

Selecting Appropriate Markers for Digestibility Studies

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Markers provide a method for indirect quantitation of digestive parameters. Gastrointestinal physiology and kinetics show a tremendous variation among species that is undoubtedly a reflection of evolution and dietary adaptation. Therefore, markers used in nutrition research must be appropriately selected for each species and each circumstance. Markers serve to facilitate quantification of passage rate and digesta fill as well as the relationship between intake and diet digestibility. They are generally administered by one of two techniques, either as a constant level in the diet or as a Pulse (or bolus) dose. By definition, markers are closely associated with the fractions of the digesta, are non-absorbable by the animal, and do not interfere with the normal function of the gastrointestinal tract. Additionally, it is a general assumption that the marker is in equilibrium with the pool of the fraction that it labels and that it is recoverable. Digesta may be divided into two fractions, a liquid phase and a particulate phase. Appropriate markers for most circumstances are those which are specific to a particular phase of the digesta. However, multiple marker systems in which both liquid and particulate fractions may be monitored simultaneously have been developed and successfully utilized in a number of species. Unfortunately, a perfect marker or marker system for all species does not seem to exist. Sensitivity to particular markers may cause a gastrointestinal upset that alters transit time, and in turn digestibility, eliminating the possibility of collecting valid data.

Key words: markers, digestibility, passage rate, digesta

INTRODUCTION

Gastrointestinal physiology and kinetics show a tremendous variation among species that is undoubtedly a reflection of evolution and dietary adaptation. These variations can pose any number of challenges for a researcher studying nutrition and digestive efficiency, particularly of non-domesticated animals. Markers provide a method for indirect quantitation of digestive parameters. Therefore, markers used in nutrition research must be appropriately selected for each species and each circumstance. They serve to facilitate quantification of passage rate and digesta fill as well as the relationship between intake and diet digestibility.

There is a large variety of markers that may be utilized for digestibility studies. The criteria characterizing ideal markers were summarized first by Faichney [1975], as follows :

- 1) The marker must be strictly non-absorbable.
- 2) The marker must not affect or be affected by the gastrointestinal tract or its microbial population.

3) The marker must be physically similar to or intimately associated with the material it is to mark.

4) The method of estimating the marker in digesta samples must be specific and sensitive and it must not interfere with other analyses.

While it may be difficult (or nearly impossible) to find an ideal marker for every situation, we must strive to select the most appropriate marker that will not influence the outcome of the data. A thorough and relatively recent review [Owens and Hanson, 1992] specifically discusses the shortcomings of numerous markers and various sources of error in ruminant animals. Our goal is to briefly summarize the use and types of most commonly used markers and to explore possible limitations and inadequacies that may become more apparent when studying non-domestic species.

Markers: A Brief Summary of Techniques, Types and Limitations

In general, digesta may be divided into two fractions, a liquid phase and a particulate phase. It is assumed that an appropriate marker is in equilibrium with the pool of the fraction that it is intended to label and that it is recoverable. Appropriate markers are those which are specific to the particular phase of digesta that is to be studied. Markers are generally administered by one of two techniques, either continuously at a constant level in the diet or as a pulse (or bolus) dose. Using these methods individually or in combination can provide an abundance of data, including information on passage rate, gastrointestinal volume or fill, fecal output, retention time, and the relationship between intake and diet digestibility.

Digesta markers may be lumped into one of two broad categories, internal markers or external markers. Internal markers are those which are inherent in feeds, such as silica, acid insoluble ash or lignin. Each of these feed fractions does have limitations in usefulness. Lignin is a theoretically indigestible fraction of the plant cell wall. There are, however, reports of lignin disappearance during digestion which are discussed by Merchen [1988]. Use of acid insoluble ash or silica each present a problem opposite to that of lignin and may result in skewed results if there is soil contamination of feeds or if soil or bedding are consumed [Merchen, 1988].

External markers are unreactive preparations which may be administered or added to the feed. Insoluble metal oxides or rare-earth elements have been used to investigate digestibility and digesta flow and feed particles have been dyed or stained. Polyethylene glycol (PEG) is often used as a liquid phase marker, although, there is some question as to its absorption and imprecise methods of analysis. Additionally, various inert plastic markers have been used successfully by a number of researchers. Welch [1990] summarized data from a number of these studies and discussed their potential uses and limitations. Among the advantages of plastic markers is their flexibility in size and specific gravity that allows them to be associated with different particle sizes in the digesta. In ruminants, for instance, if particle sizes are small enough, particles can be regurgitated during normal rumination allowing the researcher to follow these patterns. However, plastic markers do not undergo the hydration, density and size changes that occur with normal feed particles [Welch, 1990].

