = 0.84$ ) and NDF nitrogen content ( $r = 0.58$ ). From a binding capacity study of Yb [1], it is clear that polyphenolic and carboxylic acid groups are involved in the majority of RE binding by feedstuffs. In fact, proteins, starch and cellulose from feedstuffs bind less than 80  $\mu\text{mol Yb}\cdot\text{g}^{-1}$  of dry matter (DM), while p-coumaric acid, polygalacturonic acid and tannic acid bind respectively 755, 1588 and 1986  $\mu\text{mol Yb}\cdot\text{g}^{-1}$  DM. Moreover, when uronic groups of galacturonic acid are methylated, binding capacity decreases from 1588 to 123  $\mu\text{mol Yb}\cdot\text{g}^{-1}$  DM, indicating the importance of these groups in forming ligands [1]. The affinity for Yb of some studied soluble organic compounds ranked from greatest to lowest are as follows: lactate, acetate, lysine, glucose, glycine and sucrose [52]. This means that carboxyl and hydroxyl groups as well as amino acid nitrogen are functional groups involved in solute-binding of Yb. Moreover, it is possible to use RE in their chelate forms, since RE elements have a strong ability to bind chelating agents [2, 19, 49]. This was investigated by

Bernal-Santos et al. [8], who evaluated praseodymium chelated to ethylene diamine tetraacetic acid (Pr-EDTA) as a marker of the rumen liquid phase in replacement of Co- and Cr-EDTA. The authors underestimated the liquid turnover rate by using Pr-EDTA probably because of its poor distribution within the rumen liquid phase caused by its preferential association with the particulate matter functional groups and its precipitation under rumen conditions.

The uniformity of distribution throughout the feed fraction to which the RE are attached is another required property of RE. Concerning this point, little has been reported in the literature. One author [9] found that Yb-labelled in the NDF fraction of hay decreases as particle size decreases (from 1-cm chopped hay to 4- and 1-mm ground hay), as a consequence of its associated increase in NDF content together with a decrease in N content. Conversely, other authors [22] observed an irregular distribution of Yb in different cell wall particle sizes of feeds with a preferential accumulation of the marker upon the small particle fraction (< 600  $\mu\text{m}$ , separated by dry-sieving procedure). This result is probably due to the greater surface area per unit mass with such small feed particles, making more potential binding sites available per unit mass in small sieve size fractions.

These data indicate that in rate of passage experiments, marking whole forages with Yb would introduce a bias in passage measurements because of a differential distribution of the marker among particle pool size. Moreover, nothing is known about the effect of RE labelling on specific density of particles. An increase in specific density of particles labelled with mordanted Chromium, and consequently an increase in the mean retention time in the gastrointestinal tract, has, however, been reported [17].

*The cation exchange capacity of plant cell walls, which depends on the composition of their functional groups, is the property used to label cations on feedstuffs and par-*

*ticularly RE. Feedstuffs exhibit both weak and strong binding sites for RE. The functional groups involved in RE binding have been characterized as carboxyl, hydroxyl and amino acid nitrogen groups.*

#### **4. STABILITY OF RE-FEEDSTUFF COMPLEXES IN GASTROINTESTINAL CONDITIONS**

It has been reported that RE may be removed from feedstuff particles to which they were initially bound, in gastrointestinal tract conditions and particularly within the rumen. Even when dialysed against water, Yb complexes dissociate in Yb-labelled feedstuffs. This dissociation corresponds to the removal of 0.2 to 0.51%·h<sup>-1</sup> of the initially bound Yb in 24 h ([52]; (Tab. I). When estimated in vitro in rumen fluid, RE migration rates either rise up to 3.9%·h<sup>-1</sup> [14] or are low and close to values obtained in situ using nylon bags placed in the rumen (0.21%·h<sup>-1</sup>; [53]). No RE migration rate has ever been reported to exceed 0.89%·h<sup>-1</sup> ([7]; (Tab. I). These values obtained in situ using nylon bags, probably underestimated RE migration rate because they do not take into account the part of the removed marker which binds to another particle or precipitate as phosphates or hydroxides remaining in the bag, as it may occur in vivo. Bound Yb recovered after 48 hours of incubation in nylon bags in the rumen was 57.5% [7], indicating that much of the Yb either migrated or was associated with the digested particulate matter. Other studies reported a recovered fraction of 81% and even 93% of the initially bound Yb after 24 hours of incubation in nylon bags in the rumen ([9, 29]; (Tab. I). The results reported by Combs et al. [14] on the effects of fresh or autoclaved rumen incubation media on the retention of Yb- and Ce-labelled hay exhibited low recovered bound RE after 20 hours: 65 and 51.2% of the initial amount of Yb and Ce, respectively for autoclaved rumen fluid

