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The Quantitative Determination of Chromic Oxide in Feeds and Feces

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ABSTRACT

The main purpose of this investigation was to develop an accurate and precise analytical method for the determination of chromic oxide in feeds and feces applicable in digestibility studies with farm animals using the chromic oxide ratio technique.

A discussion has been given covering, in a rather general manner, the use of reference substances in digestibility studies.

In preliminary experiments it was found necessary to overcome several major difficulties in the determination of chromic oxide. A presentation of these difficulties and the methods whereby they were solved has been made.

A reliable, precise, and accurate quantitative method for the determination of chromic oxide (Cr_2O_3) incorporating the results of this investigation has been described.

Known quantities of "purified" chromic oxide were added to samples of feeds and feces and analyses were made of the Cr_2O_3 content. Analyses were also made of samples of "purified" chromic oxide for the Cr_2O_3 content.
The statistical analysis of the results of these chemical analyses indicate the precision and accuracy of the analytical method for chromic oxide developed in this laboratory.

The accuracy and precision of the analytical method for chromic oxide and the high recovery of added Cr_2O_3 from feeds and feces demonstrate the applicability of this method to digestibility studies with farm animals in which the chromic oxide ratio technique is employed.

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THE QUANTITATIVE DETERMINATION OF CHROMIC OXIDE IN FEEDS AND FECES

An Improved Method Applicable in Digestibility Studies with Farm Animals

CHARLES W. GEHRKE, DENNIS T. MAYER, EDWARD E. PICKETT AND CHARLES V. RUNYON

INTRODUCTION

In the past the measurement of digestibility and rate of consumption of the principal constituents of the rations of farm animals has been a laborious and time-consuming procedure. The older methods, as generally employed, necessitated the collection of the total quantity of feces excreted by the experimental animal during a 24-hour period. The bulky and contaminated fecal material then had to be mixed carefully so that representative samples could be taken for the chemical analyses which are used as a basis for calculating the digestibility of the various dietary principals. This method usually has been designated as the total collection method.

In recent years, several rather simple indirect methods have been proposed. These methods eliminate the long, tedious and expensive digestion trials of the total collection method. In general, the indirect methods utilize reference substances as "indicators". The use of reference substances eliminates the necessity of collecting the total feces excreted. Instead, a small proportion of the excreta are obtained as "grab" samples and utilized for the analytical determinations. However, Kane *et al.* (1950a) show that the time of sampling and the number of samples taken are important when the ratio technique is used. The reference substance may be a natural plant substance such as lignin or plant chromogens, or some inert chemical substance added in known quantities to the feed.

The possession of definite characteristics determines whether a particular substance may be used as an indicator or reference substance. Reference substances must be inert, insoluble, and nontoxic materials which pass through the digestive tract without being absorbed, digested, or altered in any way. In addition, it is essential that the reference substances be one for which simple and accurate quantitative analytical procedures are available for its determination in feeds and feces. Lignin, plant chromogens, chromic oxide, iron oxide, and several other materials have been used as reference substances; and they possess in varying degrees the characteristics of the ideal reference substance. Barnicoat (1945-1946) has reviewed the earlier literature pertaining to the use of reference substances in digestibility studies.

The use of reference substances in digestibility studies has been designated the "ratio technique" by Kane, *et al.* (1950a). Briefly, the method involves the determination of the quantity of the reference substance in the feed of the experimental animals. Representative "grab" samples of the feces are then analyzed for the reference substance. The ratio of the chromic oxide in the feed to that found in the feces can be ascertained and used as a factor by which the ratio between the concentration of a particular nutrient in the feces and the feed is multiplied to obtain the digestibility of a particular nutrient. The complete equation for the calculation of digestibility determined by the chromic oxide method is given below:

$$\text{Digestibility} = 100 - 100\left(\frac{\%\ Cr_2O_3 \text{ in feed}}{\%\ Cr_2O_3 \text{ in feces}}\right) \times \left(\frac{\%\ \text{of nutrient in feces}}{\%\ \text{of nutrient in feed}}\right)$$

The percentage of protein, or fat, or of carbohydrate may be substituted in the above equation and the digestibility of any of these particular nutrients thus determined.

