X-Ray Spectrographic Determination of Chromic Oxide in Steer Feces

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The x-ray spectrographic procedure is based upon the linear relationship of the counting rate at the chromium K_α line to the chromic oxide concentration. The working curve is based on the chemically determined chromic oxide concentration in selected fecal samples. The method is useful for the routine rapid determination of chromic oxide.

Chromic oxide has gained widespread use as an external fecal indicator since it was first suggested by Edin in 1918 (*7*). It is one of several indicators which may be employed to estimate the fecal output of animals in feeding and grazing trials. Its use for this purpose has been adequately reviewed by Reid (*10*).

Several procedures for the chemical determination of chromic oxide in feeds and feces are available. Colorimetric (*1, 3, 11*), volumetric (*8*), and atomic absorption (*14*) methods, among others, have been described. Some of these have been compared by Stevenson and De Langen (*12*). Methods currently available are time-consuming and some of them present inherent difficulties in obtaining reproducible results (*5, 12*). This paper reports an x-ray spectrographic technique for the determination of the chromic oxide concentration in feces of grazing steers.

Equipment

A Philips standard x-ray spectrograph with full wave rectification and current stabilization was used. A tungsten-target FA-60 x-ray tube was operated at 19 kv. and 18 ma. A lithium fluoride crystal, $2d$ spacing of 4.0276 A., was used in an air path. The collimation system consisted of one 4- × 1/8-inch and one 4- × 0.005-inch Soller slit assemblies. The scintillation detector, with a thallium-activated sodium iodide crystal, was operated at 1050 volts. The modified sample holder and the Mylar-backed aluminum alloy frames have been described (*2*).

Procedure

The samples were oven-dried at 70° C., ground to pass a 20-mesh screen, and stored in capped bottles. The determinations were made on about 0.5-gram subsamples evenly spread in the frames described. As previously reported, it was not necessary to pack weighed quantities of the samples into the frames (*2*). The feces samples were collected twice daily according to the experimental design and with the cooperation of the Holstein-Friesian steers that were grazing on experimental alfalfa-grass pastures. The animals were fed 1 pound of pelleted corn meal containing 20 grams of chromic oxide every morning. The chromic oxide was added to the corn meal in the form of finely ground chromic oxide "bread" prepared in a manner similar to that described by Kane, Jacobson, and Moore (*8*).

The working curve is based upon selected samples of feces which were analyzed chemically for chromic oxide according to the colorimetric method of Brisson (*3*). The curve is obtained by plotting the counting rate at the chromium K_α line (λ = 2.291 A, 2θ = 69.34°) as a function of the chromic oxide concentration in the samples. The counting rate, based on the time required to record 25,600 events, was found to be directly proportional to the concentration of chromic oxide. The equation of the line for this relationship was calculated by the method of least squares (*4*). The concentration of chromic oxide in the unknowns was then calculated from the above equation. (The concentration of chromic oxide may also be read directly from the working curve.)

Results and Discussion

X-ray spectrographic data may be related to the concentration of an element in a given sample by several means. Three of these were used to relate data obtained in this study to the concentration of chromic oxide in the fecal samples.

F. The counting rate at the chromium K_α line.

$F - S$. The counting rate at the chromium K_α line less the counting rate at background (λ = 2.164 A., 2θ = 65.00°).

F/S. The ratio of the counting rate at the chromium K_α line to the counting rate at the background.

Measure F was found to be as good as either $F - S$ or F/S (Table I). Studies already reported showed that the ratio of the counting rate at the K_α line to the counting rate at background was a good measure of the concentration of an element in the parts per million range (*2, 9, 13*). The chromic oxide concentration in the samples being analyzed, however, was expected to be between 0.2 and 1.0%. With this chromic oxide concentration, a relatively low power setting gave the desired counting rate at the chromium K_α line while maintaining nearly constant the counting rate at the background.

The feasibility of utilizing synthetic reference samples to develop a working curve was investigated. Stock synthetic reference samples of feces containing 1.00 and 2.00% chromic oxide were prepared, and portions were added to feces free of chromic oxide to provide samples with a range of 0.00 to 1.20%. The curves based on this set of synthetic reference samples

Table I. Comparison of Three Possible Measures of Chromic Oxide Concentration Based on Chemical Analysis of 30 Samples

Measure	Constants of Working Curves	Correlation with Chemical Determination[a]	Mean and Standard Deviation from Working Curve[b]
F[c]	$F = 24.6 + 227.5x$[d]	0.997	0.019 ± 0.021
$F - S$[e]	$F - S = 4.1 + 226.8x$	0.997	0.019 ± 0.021
F/S	$F/S = 1.240 + 10.726x$	0.995	0.025 ± 0.022

[a] Correlation coefficient $r = \frac{\Sigma xy}{\sqrt{\Sigma x^2 \Sigma y^2}}$ (*6*).