**Table I.** Migration rates of rare earth (RE) elements from feedstuff under ruminal conditions.

Feedstuff	Treatment	RE	Incubation time (h)	Migration rate (%·h <sup>-1</sup> )	Reference
Cracked corn	Dialysed/water	Yb	24	0.51	[52]
Alfalfa hay				0.26	
Cottonseed hulls				0.20	
Prairie hay				0.22	
Cracked corn	In vitro rumen fluid	Yb	48	0.21	[53]
Bromegrass hay	In vitro:				
	Fresh rumen fluid	Yb, Ce	20	3.9	[14]
	Autoclaved rumen fluid	Yb, Ce		2.1	
Whole shelled corn	Rumen nylon bag	Yb	24	0.19	[35]
Roughage	Rumen nylon bag	Sm, Ce, La	24	0.04	[29]
Grain				0.31	
Concentrate	Rumen nylon bag	La, Sm	12	0.58	[15]
Silage	Rumen nylon bag	Yb	48	0.89	[7]
Roughage	Rumen nylon bag	Yb	24	0.79	[9]

incubation, and 24 and 19% of the initial amount of Yb and Ce, respectively for fresh rumen fluid incubation. These results underlined the role of rumen microbes in displacing RE from labelled feeds. Even if some migration of RE has been shown for labelled particles during ruminal fermentation, the majority of migration occurs when labelled feedstuffs encounter the acidic conditions of the abomasum. Moreover, migration of the marker is much greater as acidic conditions increase [14, 16, 53]. When pH of the incubation media decreases from 4.9 to 1.2, migration of bound feedstuff-Ce increases from 5.4 to 18.2% [16]. Allen [2] reported RE recoveries from alfalfa hay of 44.5, 32.5 and 7.7% following a wash treatment in HCl solutions at respectively pH 4.0, 3.0 and 2.2. Among RE elements, recoveries vary cyclically as pH of wash solutions decline [1, 4]. A greater migration of markers from labelled hay was also observed in an acid incubation study [14] when the pH media of either fresh or autoclaved rumen fluid, was reduced from 6.5 to 2.2 (Tab.II). When concentrate feedstuffs

labelled with La and Sm were incubated in sacco in the rumen for 12 h followed by a 3 h in vitro incubation in abomasal fluid (pH 2.5–3.5), 23 and 38% removal of the markers La and Sm were respectively reported following this treatment ([15]; Tab. II). This indicates that both markers tend to become more mobile under acidic conditions, with La being somewhat more tightly bound to feed particles than Sm, in disagreement with results from [1]. A higher migration rate of the marker in concentrate feedstuffs was obtained when the incubation was performed both in sacco in the rumen and in vitro in abomasal fluid (7.7 to 12.6%·h<sup>-1</sup>) rather than with rumen incubation alone (0.58%·h<sup>-1</sup> [15], Tabs. I and II). Other authors [35] using the same dual procedure (rumen nylon bag incubation followed by an in vitro abomasal incubation) to investigate Yb labelled whole shelled corn, reported lower migration of Yb than those reported in [15]. This result is partly explained by the difference in the pH value of the fluid incubation medium used in this assay (Tab. II) compared to study [15]. With