Certain inherent difficulties in the analytical determination of lignin in plant materials makes its utilization as a reference substance less desirable than that of an inert chemical substance, e. g. chromic oxide.

Reid, *et al.* (1950) suggest the use of natural plant pigments or chromogens as reference materials and describe a reliable method for their quantitative determination in feeds and feces. The method, however, is only practicable in digestibility studies on those animals in which the ration consists primarily of plant materials. It is especially adaptable to digestibility studies of farm animals on pasture.

In 1945 Edin, *et al.* published a summarized description of "Edin's Indicator Method"; one of the first to use chromic oxide as a reference substance. The addition of known amounts of chromic oxide to the feed as a reference substance in digestibility studies is now designated as the chromic oxide technique or method. In general, this method has several advantages over the methods previously mentioned. In this laboratory it has been found that chromic oxide can be mixed directly with swine rations in which it is evenly dispersed and from which it does not settle over long periods of time. Chromic oxide can also be used in digestibility studies on other farm animals. With the coarse dry feeds provided for cattle or sheep, it is necessary to mix the reference substance in a carrier consisting of moistened flour, corn meal, etc., which is then dried and ground. Edin (1945) in his method used a moistened macaroni paste as a carrier. However, in digestibility studies of animals on pasture the chromic oxide method is not feasible, unless certain additional adaptations are employed.

There are several oxides of chromium of which chromic oxide (Cr_2O_3) is the only one utilizable as a reference substance. Chromic oxide is insoluble, inert, and non-toxic. and is neither digested, altered. nor absorbed in the di-

gestive tract. It has the added advantage of absorbing only small amounts of water from the air, hence can be accurately weighed prior to its addition to the experimental feed mixture.

However, this laboratory and several others (personal communications) have experienced difficulties in attempting to determine Cr_2O_3 in feeds and feces by the standard procedures recommended for its determination in non-biological materials, viz. in ores and minerals.

The purpose of the present paper is to describe, in detail with precautions and pertinent suggestions, a simple, accurate and reproducible procedure for the quantitative determination of chromic oxide in feeds and feces.

EXPERIMENTAL

Approximately one-pound samples of thoroughly mixed swine feces* were submitted for chemical analysis to the Department of Agricultural Chemistry. Samples of the feed used in this study were also submitted for analysis. The preliminary handling of the feed and fecal samples prior to making the actual analyses differed; therefore, the preparation of the samples for the chromic oxide analysis will be discussed separately. The usual precautions must be exercised in sampling and in the preparation of the feed and fecal samples for chemical analysis.

Feeds

Drying and Grinding. The feed samples were ground to pass a 1 mm. screen. Representative samples of the feed were taken, spread thinly on large sheets of paper and allowed to come into equilibrium with the moisture of the air, then placed in tightly closed screw cap bottles, and stored for chemical analysis. The analytical determinations were all completed in a 3-month period.

Chromic oxide determinations were made singly on all of the feed samples submitted by the Department of Animal Husbandry. A second determination was made at a later date, and in some cases a third analysis was made. The replicate results agreed closely indicating that, in the ration used, very little segregation of the feed-chromic oxide mixture occurred.

Feces

Drying and Grinding. Some difficulty was experienced in vacuum drying of the fecal samples. The procedure finally adopted was simply that of placing thin layers of fecal material in large flat-bottom evaporating dishes. The dishes were placed in a forced draft oven at 65° C., and held at this temperature until dry. The fecal samples were effectively dried in this manner in about 12 to 18 hours. After drying, the fecal material was ground in the Bird Impact Mill to pass a 1 mm. screen. Representative samples were taken and air dried overnight, then stored in tightly closed screw cap bottles for chemical analysis.

Determination of Chromium Oxide

The analytical procedure which was adopted is presented under RESULTS below. Some of the factors considered in the development of this procedure

*Digestibility studies, Department of Animal Husbandry, in which chromic oxide (Cr_2O_3) was added to the feed as a reference substance.

will now be discussed. A few of them comprise essential details of the procedure and should be borne in mind when using the method as described.