[b] Mean $\left(\bar{X} = \frac{\Sigma X}{n}\right)$ with standard deviation $\left(s = \sqrt{\frac{\Sigma x^2}{n-1}}\right)$ (*6*) of absolute differences between concentrations determined chemically and calculated from equations of working curves.

[c] F. Counting rate, c.p.s., at chromium K_α line, λ = 2.291 A., 2θ = 69.34°.

[d] x. Chromic oxide concentration ranging from 0.01 to 1.19 %.

[e] S. Counting rate, c.p.s., at background, λ = 2.164 A., 2θ = 65.00°.

Table II. Constants for Equations of Lines ($y = b + mx$) and Correlation Coefficients for Five Sets of Reference Samples

Base of Reference Sample	F			F − S			F/S		
	b	m	Correlation coeff.	b	m	Correlation coeff.	b	m	Correlation coeff.
Chemical determination	24.6	227.5	0.997	4.1	226.8	0.997	1.240	10.726	0.995
Dilution of stock chromic oxide synthetic reference samples	24.6	279.9	0.999	3.4	278.5	0.999	1.233	12.492	0.997
Addition of									
Chromic oxide powder	29.2	267.2	0.998	7.3	268.7	0.998	1.095	13.384	0.998
Sodium dichromate solution	27.3	283.8	0.999	6.2	283.3	0.999	1.444	12.911	0.998
Chromic oxide bread	27.5	165.1	0.999	6.5	165.7	0.999	1.271	8.183	0.999

Table III. Comparison of Calculated and Duplicate Chemically Determined Chromic Oxide Concentrations in Selected Synthetic Reference Sample

Samples Prepared by	Chromic Oxide Concentrations, %		
		Chemical	
	Calcd.	I	II
Dilution of chromic oxide stock	0.50	0.47	0.46
Addition of			
Powdered chromic oxide	0.50	0.47	0.48
Sodium dichromate solution	0.50	0.48	0.48
Chromic oxide bread	0.80	0.75	0.80

had the same intercepts as the curves based upon the chemically analyzed samples, but their slopes were appreciably steeper (Table II).

The curves based upon synthetic reference samples prepared by adding increments of chromic oxide (finer than 80-mesh) to weighed portions of dried ground feces were similar to the curves based on the first set of synthetic reference samples. Furthermore, curves based upon synthetic reference samples prepared by adding equivalent amounts of chromium as an aqueous solution of sodium dichromate to dried ground feces previously moistened with acetone were similar to the above curves.

A set of curves with markedly different slopes, however, was obtained when increments of the chromic oxide "bread" were added to portions of dried ground feces. The curves based on this set of synthetic reference samples had about the same intercepts for all three measures as those obtained on the other sets of reference samples, but they had slopes which were considerably lower than the slopes of the other curves.

A study of these curves indicates that the addition of chromium as powdered chromic oxide, as an aqueous solution of sodium dichromate, or as chromic oxide bread results in a set of synthetic reference samples whose curves differ markedly from those based on the chemically determined chromic oxide concentration in selected samples (Table II). The use of the curves based upon synthetic reference samples resulted in differences of 20 to 30% in the chromic oxide concentrations.

The magnitude of these differences led the authors to check chemically the chromic oxide concentrations in the synthetic reference samples. Chemical chromic oxide determinations (*3*) on selected synthetic reference samples showed that there was little possibility of major error in the adding and mixing of chromic oxide or its equivalent as an aqueous solution of sodium dichromate with portions of the dried ground feces. The chemically determined chromic oxide concentrations were within 8% of the calculated values (Table III).

A study of the reproducibility of the chromic oxide determinations indicated that the error due to subsampling was about the same as the error expected in counting 25,600 events when measure F or $F - S$ was used. The error due to subsampling was about three times the error expected in counting 25,600 events when measure F/S was used. The error in the determination of the chromic oxide concentration based upon single observations on each of ten subsamples was slightly higher than that based upon ten observations on a given subsample which had not been removed from the sample chamber between observations (Table IV).

The discrepancies in the slopes of the working curves based on the synthetic reference samples when compared to the slopes of the working curves based on the set of selected chemically analyzed samples are difficult to explain. From considerations of the path followed by the feed concentrates containing chromic oxide and the roughages obtained by grazing, it would be logical to assume that the digestive process of the steers is a major factor. The authors have been unable to duplicate the effects of this process in vitro. It is obvious from the results tabulated in Table II that the addition of chromic oxide as a powder, as the chromic oxide bread, or as the equivalent amount of sodium dichromate in an aqueous solution to portions of dried ground feces will not duplicate the state of the chromic oxide in the samples collected from grazing steers. Furthermore, the counting rates obtained on a small number of chemically analyzed excreta of chicks fed a semipurified diet containing chromic oxide differed markedly from the counting rates obtained on chemically analyzed (*3*) fecal samples from grazing steers.