**Table II.** Migration rates of rare earth (RE) from feedstuff under acidic conditions.

Feedstuff	Treatment	RE	Incubation time (h)	Migration rate (%·h <sup>-1</sup> )	Reference
Concentrate	Abomasal fluid, pH (2.5–3.5)	Sm	3	12.6	[15]
		La		7.7	
Corn	Acid solution, pH 3.0	Yb	12	5.3	[52]
	Acid solution, pH 1.5			7.7	
Whole shelled corn	Abomasal fluid, pH 4	Yb	3	NS	[35]
	Abomasal fluid, pH 3			0.06	
	Abomasal fluid, pH 2			1.20	
Cracked corn	Acid-pepsin, pH 2	Yb	48	1.2	[53]
Roughage	Abomasal fluid, pH 2.5	Yb-citrate	3	3.0	[9]
		Yb-acetate		7.8	
Bromegrass hay	Fresh rumen fluid, pH 6.5	Yb/Ce	20	3.8/4.0	[14]
	Fresh rumen fluid, pH 2.2	Yb/Ce		4.8/4.4	
	Auclaved rumen fluid, pH 6.5	Yb/Ce		1.8/2.4	
	Auclaved rumen fluid, pH 2.2	Yb/Ce		4.9/4.6	

concentrate diets [15] compared to forages, a more important migration rate of RE could be expected regarding the lower lignin and NDF content, and consequently the lower cation exchange capacity than forages.

*Even if some migration from labelled particles occurs during ruminal fermentation because rumen microbes displaces RE from labelled feeds, it is clearly established that acid conditions cause most of the marker migration. Under acidic conditions, RE element recoveries vary cyclically with increasing atomic number while under rumen conditions no differences among RE have been reported.*

## 5. DIGESTIVE MODIFICATION INDUCED BY RE LABELLING

Several authors showed that the fermentation of feedstuffs is affected by the binding of RE elements, as digestibility of labelled feedstuff decreases. A significant decrease of extent of in vitro DM degrad-

ability of Yb-labelled feedstuffs was observed, with reduction of both rates of DM disappearance and extent of digestion ([52]; Tab. III). A reduction from 48.0 to 35.5% (26.0%) of dry matter disappearance in situ of Yb-labelled NDF silage was reported ([7]; Tab. III). Bernard [9] reported that the higher the level of Yb bound to feedstuff, the lower the microbial colonisation estimated using the <sup>15</sup>N technique, suggesting that RE bind to feedstuffs on the same sites as microbes. As a consequence, a significant decrease in digestibility of Yb-labelled feedstuff is observed when there are high levels of bound Yb (Tab. III). Among several studies, only one study [35] reported no influence of Yb labelling on in situ DM digestibility.

From these observations, it may be recommended to bind no more than the level of RE sufficient for dosing.

*Several studies reported that RE labelling decreases feed digestibility. This decrease is larger as the level of bound RE increases, probably because of a negative interaction*

**Table III.** Decrease in DM disappearance following RE labelling of feedstuffs under ruminal conditions.

Feedstuff	RE salt/ Labelling procedure	Bound RE (mg·g <sup>-1</sup> DM)	Incubation time (h)	Variation of DM disappearance <sup>a</sup> (%)	Reference
Cereal grains	Yb-Cl <sub>3</sub> /Soaking	4.3	24	-3.9	[52]
		↓	(in vitro)	↓	
Roughage		20		-5.8	
Hays	Yb-Cl <sub>3</sub> /Soaking	5	24	-7.0	[11]
		↓	(in vitro)	↓	
		40		-30.8	
Wheat forage	Yb-Cl <sub>3</sub> / Soaking	15.6	24	-15.1	[36]
	Yb-Cl <sub>3</sub> / Spray	48.6	(in vitro)	-18.0	
Corn	Yb/Soaking	10.80	48	No effect	[35]
			(in situ)		
Silage	Yb-acetate/Soaking	35	48	-26	[7]
			(in situ)		
Hay	Yb-citrate/Soaking	2.15	24	-22.4	[9]
	Yb-acetate/Soaking	19.74	(in situ)	-43.7	

<sup>a</sup> Calculated as: (DM disappearance of RE-labelled feed – DM disappearance of unlabelled feed) × 100/DM disappearance of unlabelled feed.

with microbe attachment as demonstrated by Bernard [9].

## 6. FEEDSTUFF LABELLING PROCEDURE

Various binding procedures have been proposed for labelling RE elements of feedstuffs. Feedstuff preparation, solution concentration, time, temperature, pH, RE salt, and application procedure (spraying or soaking) are factors which play important roles in the final concentration and the stability of bound RE.

