Determination of Marker Chromium in Faeces Using Inductively Coupled Plasma Emission Spectrometry

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Chromium is determined in chromium(III) oxide-labelled faeces using an inductively coupled plasma source and emission spectrometry. A nitric acid - perchloric acid mixture is used to destroy organic matter in the faecal material and to dissolve the chromium(III) oxide. The concentration of chromium in the diluted digest is determined using the 358-nm line. The procedure is manipulatively simple, requires minimum supervision and is suitable for high-throughput applications. The precision for a single determination is ±5.4%.

Keywords: *Marker chromium determination; chromum(III) oxide; faeces; inductively coupled plasma emission spectrometry*

Chromium(III) oxide (Cr_2O_3) is used as a marker substance to determine the feed intake of grazing animals, a constant oral infusion of chromium(III) oxide in glycerol is usually used. Chromium(III) oxide mixes uniformly with ingesta[1] and is inert to absorption by the animal,[2] and its faecal concentration depends on the concentration of the marker substance in the glycerol support medium, the rate of infusion of the marker substance and the digestibility and amount of the feed ingested by the animal. Labelled faeces may contain in excess of 2 000 μg g^{-1} of marker chromium. If digestibilities are known, intake can be calculated from the concentration of marker chromium in the faecal material.

A rapid procedure for the determination of marker chromium in faeces was required as part of an exploratory comparison[3] of the chromium(III) oxide - faecal output - forage indigestibility and the bite size - bite number methods for determining forage intake in cattle.

The convenience of simply pressing a pellet of the finely ground, labelled faeces with a boric acid backing coupled with the use of X-ray fluorescence spectrometry was most appealing. However, massive spectral interferences, particularly from iron, were observed with our spectrometer, which precluded the use of this technique.

Marked interferences have been encountered in the spectrophotometric and flame atomic-absorption spectrophotometric (AAS) determinations of chromium in solution. Preliminary testing of a spectrophotometric method[4] for the determination of chromium gave erratic and unreliable results for multiple re-analyses of the same sample material. Inductively coupled plasma emission spectrometry (ICPES) has good sensitivity and a wide dynamic range and is relatively free from chemical interferences.

Alkali fusion procedures[5] commonly used to solubilise chromium(III) oxide are slow and tedious. The simple, but not often used, perchloric acid procedure[6] for dissolving chromium(III) oxide was combined with ICPES to develop a simple, rapid and reproducible method for the determination of marker chromium in faeces.

Experimental

Instrumentation

A Labtest Plasmascan 700 inductively coupled plasma emission spectrometer was used. Its scanning monochromator had a focal length of 0.35 m and a grating blazed at 250 nm first order with 1 180 rulings mm^{-1}. The nebuliser was a modified cross-flow using the V-groove principle of Suddendorf and Boyer.[7] The plasma was run at 1.1 kW incident power and the viewing height was 11 mm above the load coil. An IP28 photomultiplier was used at 0.75 kV. The argon flow-rates were coolant gas 10.5 l min^{-1}, sample gas 0.9 l min^{-1} and plasma gas zero. The sample solution pump rate was 1.8 ml min^{-1}. A slit width of 0.1 mm was used with a wavelength of 358 nm.

Apparatus

Pyrex tubes (24 × 150 mm) calibrated with a 20-ml mark were used for sample digestion together with a 60-place aluminium heating block drilled to suit the digestion tubes and thermostatically controllable to 300 °C.

Reagents

Unless otherwise specified, all reagents were of analytical-reagent grade and the term "water" implies de-ionised or distilled water.

Nitric acid, concentrated, 70% *m/m.*

Perchloric acid, 70% *m/m.*

Potassium dichromate. Recrystallised three times from water.

Chromium(III) oxide.

Reagent blank solution. Dilute 66 ml of perchloric acid to 1 000 ml with water.

Standard solutions

Stock chromium solution. A solution containing 1 000 μg ml^{-1} of chromium was prepared using recrystallised potassium dichromate and the reagent blank solution as solvent.

Calibration standards. Prepare solutions containing 0, 25, 50, 75, 100 and 200 μg ml^{-1} of chromium in reagent blank solution from the stock chromium solution. An extended range of calibration standards up to a maximum of 500 μg ml^{-1} of chromium can be used if the marker chromium concentrations in the samples are exceptionally high.