Ethylenediamine tetraacetic acid (EDTA) complexes and mordanted plant cell walls in combination constitute perhaps one of the best marker systems currently used for domestic ruminants, allowing for tracking of both the liquid and solid phases simultaneously. Uden et al. [1980] demonstrated that plant cell walls were rendered indigestible by mordanting with chromium (Cr) and that the Cr remained associated with the fiber during digestion. The usefulness of Cr-EDTA as a liquid phase marker was first demonstrated by Downes and McDonald [1964]. Both Cr-EDTA and cobalt (Co)-EDTA are seemingly appropriate liquid phase markers which produce similar results [Uden et al., 1980]. Further, Uden et al. [1980] demonstrated that Co-EDTA and Cr-mordant could be used simultaneously with good results.

Marker Problems in Chevrotain

The larger Malayan chevrotain (*Tragulus napu*) is among the smallest of the artiodactyls. While this tragulid does ruminate, it has an apparently rapid digesta transit time which has led to much debate regarding proper diet for this species. Thus, a study was undertaken to examine the ability and efficiency of adult chevrotain to utilize complete, pelleted diets with differing fiber levels [Bernard et al., 1994]. Initially, digestive parameters were to be determined by the simultaneous utilization of two digesta markers, Co-EDTA and Cr-mordant prepared by methods of Uden et al. [1980].

Markers were administered as a pulse dose to each animal at the rate of 15 mg Cr as Cr-mordant and 20 mg Co as Co-EDTA. They were prepared in empty gelatin capsules (size 00) and individually orally dosed. Within 10 hours of the dosing, all animals showed evidence of diarrhea. After careful examination of protocol, it was determined that the probable reason for diarrhea was inadequate binding of the Co-EDTA complex. Another batch of Co-EDTA was prepared by an experienced dairy nutrition laboratory. This batch had been tested on a number of domestic ruminants. The new batch of Co-EDTA was then prepared in gelatin capsules, this time without the Cr, and administered. Again, within 10 hours of dosing, diarrhea was apparent. A bolus dose of 20 mg Co-EDTA is equivalent to about half the dose, in relation to body weight, often used for domestic ruminants [M. Allen, Personal Communication]; yet the chevrotain still had diarrhea within 10 hours of dosing. Next, we systematically began reducing the cobalt dose in an attempt to determine an appropriate level which would not induce diarrhea. At 10 mg Co as Co-EDTA, onset of diarrhea was within 14 hours, at 5 mg 19 hours and at 2.5 mg 24 hours. Cr-EDTA was prepared [Uden et al., 1980] and tested next. There was no evidence of diarrhea or even loose or clumpy stools using the Cr. Subsequently, it became necessary to complete two studies to get the entire picture (since two markers utilizing Cr could not be administered simultaneously). The first study utilized Cr-mordant and the second Cr-EDTA. We are unable to explain why chevrotain are sensitive to so low a dose of Co-EDTA, but it is important to note that chevrotain DO NOT tolerate even relatively small doses, so cobalt should not be used as a marker in studies with chevrotain until suitable forms are identified.

CONCLUSIONS

Research data collected utilizing markers can be invaluable in filling gaps in nutrition knowledge, particularly for non-domestic species about whom we have little information. Unfortunately, a perfect marker or marker system for all species does not exist at this time. Sensitivity to particular markers may cause a gastrointestinal upset. If the gastrointestinal tract is not functioning

normally, transit time will be altered, and in turn digestibility will be affected, eliminating the collection of valid data. Therefore, it seems prudent to test and evaluate any marker that is to be used. This is particularly crucial if a digestibility study is to be completed on a rare, unusual, or expensive species on which the particular marker has not been adequately demonstrated to be safe and effective. When selecting and evaluating markers for a study, be certain to remember the characteristics of the 'ideal' marker, discover as much information as possible about the chosen marker, and be certain to safely test the marker before beginning the project.

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