### 6.1. Feedstuff preparation

Because specific binding sites of RE are not well known, it seems reasonable to confine binding to specified, less digestible enti-

ties such as the cell wall contents. Therefore, it is recommended to bind RE to feedstuff cell walls and to prevent the formation of complexes which will be rapidly dissociated from the feed in the gastrointestinal tract [1, 21]. Ellis et al. [21] proposed to extract the cell wall content with a pH 9 phosphate buffer containing 3% sodium lauryl sulphate rather than with the neutral detergent solution [56], because the detergent solution contains EDTA which is a chelating agent difficult to rinse away. Nevertheless, phosphate salt of RE presented the same disadvantage as EDTA-RE. Therefore, the “classical” extraction of the NDF fraction is the more widely used method.

*As previously proposed in several studies, it is retained to extract the NDF residue of feedstuffs, in order to remove easily digested fractions, prior to labelling, since the NDF is the fraction of interest.*

## 6.2. Application procedure of RE solution on feedstuff

The binding procedure differs in the RE application methods used to label feedstuff residues. These application methods include dosing in the rumen RE salt gelatin capsules [40, 41], spraying RE solutions onto the feedstuff to be labelled, followed or not by drying [13, 20, 29, 44], and soaking feedstuff in RE solutions followed by washing with water [10, 52] or an acidic solution [2] to eliminate unbound RE. Labelling the whole rumen content using RE salt gelatin capsules is not advised, as insoluble phosphate or hydroxyde salt formation may occur [30, 31] and sink to the ventral sac of the rumen. When compared to spraying, the soaking procedure for RE labelling leads to both a smaller ruminal turnover rate and a longer time before the first appearance of the marker in the faeces [36], suggesting that more precipitation of RE as RE phosphate and/or more migration towards new feed entering the rumen occurs when RE is applied by spraying. Other authors [14] did not find any differences in migration rate of Yb- and Ce-labelled bromegrass hay, whatever the labelling procedure (spraying or soaking). This controversy can be partly explained by the rinsing treatment following the soaking procedure: in the former [36], after soaking, the RE-labelled feed was rinsed in distilled water two to three times for 4 to 6 h; in the latter [14], it was rinsed 6 times with distilled water with a 1 h soak. The difference can also be explained by the different labelling conditions and by the different approach of the two studies: the former reported mean retention time data, while the latter used an *in vitro* system to observe the direct role of rumen microorganisms in displacing the marker from labelled feed. It is therefore difficult to compare the respective conclusions of these studies. To allow the removal of a greater proportion of potentially exchangeable RE elements, even when using the soaking technique, a careful rinsing of excess marker is recommended. At

least two rinsing procedures with distilled water with a 1 h soak between each are necessary [9].

Binding strength of RE is sensitive to acidic conditions encountered in the abomasum, because of competition between the  $H^+$  cation and RE for feedstuff binding sites. This can lead to unreliable data, in ruminant dynamic process studies, as sampling is performed after the abomasum. Therefore, after saturation of feedstuff binding sites by labelling with the soaking technique, loosely bound RE should be removed by washing with an acidic solution ( $pH < 2$ , as in [1]), assuming that remaining marker will be resistant to acidic removal. Another strategy is to allow a selective binding procedure based either on competition during labelling between the RE ligand and the binding site of the feedstuff, or on the dissociation of the RE-feedstuff complexes, afterwards, by a soluble ligand having a higher association constant than the RE-feedstuff complex [19]. Then, it may be proposed to use the soluble EDTA ligand (or Citrate as in [26]) to selectively bind Yb to those sites because of the existence of high affinity binding sites on forages for Yb which equal and exceed that of EDTA for Yb. This will allow for a high or total resistance of Yb to dissociation and particularly to the displacement of  $H^+$  in acidic conditions. This method is advantageous compared to the acid washing procedure in that the integrity of the feedstuff cell wall is not affected. Nevertheless, RE-labelled feedstuffs prepared by saturating all binding sites, followed by washing with either acid or different chelating agents does not lead to a substantial increase in the resistance of bound RE to subsequent losses under acidic conditions, such as those found in the abomasum [2]. Fortunately, the major site of retention of feedstuffs within the gastrointestinal tract is the rumen, in which pH conditions are close to neutrality (retention time in the rumen represents more than 50% of total mean retention time and more than 85% of stomachs mean retention time).

