YTTERBIUM LABELING OF GRAIN: EFFECT OF GRAIN PROCESSING, PARTICLE SIZE AND EXTRACTION METHOD

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Story in Brief

Five types of grain (corn, wheat, milo, barley and oats) subjected to one of six processing methods [high moisture ensiling (25% and 35% dry matter), steam flaking, whole, rolling or steam rolling] were labeled with ytterbium. Ytterbium content was analyzed following either extraction by EDTA or ashing. Ytterbium concentrations determined after ashing were an average of 21% higher (5.88 vs 4.84 mg/g DM) and more repeatable (SE = .28 and .53) than after EDTA extraction. Form of grain processing and particle size affected both the amount of feed recovered after labeling and washing (whole > rolled > steam flaked) and the quantity of ytterbium bound (steam flaked and steam rolled > rolled > whole). Differences in grain recovery and extent of labeling with ytterbium are related partially to differences in the particle size.

(Key Words: Ytterbium Extraction, Grain Processing, Particle Size.)

Introduction

Rare earth elements, such as ytterbium (Yb) have an affinity for plant cell walls and once attached can be used to study the rate of passage of particulate digesta in ruminants (Hart and Polan, 1984). However, the binding affinity or capacity of particulate matter for Yb varies with the type of feed indicating that (1) the functional groups that bind Yb vary with the feedstuff, (2) the molecular environment of functional groups varies with the feedstuff or (3) some types of particulate matter form multiple bonds with Yb (Teeter et al., 1984). With the immersion-washing procedure, feed composition is modified somewhat by the removal of soluble components.

Daily intakes of .8 g Yb per steer are recommended (Karimi et al., 1987) to achieve 2 ppm in the final fecal extract for analysis by atomic absorption. Although information concerning binding capacities of various roughages have been published (Teeter et al., 1984), values of the Yb binding capacities of grains and the percentage of grain lost during the immersion-washing procedure are lacking.

Two methods for preparation of samples for Yb analysis have been proposed. One method (Ellis et al., 1982) is quite time consuming, involving ashing a dry sample and extraction of Yb from the ash with acid. A second method (Hart and Polan, 1984) is rapid, consisting simply of extracting Yb with an EDTA solution. These two methods also vary in the size of the sample employed, with the ash method using 1 to 2 grams of sample compared with only .2 g with the EDTA extraction. The smaller the sample size, the greater the error in representative sampling.

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Yb concentrations in feed also may differ depending on the extraction method employed. Higher values have been suggested with the ashing procedure. However, Karimi et al. (1987) noted no reduction in the reliability of measurement with the EDTA extraction.

The objectives of our research were: 1) to determine the relationship between the method of grain processing and the binding capacity for Yb, 2) to investigate the effect of grain processing method on recovery of Yb-labeled product and 3) to compare results from these two methods for Yb extraction.

Materials and Methods

Five types of grain (corn, wheat, milo, barley and oats) subjected to one of six processing methods [high moisture ensiled (25% and 35% dry matter), steam flaked, whole, rolled or steam rolled] were treated with Yb to study passage rate. Grains were processed commercially from a single batch of each grain. Two and one-half grams of $YbCl_3 \cdot 3H_2O$ were dissolved in one liter of distilled water, poured onto 50 grams of air dry feedstuff and allowed to soak for 48 hours, during which time the mixture was stirred three to four times each day (Galyean, 1984). The grain solution then was filtered through a 250 micron screen and washed six times with distilled water over a six hour period. The labeled grain was dried at 60 C. Percentage recovery of the grain was expressed as final dried weight of the grain after the immersion-washing procedure divided by the initial as-fed weight of the grain before labeling with Yb.

The grains were characterized before immersion by dry sieving through a series of screen sizes (8mm; 4mm, 2mm, 1mm, 500 microns, 250 microns and 125 microns) to attain a particle size distribution (table 1).

Yb was extracted from samples of each batch of labeled grain by the EDTA extraction of Hart and Polan (1984) and by ashing plus acid extraction (Ellis et al., 1982). Yb concentrations of the extracts were

Table 1. Particle size distribution of processed grains.