Calibration

The Plasmascan employs a two-point calibration procedure. The upper calibration point was set using the most concentrated chromium standard solution in the selected set of standards. The reagent blank solution or, alternatively, the 25 μg ml^{-1} chromium standard solution was used to set the lower calibration point.

Intermediate standards were used to confirm the linearity of calibration over the working range of concentrations and as regular checks for instrument drift during the reading of sample digest solutions.

Procedure

Oven dry the sample overnight at 105 °C. Weigh 1.000 g of finely ground sample into a digestion tube, add 10 ml of nitric acid, ensuring that the acid wets the entire sample, and allow to stand overnight. Add 2 ml of perchloric acid and a few silica boiling chips, place the tube in a cold heating block, set the block thermostat to 50 °C and turn the mains power on. Heat the tubes at 50 °C for about 30 min, which allows initial frothing to subside.

Place a small glass funnel, about 30 mm in diameter, in each tube and increase the thermostat setting to 160 °C. Heat the samples at this temperature for 3–4 h, occasionally using small volumes of nitric acid to wash any chromium(III) oxide adhering to the upper walls of the tube down into the boiling solution, then increase the thermostat setting to 200–210 °C. Digestion is completed when all the nitric acid has boiled off (dense white fumes of perchloric acid fill the tube) and all green chromium(III) oxide has been converted into the soluble, orange, dichromate form. Remove the tubes from the block and, when they have cooled sufficiently, dilute the digest to the 20-ml mark with water. Mix the contents well and allow any insolubles to settle. After calibration of the plasma unit, analyse the diluted digests for chromium in solution using the previously described instrument operating parameters.

This procedure requires minimum operator supervision. The time required for digestion may be reduced considerably by boiling the solutions at 160 °C for only 2–3 h before raising the thermostat setting to 200–210 °C. If the reduced boiling time is used then regular supervision must be maintained once the thermostat setting has been raised to ensure that the solutions do not boil over. A small amount of nitric acid must be added to the digest if charring begins. The onset of charring is marked by a chocolate brown curdling effect in the solution.

Caution. The possible danger of explosion always exists with perchloric acid digests. All necessary precautions should be taken, a comprehensive list of which appears in reference 8.

Performance Characteristics of the Method

Samples

Marked and unmarked faecal samples were used to assess the performance characteristics of the method. These were collected from grazing cattle, some of which received a continuous oral infusion of chromium(III) oxide in glycerol.

Linearity of instrument response

Two separate sets of extended range calibration solutions containing 0, 25, 50, 75, 100, 150, 200, 250, 300, 350, 400, 450 and 500 μg ml^{-1} of chromium were prepared on each of two different days.

The linearity of the instrument response was assessed on each of the two days using the entire set of standard solutions. All combinations of low-point (reagent blank, 25 and 50 μg ml^{-1}) and high-point (200, 300, 400 and 500 μg ml^{-1}) calibrations were used. Duplicate instrument readings were made on each solution in the full standard set for each of the 12 different combinations of low-point - high-point calibrations. For each combination, a linear regression of instrument response on concentration of chromium in solution (μg ml^{-1}) was used to assess the linearity of instrument response. A lack-of-fit *F*-test was used with each assessment to test the suitability of the linear models.

Limit of quantitation

The detection limit determined from ten successive 10-s integrations of a near blank chromium solution was used to calculate the limit of quantitation,[9] which is defined as five times the detection limit.

Interferences

Spectral and ionisation effects due to 1 000 μg ml^{-1} of sodium, 1 000 μg ml^{-1} of potassium, 100 μg ml^{-1} of iron, 200 μg ml^{-1} of calcium and 100 μg ml^{-1} of magnesium in the presence of 200 μg ml^{-1} of chromium were monitored. These levels of interferents were much higher than the average levels found in faecal digest solutions.

Recovery of added chromium(II) oxide

Faecal material containing no marker chromium was ground in a beater cross mill fitted with a 1-mm sieve and the ground material was well mixed. Additions of 0, 4, 8, 12 and 16 mg of chromium as chromium(III) oxide were made to separate 10-g portions of ground faecal material. Each addition was replicated four times and then ground separately for 3 min in a large cast iron cup on a shatterbox mill. Four 1-g portions of each finely ground material were analysed for chromium. The recovery of added chromium was calculated from the slope of the linear regression of total chromium found (μg) on chromium added (μg). An *F*-test for the lack-of-fit of the linear model was also made using the regression data.