*Soaking of the feed fraction that is to be labelled in a RE-chelate solution followed by a careful rinsing procedure, to remove unbound or loosely bound markers, is the advised labelling method. The nature of the chelate used will depend on both desired quantity of RE bound and on the partition between weak and strong sites for RE on feedstuff.*

### 6.3. Labelling solution

Allen et al. [5] have widely reviewed the effect of solution concentration, duration and temperature on the binding capacities of two RE, Pr and neodymium (Nd), on hay and bran. These authors reported that, for both feeds, bound Pr increased with solution concentration passing from 0.005 to 0.008 M. Similarly, bound Nd continued to increase, but in a lesser extent, with solution concentration up to 0.016 M. So, a solution concentration of 0.02 M will allow both a sufficient labelling and a good binding rate of RE (50% as reported in [9]). The labelling duration affected binding similarly: binding with Pr equilibrated within 48 h, while with Nd it continued to increase up to 96 h. This illustrates that even if RE exhibits similar properties in their group, they have distinct individual binding characteristics. Binding of both Pr and Nd increased linearly with temperature ranging from 5 to 65 °C, with a binding increase of 27 for Pr and 41% for Nd [5]. Moreover, heat treatment from 22 and 60 °C increases the binding capacity of alfalfa and timothy hay for RE elements (Pr, Sm, Ho, Tm) by 15% [2]. This increased binding capacity may be due to either an increased physical access to sites, or to formation of new binding sites. Nevertheless, heat treatment did not reduce the observed removal of RE in acidic conditions.

According to reported cation-exchange capacities of 12 different feedstuffs with Pr in solution at pH 3.5 and 7.0 [39], it was calculated that the amount of Pr bound at

pH 3.5 ranges from 30 to 65% of the level bound at pH 7.0; this increases with lignin content of feedstuffs. In fact, the cation-exchange capacity of NDF residues of feed changes with pH: at pH 7 the binding capacity of the feedstuff is higher than that observed at pH 3.5, because a decrease in pH reduces the degree of ionisation of constituents of the fiber matrix. PH is thus an important characteristic of the labelling solution. This is however rarely mentioned in the literature.

After application of an RE labelling procedure onto forages, Allen et al. [2] performed different acid washes (at pH 4.0, 3.0 and 2.2). Remaining bound RE decreased as pH of the wash solution was more acid. The RE recoveries from forages following the three acid washes varied cyclically with increasing atomic number, in a similar pattern as the stabilities of RE gluconates, acetates and propionates [50].

As discussed above (see 6.2), some workers [15, 29] labelled feedstuffs by spraying with an RE solution at pH 1, while others [52] soaked feedstuffs in RE solution at pH 3.8. Utilisation of less acidic (pH > 4) and alkaline solutions was not advisable for binding of RE, due to the formation and precipitation of insoluble hydroxides. Nevertheless, different RE salts have been used to bind RE to feedstuffs and particularly RE acetates which are more stable to hydroxide formation, and are therefore more available to specifically bind to feedstuff [1]. Consequently, RE acetate solutions have been used in studies where an accurate mean retention time measurement was required [7, 10].

*A concentration of RE labelling solution of 0.02 M is convenient to label feedstuffs; the duration of soaking depends on the required amount of bound RE and on the different RE elements themselves as binding curves differ among RE (different durations are necessary to reach the plateau of RE binding). An increase in labelling temperature increases the amount of labelled*

*RE without any improvement in binding strength. The pH of the labelling solution is intimately linked to the RE-salt used, since their solubility depends on it. Nevertheless, RE-acetate solutions are recommended to label feedstuffs since they are more stable to hydroxyde formation and may be used at neutral pH.*

## 7. CONCLUSION

With regards to the evidence that migration of RE elements from originally labelled feedstuffs may occur under gastrointestinal conditions and may be related to application procedure, much care must be taken in the preparation of RE-labelled feedstuffs and in the evaluation of RE migration data mentioned in the literature. The soaking procedure is advised to bind RE to cell wall residues of feedstuffs, followed by a careful washing procedure to remove unbound or loosely bound markers. Both cell wall binding capacity of the feedstuff and nature of the RE-salt will determine the quantity of labelled RE. An increase in bound RE leads to a decrease in DM digestibility caused by a decrease in microbial adhesion. This result suggests that the presence of RE in decreasing microbial degradation reduces RE migration due to feed degradation. Therefore, if digestibility of labelled feed is not of main importance, a strategy could be to increase bound RE by using RE-acetate to label feedstuff, leading to a smaller error in mean retention time measurement than if RE migration occurs from initially bound particles to other ones or to the liquid phase. Nevertheless, acidic conditions remain the major cause of RE migration: the longer the period of contact with acidic conditions, the more RE migration occurs.