	Size of sieve openings							
Grain and form	8mm	4mm	2mm	1mm	500um	250um	125um	Pan
	---- Percentage of grain remaining on screen ----							
Steam flaked corn	28.9	38.5	18.8	7.9	3.6	1.6	.2	.7
Whole shelled corn	25.8	73.4	.8	--	--	--	--	--
Rolled corn	3.2	49.4	40.6	6.0	.4	.2	.2	--
25% High moisture corn	4.6	37.4	39.9	11.3	4.8	1.1	.2	.9
35% High moisture corn	5.0	36.2	42.8	13.0	2.2	.3	--	.6
Rolled wheat	--	6.3	79.0	13.5	.8	.1	.3	.1
Steam flaked wheat	--	31.5	55.0	11.8	.9	.2	.2	.4
Rolled milo	--	--	57.6	41.7	.6	--	.1	.1
Steam flaked milo	--	9.7	43.7	30.0	10.1	4.8	.7	1.1
Rolled barley	--	30.3	67.0	2.6	.1	--	--	--
Steam rolled barley	--	40.8	58.8	.3	--	--	--	--
Rolled oats	--	16.0	77.2	4.6	1.1	.4	.4	.4
Whole oats	--	14.9	82.2	2.7	.1	--	--	--

measured by atomic absorption spectrophotometry using a nitrous oxide flame. To determine potential differences in Yb binding, statistical analysis of the data involved a one way classification of each labeled grain with replication within grain serving as the error term. Differences between methods of processing within grain and amount of Yb bound were estimated. Simple linear regression was used to compare the two methods of Yb extraction.

Results and Discussion

Mean ytterbium concentrations for the 13 feeds are reported for the EDTA and ashed extraction methods (table 2). Ytterbium concentrations were greater for 12 of the 13 feeds tested by acid extraction of ash than by the EDTA extraction method. Averaged across feeds, the ash extraction procedure proved more repeatable than the EDTA extraction method (SE = .28 and .53, respectively). These variations might be due to the fact that the zero solutions and standards were not prepared using an identical matrix as the samples; this could alter the analytical estimates of Yb concentrations in the samples. Hart and Polan (1984) and Karimi et al. (1987) reported previously that extraction with a chelate (EDTA) simplified analysis of digesta samples without reducing the reliability of the measurement. In our study, the correlation between the two methods was high (r^2=.83; P<.0001) but EDTA extraction tended to underpredict the Yb concentration. This difference may be important when choosing an extraction method for samples having low Yb concentrations.

The intercept and slope of the regression between the two extraction methods were estimated to be 1.47 mg/g DM and .91, respectively (SE = .68 and .12; P<.05 and <.0001). The non-zero intercept indicates that passage rates estimated by the regression of the natural logs of

Table 2. Ytterbium concentrations of feeds.

	Yb concentration (mg/g DM)			
Feed	EDTA extraction	SE	Ashed extraction	SE
Steam flaked corn	4.88	.66	6.16	.07
Whole shelled corn	1.20	.06	1.53	.00
Rolled corn	3.40	.07	4.52	.46
25% High moisture corn	2.23	.13	3.26	.11
35% High moisture corn	2.52	.13	3.19	.11
Rolled wheat	5.18	.51	5.72	.72
Steam flaked wheat	11.59	1.21	10.35	.02
Rolled milo	5.64	1.06	5.92	.00
Steam flaked milo	6.45	.38	7.13	.21
Rolled barley	5.93	.40	9.05	.12
Steam rolled barley	6.85	.25	9.88	.35
Rolled oats	4.51	.06	5.77	.28
Whole oats	2.60	.07	3.94	.00
Average of all feeds	4.84	.53	5.88	.28

the Yb concentration will not be equal. Hence, passage rates determined by the EDTA extraction method will not be the same as with the ash-acid extraction method. If the regression intercept is not zero, even though the feed/digesta extraction ratios are similar, extrapolation to zero time will give different estimates of rumen fill, with the EDTA extraction method predicting smaller rumen volumes.

The dry matter content of the labeled feeds and the percentage feed recovered following immersion-washing varied among the feeds tested (table 3). Form of processing affected the amount of feed recovered, with whole > rolled > steam flaked. This trend can be related primarily to differences in particle size. However, the type of feed and solubility of its components were important. Additional amounts of high moisture corn and rolled wheat had to be labeled because of loss of very small particles and(or) soluble components.