Conclusions

The x-ray spectrographic technique described is rapid (as many as 10 determinations an hour), requires a minimum of sample pretreatment, and is sufficiently accurate for all but the most exacting kind of work. However, the x-ray spectrographic data obtained

Table IV. Means and Standard Deviations[a] of Chromic Oxide Concentration Based on Single Observations on Each of 10 Subsamples and 10 Observations on One Subsample

Sample No.	No. of Subsamples	No. of Observations	Measure F	Measure F − S	Measure F/S
1	10	1	0.476 ± 0.007	0.477 ± 0.007	0.491 ± 0.011
	1	10	0.468 ± 0.005	0.471 ± 0.005	0.490 ± 0.017
2	10	1	0.748 ± 0.010	0.750 ± 0.008	0.770 ± 0.008
	1	10	0.743 ± 0.006	0.745 ± 0.006	0.762 ± 0.020

[a] Mean: $\bar{X} = \frac{\Sigma X}{n}$.

Standard deviation: $s = \sqrt{\frac{\Sigma x^2}{n-1}}$ (*6*).

on samples being analyzed must be compared to x-ray spectrographic data obtained on similar reference samples analyzed chemically for chromic oxide. Furthermore, the results of a limited study showed that x-ray spectrographic data obtained on reference samples of feces from grazing steers differed markedly from those obtained on samples of excreta from chicks fed a semipurified diet containing chromic oxide.

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PLANT UPTAKE OF MINERALS

Effect of Residual Lime in Soil on Minor Elements in Plants

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Residual lime in the soil resulted in significant changes of the plant composition of peanut foliage, soybean foliage, and orchard grass 7 and 9 years after lime application. Copper, cobalt, manganese, iron, and zinc were significantly lowered in each plant. Molybdenum was significantly increased.

LIME has long been used as a fertilizer and for correcting and controlling soil acidity. The effect of soil acid conditions can be observed by examining plants grown on soils which have incremental dressings of lime. One such study, which illustrates the depressing effects of lime on the levels of cobalt, nickel, and manganese in pasture herbage and its enhancing effect upon molybdenum, was conducted by Mitchell (*9*). He used the equivalent of 6 and 12 tons of $CaCO_3$ per acre with the pH of the soils at 5.4 for control and 6.1 and 6.4, respectively, for the levels of $CaCO_3$. He found marked depression in cobalt, nickel, and manganese content with decided increases in molybdenum. Taylor (*15*) and coworkers found that lime significantly decreased the manganese content of plants, significantly increased the molybdenum content, but normally has little influence on the level of copper. Fiskell (*7*) showed that liming reduced intake of manganese content of blue lupines after 4 years' application and that symptoms of a copper deficiency in ladino clover were intensified by a heavy liming program. Robinson's (*14*) work under greenhouse studies showed that the molybdenum content of alfalfa, crimson clover, and Austrian winter peas grown on acid soils high in molybdenum may be increased by heavy liming to a point where these crops are toxic to cattle. Other workers (*1, 2, 4, 8, 11, 13, 16, 17*) confirm the effects of soil liming on the composition of plants and the uptake of minerals.

The capacity of pastures to supply the mineral needs of grazing stock in adequate amounts, in proper proportions, and without harmful excess depends upon many interrelated factors. Controlled experiments (*10*) in 1953 and 1954 over wide areas of the state provided opportunity to study residual lime effects for periods up to 7 or 9 years after application. The literature does not report the effects of soil lime treatments on the minor element uptake of plants after years of application.

Procedures

Samples were collected from lime plots in three areas of the state: peanut tissues (leaves) from Norfolk loam fine sandy, deep phase, near the Holland Experiment Station; soybean tissues (leaves) from Sassafras sandy loam, near the Warsaw Experiment Station; and orchard grass from Lodi loam, near Abingdon, Va. The peanut and soybean tissues were collected 7 years and the orchard grass 9 years after lime treatment. The experimental sites were located on private farms. After the lime was applied, the owners continued to crop the areas with normal practices, except that no additional lime was applied. Fertilization varied, but in most cases the rates used were less than those recommended; and it is believed that neither crops nor fertilization materially influenced pH or base relationships in the soil. No crop yield response was noted except in the case of soybeans, where approximately a 25% increase was obtained from the use of lime compared to no lime. No grazing was done on the peanut or soybean soils, but the orchard grass was in continuous pasture for dairy cattle.

Mineralogically, the three soils were composed primarily of vermiculite (70 to 75%) and contained smaller quantities of illite and kalonite (10 to 25%). Total exchangeable cations present in the unlimed soils were 5.6, 5.9, and 9.6 meq. per 100 grams of soil for the peanut, soybean, and orchard grass soils, respectively. Soil pH was determined on a Model G, Beckman pH meter, using a 1 to 1 soil-water ratio and a half-hour equilibration period. Other soil properties are discussed fully by Moschler (*10*).

Cobalt, copper, iron, manganese, and zinc were determined according to official methods of the AOAC (*3*). Molybdenum was determined by the thiocyanate stannous chloride method of Evans (*6*). The analyses are reported on a dry weight basis, and wet digestion (nitric and perchloric acids) was used instead of dry ashing.