## REFERENCES

- [1] Allen M.S., Investigation into the use of rare earth elements as gastrointestinal markers, MS thesis, Cornell University, Ithaca, New York, 1982.
- [2] Allen M.S., Methodologies for studying the dynamic of rumen function, Ph.D. thesis, Cornell University, Ithaca, New York, 1986.
- [3] Allen M.S., van Soest P.J., Comparative affinities of rare earth elements for plant cell walls, *J. Anim. Sci.* 55 (Suppl. 1) (1982) 403.
- [4] Allen M.S., Van Soest P.J., Periodicity and variation in the binding of rare earth ions to plant cell wall particles, in: Kennedy P.M. (Ed.), *Techniques in Particle Size Analysis of Feed and Digesta in Ruminants*, Can. Soc. Anim. Sci., Edmonton, Alberta, Canada, 1984, pp. 180–183.
- [5] Allen M.S., McBurney M.I., van Soest P.J., Cation-exchange capacity of plant cell walls at neutral pH, *J. Sci. Food Agric.* 36 (1985) 1065–1072.
- [6] Balch C.C., Campling R.C., Regulation of voluntary food intake in ruminants, *Nutr. Abst. Rev.*, Serie B 32 (1962) 669–686.
- [7] Beauchemin K.A., Buchanan-Smith J.G., Evaluation of markers, sampling sites and models for estimating rates of passage of silage or hay in dairy cows, *Anim. Feed Sci. Technol.* 27 (1989) 59–75.
- [8] Bernal-Santos G., van Soest P.J., Sniffen C.J., Thonney M.L., Evaluation of praseodymium as a liquid phase marker in rumen turnover studies, *Anim. Feed Sci. Technol.* 63 (1996) 201–209.
- [9] Bernard L., Étude de la dynamique des particules et des liquides dans le réticulo-rumen, chez le mouton recevant une ration de foin de Dactyle sous forme hachée et broyée en différentes proportions, Thèse, Université de Clermont-Ferrand II, France, 1992.
- [10] Bernard L., Chaise J.P., Delval E., Poncet C., Validation of the main modeling methods for the estimation of marker mean retention times in the different compartments of the gastrointestinal tract in sheep, *J. Anim. Sci.* 76 (1998) 2485–2495.
- [11] Coleman S.W., Evans B.C., Horn G.W., Some factors influencing estimates of digesta turnover rate using markers, *J. Anim. Sci.* 58 (1984) 979–986.
- [12] Combs D.K., Satter L.D., Sequential determination of cobalt, chromium, lanthanum, samarium, europium and ytterbium in digesta by direct current plasma emission spectroscopy, *J. Dairy Sci.* 68 (Suppl. 1) (1985) 128.
- [13] Combs D.K., Shaver R.D., Singh N., Satter L.D., Effects of application procedure, pH and microbial growth on retention of Ytterbium or Cerium by brome hay, *Can. J. Anim. Sci.* 64 (Suppl. 1) (1984) 66–67.
- [14] Combs D.K., Shaver R.D., Satter L.D., Retention of rare earths by hay particles following incubation in fresh or autoclaved rumen fluid, *J. Dairy Sci.* 75 (1992) 132–139.