Size of Sample. The rations contained 5 mg. of the "purified" chromic oxide per gram. Ten gm. samples, containing 50 mg. of chromic oxide, were ashed as described below. It was found that the fecal samples contained about five times as much chromic oxide per unit weight as the feed samples. Because of this greater concentration of chromic oxide in the feces, it is more convenient to reduce the sample size to 5 gm. of fecal matter.

Ashing of Sample. Approximately 10 gm. of dried feed or 5 gm. of dried fecal matter were weighed into 145 ml. porcelain crucibles and placed in a cold muffle furnace which was then heated to a temperature of 500-550° C., and held there overnight. A uniform light gray ash was obtained.

Fusion and Solution of the Ashed Sample. Conversion of the chromic oxide to a soluble chromate prior to its determination was found to proceed best by a sodium peroxide fusion method. Various wet ashing procedures, involving treatment with hot concentrated acids and oxidizing agents, gave very incomplete conversion or undesirable products. Although fusion methods are often rather unsatisfactory, the sodium peroxide fusion method presented here is quite simple and convenient.

The choice of the best type of crucible for the fusion presented some difficulties. Barnicoat (1945-1946) used nickel crucibles for the fusion with sodium peroxide. Later the nickel taken into solution in the melt had to be removed by precipitation as NiO, entailing approximately a 2 per cent loss of Cr_2O_3 by adsorption. Much the same difficulties were encountered with iron crucibles. Platinum will not stand the action of molten sodium peroxide, and silver melts at a rather low temperature. Porcelain crucibles were used and proved to be acceptable.

In the fusion process finally adopted the ashed sample, in the 145 ml. crucible, was thoroughly mixed with 20 gm. of C.P. sodium peroxide. Only relatively fresh, dry sodium peroxide should be used. The sample was then fused in the covered crucible by gradually heating over a Fisher burner. It is imperative that the mixture be heated slowly, with a gradual increase in the temperature, until the mixture is molten. If spattering occurs during the fusion, the heating is being conducted too rapidly. When the mixture is completely molten it is removed from the flame. The time required for the fusion is 15-25 minutes. The temperature reaches 1000-1200° C. All organic matter must be oxidized in the ashing process before the fusion is started; otherwise an explosion will result and the peroxide-sample mixture will "burn" through the bottom of the crucible. When the proper technique is followed, the fusion proceeds smoothly and a quantitative conversion of the Cr_2O_3 to the chromate occurs.

It was found that the sodium peroxide attacks the porcelain crucible during the fusion. However, if care is exercised in heating the sample, the crucible may be used for three or four determinations.

A quantitative conversion to the chromate was obtained when 125 mg. samples of chromic oxide, sesqui, "purified", obtained from the Allied Chemical and Dye Corp., were fused by this process.

The chemical reaction for the conversion of the chromic oxide to chromate is as follows:

$$Cr_2O_3 + 3\ Na_2O_2 \rightarrow 2\ Na_2CrO_4 + Na_2O.$$

Other Fusion Methods. The research laboratories of the Allied Chemical and Dye Corporation conduct the chromic oxide fusion as follows: A 0.100 gm. sample is heated with 5 ml. of $HC10_4$ and allowed to fume until the chromic oxide is completely oxidized. The sides of the flask are washed down with water, and the solution is evaporated to fumes of $HC10_4$, cooled, water added, and the chlorine boiled out.

Edin, *et al.* (1945) reported a method for the analysis of chromic oxide in feeds and feces. A rather large number of analytical steps were involved in their method.

Kane, Jacobson, and Moore (1950b) reported on a comparison of techniques used in digestibility studies with dairy cattle. They described a fusion method for the determination of chromium oxide which was a slight modification of Edin's fusion method (Edin, *et al.* 1945).

A previous reference has been made to the fusion process of Barnicoat (1945-1946) in which Na_2O_2 was used.

Some of the reported fusion processes have the disadvantage of complexity, and the introduction of additional steps in the analytical procedure. Some of these disadvantages are avoided in the fusion method developed in the laboratories of the Missouri Station.