Repeatability and reproducibility

Three different faecal samples, each from different cattle treated with a continuous infusion of chromium(III) oxide, were analysed in triplicate on each of three days. A two-way analysis of variance of the data was used to obtain precision estimates for the method.

Results and Discussion

Linearity of Instrument Response

The instrument response was linear over the range 0–500 μg ml^{-1} of chromium for each of the low-point - high-point calibration combinations tested. The lack-of-fit *F*-test associated with each linear regression of instrument response *versus* concentration of chromium in solution (μg ml^{-1}) was not significant.

The variance of instrument response for the reagent blank solution was higher, but not significantly higher, than that for either the 25 or 50 μg ml^{-1} chromium solution. For this reason, the reagent blank solution is rarely used to set the low calibration point.

Limit of Quantitation

The detection limit was found to be 0.013 μg ml^{-1} of chromium. From this the limit of quantitation was calculated to be 1.4 μg g^{-1} of chromium.

Interferences

Duplicate determinations were used with each added interferent and the results are given in Table 1.

There were no significant spectral or ionisation effects due to the added interferents. These elements would not be expected to interfere in real samples as the level of spiking was considerably higher than that normally found in faecal digests.

Recovery of Added Chromium(III) Oxide

The linear regression of total chromium found (μg) on chromium added (μg) gave a least-squares slope of 0.993 6 with a standard deviation of 0.020 7 for 78 degrees of freedom. The recovery with its confidence interval ($P = 0.05$) of 99.4 ± 4.1% was not significantly different from 100%.

The *F*-test for lack-of-fit of the linear model to the regression data was not significant ($P = 0.05$).

Table 1. Effect of possible interferents on the determination of chromium using ICPES

Concentration/μg ml^{-1}	ICPES* result/μg ml^{-1} (duplicate determinations)
200 Cr + 1 000 Na	198.0, 198.4
200 Cr + 1 000 K	200.5, 203.7
200 Cr + 100 Fe	199.0, 199.8
200 Cr + 200 Ca	201.2, 201.0
200 Cr + 100 Mg	197.0, 199.1

* Calibration points were set using the 50 and 200 μg ml^{-1} standard solutions.

Table 2. Precision estimates for the determination of marker chromium in faeces using ICPES

(*a*) *Analytical data*—

Day	Replicated chromium/μg g^{-1} sample		
	Sample 1	Sample 2	Sample 3
1	1 240, 1 194, 1 292	1 712, 1 772, 1 732	2 212, 2 160, 2 168
2	1 270, 1 318, 1 270	1 782, 1 772, 1 780	2 226, 2 268, 2 160
3	1 244, 1 258, 1 200	1 846, 1 806, 1 826	2 192, 2 200, 2 314

(*b*) *Analysis of variance**—

Source	Degrees of freedom	Mean square	*F* ratio
Days	2	18.005 6	2.21
Samples	2	6 763.435 8	—
Day × sample interaction	4	8.156 767 5	1.62
Replicates	18	5.034 305 56	

Repeatability relative standard deviation[10] = 2.244%
Reproducibility relative standard deviation[10] = 2.678%

* A computer program was used to code the original data in (*a*) to have a grand mean of 100 and then to complete the analysis of variance on the coded data.

Repeatability and Reproducibility

Table 2 shows the replicated analyses for each sample for each day and the analysis of variance used to calculate precision estimates.

The precision, as a relative confidence interval ($P = 0.05$) about a mean determination, was calculated as ±4.7% from the repeatability relative standard deviation (18 degrees of freedom). The relative confidence interval ($P = 0.05$) about a single determination was ±5.4% calculated from twice the reproducibility relative standard deviation.

Note on Other Methods

Attempts were made to compare the ICPES method with a spectrophotometric method[4] following either perchloric acid digestion or alkali fusion of the faecal material. Erratic results were obtained with chromium-marked samples even though the calibration lines for the spectrophotometric procedures were linear and reproducible. No attempt was made to investigate the reasons for such poor sample results as the excellent recovery of the ICPES method was obtained using chromium(III) oxide mixed with faecal material in a manner similar to that of real samples.

Note on Sample Fineness

Chromium(III) oxide readily separates from dry powdered faecal material. For the highest precision, samples should be ground in a shatterbox-type mill. An acceptable but lower precision is obtained when a beater cross mill fitted with a 1-mm sieve is used.[11]

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