- [15] Crooker B.A., Clark J.H., Shanks R.D., Rare earth elements as markers for rate of passage measurements of individual feedstuffs through the digestive tract of ruminants, *J. Nutr.* 112 (1982) 1353–1361.
- [16] Ehle F.R., Some nutritional implications of the hamster forestomach, MS thesis, Cornell University, Ithaca, New York, 1977.
- [17] Ehle F.R., Influence of feed particle density on particulate passage from the rumen of a Holstein cow, *J. Dairy Sci.* 67 (1984) 693–697.
- [18] Ellis W.C., Dysprosium as an indigestible marker and its determination by radioactivation analysis, *J. Agric. Food Chem.* 16 (1968) 220–224.
- [19] Ellis W.C., Beever D.E., Methods for binding rare earths to specific feed particles, in: Kennedy P.M. (Ed.), *Techniques in Particle Size Analysis of Feed and Digesta in Ruminants*, Can. Soc. Anim. Sci., Edmonton, Alberta, Canada, 1984, pp. 154–165.
- [20] Ellis W.C., Huston J.E., Ce-Pr as a particulate digesta flow rate marker in ruminants, *J. Nutr.* 95 (1968) 67–78.
- [21] Ellis W.C., Lascano C., Teeter R., Owens F.N., Solutes and particulate flow markers, in: *Protein requirements for cattle*, Protein Symposium Oklahoma State University, Stillwater, 1982, pp. 37–56.
- [22] Erdman R.A., Smith L.W., Ytterbium binding among particle size fractions of forage cell walls, *J. Dairy Sci.* 68 (1985) 3071–3075.
- [23] Fahey G.C., Jung H.G., Lignin as marker in digestion studies: a review, *J. Anim. Sci.* 57 (1983) 220–225.
- [24] Faichney G.J., The use of markers to partition digestion within the gastrointestinal tract of ruminants, in: Mc Donald I.W., Warner A.C.I. (Eds.), *Digestion and Metabolism in the Ruminant*, University of New England Publishing Unit, Armidale, Australia, 1975, pp. 277–291.
- [25] Ferdinand W., *The Enzyme Molecule*, Wiley J. and sons Ltd, Bristol, England, 1976, pp. 1–95.
- [26] Ferlay A., Legay F., Bauchart D., Poncet C., Doreau M., Effect of supply of raw or extruded rapeseeds on digestion in dairy cows, *J. Anim. Sci.* 70 (1992) 915–923.
- [27] Galyean M.L., Krysl L.J., Estell R.E., Marker-based approaches for estimation of fecal output and digestibility in ruminants, in: Owens F.N. (Ed.), *Feed intake by beef cattle: symposium*, Oklahoma Agric. Exp. Stn. Misc. Publ. 109, Stillwater, 1987.
- [28] Harrison D.G., McAllan A.B., Factors affecting microbial growth yields in the reticulorumen, in: Ruckebusch Y., Thivend P. (Eds.), *Digestive Physiology and Metabolism in Ruminants*, Lancaster, MTP Press Ltd, 1980.
- [29] Hartnell G.F., Satter L.D., Extent of particulate marker (samarium, lanthanum, and cerium) movement from one digesta particle to another, *J. Anim. Sci.* 48 (1979) 375–380.
- [30] Kennelly J.J., Yang M.T., Milligan L.P., Attachment of rare earth markers to particulate matter, *J. Anim. Sci.* 53 (Suppl. 1) (1981) 492.
- [31] Kennelly J.J., Yang M.T., Milligan L.P., Influence of buffers, pH and rumen fluid on dysprosium precipitations in vitro, *J. Anim. Sci.* 53 (Suppl. 1) (1982) 410.
- [32] Kotb A., Luckey T.D., Markers in nutrition, *Nutr. Abstr. Rev.* 42 (1972) 813–845.
- [33] Kyker G.C., Rare earths, in: Comar C.L., Bronner F. (Eds.), *Mineral Metabolism*, Chap. 32, Vol. 2, Part B, Academic Press, New York, 1962, pp. 499–541.
- [34] Latham M.J., Brooker B.O., Pettipher G.L., Harris P.J., *Ruminococcus flavefaciens* cell coat and adhesion to cotton cellulose and cell walls in leaves of perennial ryegrass (*Lolium perenne*), *Appl. Environ. Microbiol.* 35 (1978) 156–165.
- [35] Ledoux D.R., Williams J.E., Stroud T.E., Ellersieck M., Ytterbium as a particulate phase marker for measurement of digesta passage rate in ruminants, *Nutr. Rep. Int.* 32 (1985) 111–115.
- [36] Mader T.L., Teeter R.G., Horn G.W., Comparison of forage labeling techniques for conducting rate of passage studies, *J. Anim. Sci.* 58 (1984) 208–212.
- [37] McBurney M.I., Cation exchange and buffering capacity of dietary fibers, MS thesis, Cornell University, Ithaca, New York, 1981.
- [38] McBurney M.I., van Soest P.J., Chase L.E., Cation-exchange capacity and buffering capacity of neutral detergent fibers, *J. Sci. Food Agric.* 34 (1983) 910–916.
- [39] McBurney M.I., Allen M.S., van Soest P.J., Praseodymium and copper cation-exchange capacities of neutral-detergent fibres relative to composition and fermentation kinetics, *J. Sci. Food Agric.* 37 (1986) 666–672.
- [40] Miller J.K., Byrne W.F., Comparison of scandium-46 and cerium-144 as nonabsorbed reference materials in studies with cattle, *J. Nutr.* 100 (1970) 1287–1292.
- [41] Miller J.K., Moss B.R., Byrne W.F., Distribution of cerium in the digestive tract of the calf according to time after dosing, *J. Dairy Sci.* 54 (1971) 497–502.
- [42] Morgan A., The direct uptake of fission products on Ryegrass, in: *Report on Health Physic Division*, Atomic Energy Research Establishment, Harwell, Berkshire, England, 1959.
- [43] Muecke G., Möller P., Les terres... rares ?, *Pour la Science* 125 (mars 1988) 64–71.
- [44] Olbrich S.E., Mart F.A., Vogt J.R., Hilderbrand E.S., Use of neutron activation analysis in the determination of digestibility with cerium as an inert marker, *J. Anim. Sci.* 33 (1971) 899–902.