Particle size also affected the amount of Yb bound to the feed. Rolling of oats and corn both increased ($P<.0001$) the amount of Yb bound compared to the whole forms of either grain. Teeter et al. (1979; 1984) also reported that binding capacities were greater for rolled than for whole milo and corn. Steam flaking of corn, milo and wheat further increased ($P<.001$) Yb binding (1.65, 1.21 and 4.63 mg/g DM) as compared with rolled forms of these grains. Steam rolled barley bound more ($P<.01$) Yb/g DM than dry rolled barley. Hence, binding of Yb tended to be related to the method of processing with steam rolled and steam flaked > dry rolled > whole. Differences in the amounts of Yb bound can be related to differences in the amounts of surface exposed for Yb binding.

Solubility of feed components also may be important in the binding capacity of the grain. Dry rolled corn bound greater ($P<.001$) concentrations of Yb than high moisture corn. The decreased binding of Yb with high moisture corn may be due to higher solubility of the starch in the ensiled product. However, differences in binding between the 25% and 35% high moisture corn were small ($P>.05$).

Differences in the amounts of grain recovered and labeled with Yb may be related to differences in particle size, although particle size alone cannot account for all the variability. Ellis et al. (1982)

Table 3. Dry matter and percentage feed recovered.

Feed	Dry matter of feed recovered	Percentage recovered[a]
Steam flaked corn	84.74	72.38
Whole shelled corn	90.85	97.50
Rolled corn	90.36	86.04
Rolled wheat	89.75	53.74
Steam flaked wheat	88.39	76.18
Rolled milo	97.36	83.59
Steam flaked milo	94.10	64.23
Rolled barley	95.29	72.65
Steam rolled barley	95.34	84.72
Rolled oats	96.34	80.11
Whole oats	95.84	90.13

[a] Percentage grain recovered was expressed as weight of dried grain after the immersion-washing procedure divided by the initial weight of the grain before labeling.

noted that feeds higher in fiber tended to have greater binding capacities. Starch, in contrast, has a low binding capacity suggesting that groups other than those presented by polyglucans are involved. Certain processing methods, such as ensiling and heat treatment, may alter the chemical and physical composition and solubility of components (e.g., starch, protein) which in turn will influence binding and recovery. Intracellular components of feedstuffs, such as protein and nucleic acids may physically bind Yb, but the opportunity for exposure would be much lower than for surface structures. Nevertheless, their exposure for binding should increase with processing.

Variations among grains and forms of grain both in extent of labeling and in recovery following immersion-washing are disconcerting. First, they indicate that labeled feed will differ from unlabeled feed in physical and possibly chemical properties and subsequently in ruminal kinetics (e.g., ruminal distribution, wetability, flow rate). Whether the depression in digestion rates attributed previously to Yb (Teeter et al., 1984; Mader et al., 1984) is due to altered composition (removal of small particles and soluble components) or to attachment of rare earth to microbial binding sites is uncertain. Because Yb concentrations increase during in situ incubation, reduced microbial attachment is presumed. If this reflects altered wetability and density, altered passage also might be of concern. Difference in total Yb uptake with grain processing indicate that structural components presumably differ in extent of labeling. If true, and if these components differ in extent of ruminal digestion and rates of passage, comparison among grains may prove misleading. One alternative to the immersion-washing procedure would be to dose the rare earth directly into the rumen. Unfortunately, haphazard binding to small particles and solutes (e.g., VFA) drastically complicates interpretation of data obtained by ruminal dosing.

The search for the perfect marker is likely to continue. It remains uncertain whether the ideal particle to mark should be subject to the same fermentation and density and particle size changes in the rumen as untreated feedstuffs or whether, as with mordanted fiber, that the ideal particle should be inert to such changes. Marker migration certainly is reduced by mordanting. But if digestible particles are preferentially retained in the rumen, fermentability of the particle should be retained in order to monitor and understand ruminal kinetics of dietary particles. It is unfortunate that particulate marker procedures are not checked routinely by comparing ruminal fill with duodenal flow. Despite lack of confidence in the precision of marker procedures, observed changes in passage rate due to animal, feed or feeding factors seem to be reliably and repeatably detected. Consequently, one should be less concerned about reliability of observed differences than about extrapolating marker values to in vivo conditions.

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