



Using portable X-ray fluorescence (pXRF) to determine fecal concentrations of non-absorbable digesta kinetic and digestibility markers in sheep and cattle



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ABSTRACT

Concentration of indigestible markers in feces is routinely used to estimate the digestibility or rate of passage of feed through the gut. Current procedure for measuring fecal marker concentrations is an acid digest before analysis using inductively coupled plasma optical emission spectroscopy (ICP). Portable X-ray fluorescence (pXRF) spectroscopy uses short wavelength X-rays to excite sample material and generate characteristic elemental emissions proportional to the concentration of the element present in ground fecal material. The aim of this study was to measure fecal concentrations of Co, Cr, Ti, and Yb digesta marker by both ICP and pXRF and determine any correlation between the two methods, thereby assessing the potential of using pXRF as a marker analysis method. Silica (Si) concentrations in fecal and feed samples were also measured using both methods. Cattle and sheep feces and various feed samples collected from four separate studies were analyzed for concentrations of Co (CoEDTA), Cr (Cr mordanted fibre), Ti (TiO₂), Yb (Yb(III)acetate), and Si using ICP and pXRF. Regression analysis used to assess the relationship between ICP and pXRF determined fecal concentrations indicated strong linear relationships ($P < 0.01$) between pXRF and ICP estimates of Co ($r^2 = 1.00$), Cr ($r^2 = 0.95$), Ti ($r^2 = 0.97$), Yb ($r^2 = 0.94$), and Si ($r^2 = 0.88$). All curves were validated using a second set of independent fecal samples. A full set of total collection fecal samples for six sheep were also analyzed by pXRF for Co and Cr (digesta kinetics), and Si (digestion) marker concentrations. These fecal Co and Cr concentrations were used to estimate digesta mean retention times (MRT) and compared to estimated digesta MRT from the same sheep using Co and Cr marker concentrations determined by ICP analysis. No differences in estimated MRT were found when using either analytical method. Silica concentrations from the same fecal samples as determined by pXRF were used to determine the rate of DMD for each animal. When compared to apparent DMD for the same animals as determined using feed DM intake and fecal DM output collected during the total collection, no differences were again found using either analytical method. The results indicate that pXRF can be used as an alternative to standard ICP procedure, obviating the need for acid digestion, reducing cost and time; and may also offer an alternative analytical method for determining livestock DM digestibility.

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Abbreviations: ICP, inductively coupled plasma optical emission spectroscopy; pXRF, portable X-ray fluorescence; Co, cobalt; Cr, chromium; Cr₂O₃, chromic oxide; Si, silicon; Ti, titanium; Yb, ytterbium; CoEDTA, cobalt EDTA; MRT, mean retention time; DM, dry matter; DMD, dry matter digestibility; NDF, neutral detergent fibre.

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1. Introduction

Study of feed digestion and the kinetics of digesta passage through the gastrointestinal tract of ruminants relies on the measurement of endogenous dietary markers such as silica, lignin and NDF (Fahey and Jung, 1983; Huhtanen et al., 1994; Rymer, 2000) or exogenous markers such as chromium, ytterbium, titanium, and cobalt that move in varied proportions with solid and liquid phases of the digesta respectively (Faichney, 1975; Udén et al., 1980). Analysis of these markers in the feces has typically required spectrometric analysis by atomic absorbance (Udén et al., 1980) or Inductively Coupled Plasma Emission Spectroscopy (ICP) following oxidation of samples in a furnace or a wet acid digest and solubilisation (Anderson and Henderson, 1986; Molinero et al., 1988).

Portable X-ray fluorescence (pXRF) technology is a potential alternative to traditional marker concentration analysis due to its accelerated measurement of the concentration of many minerals within soil, plant samples, and leaf litter (Piorek, 1997; McLaren et al., 2012; Tighe and Forster, 2014) with minimal sample preparation. Portable XRF measures the X-ray energy emitted by elements, its fluorescence, after a sample has been irradiated (Kilbride et al., 2006). This excess energy is emitted as X-rays and is unique for each element allowing individual concentration detection (Kalnicky and Singhvi, 2001). If pXRF could accurately determine the concentration of minerals used as markers in digestion and digesta kinetics studies without requiring sample digestion, this would substantially reduce cost, duration and inconvenience of mineral measurements in livestock research. The purpose of this study was to measure the content of endogenous and exogenous markers in feed and ruminant fecal samples using wet chemistry and assess if any association with marker concentration determined using pXRF existed. This would allow to the determination of the usefulness of pXRF as a cheaper, easier method of digestibility and digesta kinetic marker analysis.

2. Materials and methods

2.1. Fecal samples

Samples for analysis were obtained from 4 different experiments, all conducted at the University of New England animal facilities under the university's animal ethics terms of reference, and collected from both sheep and cattle. Fecal samples for Co ($n=30$), Cr ($n=30$), Si ($n=9$) (Barnett et al., 2012; Barnett et al., 2015) and Yb ($n=12$) (unpublished) analysis were collected from sheep while samples for Ti ($n=14$) analysis were obtained from cattle (unpublished).

2.2. Feed samples and chemical analysis

Eleven different feed samples ranging from grasses to chaff and grains were dried in a fan-forced oven at 80 °C until no further weight loss before being ground and passed through a 1 mm screen. All samples were dry ashed and digested using NaOH fusion (Sugar Research Australia, Indooroopilly, QLD, Australia) in duplicate before analysis of Si concentrations using Inductively Coupled Plasma Optical Emission Spectroscopy (ICP-OES; Varian Vista Radial MPX, Varian Medical Systems, Palo Alto, California, USA) with the mean values used. Silica concentrations for the 11 feed samples and 9 fecal samples ($n=20$; refer to Fecal Samples in Materials and methods) were used to develop and validate the calibration curve.

2.3. Fecal sample chemical analysis

All fecal samples were dried at 80 °C in a fan-forced oven to a constant weight then ground through a 1 mm sieve. Samples containing Cr and Co digesta markers were oxidized by modified sealed chamber digestion (Anderson and Henderson, 1986), samples containing Ti were digested in accordance with Titgemeyer et al. (2001) procedure, and samples containing Yb were digested in 15.8 N nitric acid using an ultrawave single reaction chamber microwave digestion system (Milestone, Sorisole BG, Italy) before analysis of concentrations by ICP. Samples analyzed for Si concentrations were dry ashed and digested using NaOH fusion, in duplicate (Sugar Research Australia, Indooroopilly, QLD, Australia).

2.4. Feed and Fecal sample pXRF analysis

A small amount (<10 g) of the ground sample was placed in a 22 mm × 24 mm wide necked (Nalgene; Rochester, NY, USA) sample cup and covered with a thin film of mylar plastic film. Each sample was analyzed using a Bruker Tracer III-V pXRF (aka "gun") with associated software (Bruker Corp., Billerica, Massachusetts, USA) by placing the cupped sample within the gas flow chamber directly in line with the gun's detector. The instrument used a rhodium anode miniature X-ray tube set at a resolution of 15 kV and 55 μA. Collimation of the pXRF beam covered a surface area of 7 mm². Triplicate measures of each sample for the same elements were conducted after shifting the sample cup on the surface of the pXRF gun, between measures, to create a slightly different surface area. Mean values of elements measured for each sample were reported. Sample analysis was conducted for 180 s per sample while flooding the system with high purity helium. All results were collected as raw spectra in the equipment software and converted to counts using the net area under each elemental response

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