- [45] Owens F.N., Hanson C.F., External and internal markers for appraising site and extent of digestion in ruminants, *J. Dairy Sci.* 75 (1992) 2605–2617.
- [46] Pond K.R., Ellis W.C., Lascano C.E., Akin D.E., Fragmentation and flow of grazed coastal bermudagrass through the digestive tract of cattle, *J. Anim. Sci.* 65 (1987) 609–618.
- [47] Robinson W.O., Edington G., The agricultural significance of the minor elements, *Am. Fert.* 89 (1983) 5.
- [48] Robinson W.O., Bastron H., Murata K.J., Biogeochemistry of the rare earth elements with particular reference to hickory trees, *Geochim. Cosmochim. Acta.* 14 (1958) 55–65.
- [49] Siddons R.C., Paradine J., Beever D.E., Cornell P.R., Ytterbium acetate as a particulate phase digesta-flow marker, *Br. J. Nutr.* 54 (1985) 509–519.
- [50] Sinha S.P., Complexes of the rare earths, Pergamon Press, Oxford, 1966, pp. 7–85.
- [51] Sutherland T.M., Particle separation in the forestomachs of sheep, in: Sutherland T.M., Mc Williams J.R., Leng R.A. (Eds.), *Reviews in Rural Science*, Vol. 2, University of New England Publishing Unit, Armidale, Australia, 1976, pp. 65–72.
- [52] Teeter R.G., Owens F.N., Mader T.L., Ytterbium chloride as a marker for particulate matter in the rumen, *J. Anim. Sci.* 58 (1984) 465–473.
- [53] Turnbull G.W., Thomas E.E., Evaluation of rare earth markers using an in vitro ruminal fermentation system and effect of processing method on ruminal turnover of sized corn particles, *J. Anim. Sci.* 64 (1987) 1835–1841.
- [54] Udén P., Comparative studies on rate of passage, particle size, and rate of digestion in ruminants, equines, rabbits and man, Ph.D. thesis, Cornell University, Ithaca, New York, 1978.
- [55] Udén P., Colucci P.E., van Soest P.J., Investigation of chromium, cerium and cobalt as markers in digesta rate of passage studies, *J. Sci. Food Agric.* 31 (1980) 625–632.
- [56] van Soest P.J., Wine R.H., Determination of lignin and cellulose in acid detergent fiber with permanganate, *J. Oph. Agr. Chem.* 51 (1968) 780–785.
- [57] von Engelhardt W., Radioactive reference substances in gastrointestinal studies, in: *Tracer Techniques in Tropical Animal Production*, International Atomic Energy Agency, Vienna, 1974.