Transfer and Neutralization of the Sample. The crucible containing the fused sample is next placed in a tall 600 ml. beaker. The beaker is covered with a watch glass to prevent loss of the fusion mix during the transfer. A small stream of water, a total of 100 ml. being sufficient, is projected into the crucible. Considerable heat is liberated upon solution of the mixture containing chromate salts, sodium oxide, and unreacted sodium peroxide. All of the sample is quantitatively transferred from the crucible to the beaker. Enough water is then added to bring the volume to between 200-300 ml. Excess alkali is destroyed by adding 30 grams of ammonium carbonate to the solution in the beaker. The sample in solution in the 600 ml. beaker is brought to the boiling point to expedite the reaction and dissolve all of the ammonium carbonate. It is now cooled and neutralized with 1:1 sulfuric acid. An excess of 40 ml. of the 1:1 acid is added. Following the neutralization reaction the chromate ion is converted to the dichromate ion. One can easily determine when the neutralization point is reached by simply observing the evolution of CO_2, as the sample is neutralized when the evolution of CO_2 ceases. Upon acidification the color of the solution also changes from yellowish to a reddish-orange color. The neutralization must be conducted carefully; otherwise, due to the excessive volume of CO_2 liberated, the solution will foam over the top of the beaker.

Reduction of Dichromate to Chromic Ion. A standard solution of ferrous ammonium sulfate ($FeSO_4 \cdot (NH_4)_2SO_4 \cdot 6\ H_2O$), approximately 0.1 N for feeds and 0.2 N for feces, was prepared by dissolving the reagent grade chemical in 1 molar sulfuric acid. The normality of the solution was determined by titration with standard potassium permanganate solution (standardized by titration with Bureau of Standards sodium oxalate). About 40 ml. of the reducing agent (ferrous ammonium sulfate) were used for each sample. This is enough of the standard $FeSO_4 \cdot (NH_4)_2SO_4 \cdot 6\ H_2O$ solution to give a back titration of about 20 ml. of the standard permanganate solution. To improve the sharpness of the end point, 10 ml. of 1:1 syrupy 85 per cent H_3PO_4 were added. The ionic equation for the reduction of the dichromate ion to the chromic ion by the ferrous ammonium sulfate is as follows:

$$Cr_2O_7^{=} + 6\ Fe^{++} + 14\ H^{+} \longrightarrow 2\ Cr^{+++} + 6\ Fe^{+++} + 7\ H_2O$$

Other methods are available for quantitatively determining the amount of dichromate present. The use of any particular one depends upon the analyst's choice. At first, we determined the dichromate concentration potentiometrically using platinum and calomel electrodes. Good results were obtained, but the titration took considerable time to complete.

Titration of Excess Ferrous Ion with Standard Potassium Permanganate Solution. The amount of ferrous ammonium sulfate in excess of that actually needed for the reduction of the dichromate present was determined by titration with standard potassium permanganate (0.1 N for feeds and 0.2 N for feces). The end point is detected easily despite the green color of the solution. The dark-colored $KMnO_4$ serves as its own indicator.

Calculation of Chromic Oxide Content. A number of blank determinations were made to determine the natural oxidizing agents present in the feeds, feces, and chemicals used. Representative samples of feeds and feces containing no Cr_2O_3 were analyzed. The values for these blanks are shown in the example used for the calculation of the chromic oxide content of a feed sample. With each set of 20 samples, several standards and blanks were analyzed.

Calculation (Blank).

1. ml. of 0.1033 N $FeSO_4 \cdot (NH_4)_2SO_4 \cdot 6\ H_2O$ added	20.00
2. ml. of 0.1266 N $KMnO_4$ for back titration	16.30
3. ml. of 0.1266 N $KMnO_4$ for end point	0.05
4. ml. net of 0.1266 N $KMnO_4$ used in titration	16.25
5. ml. of $FeSO_4 \cdot (NH_4)_2SO_4 \cdot 6\ H_2O \doteq$ 16.25 ml. of 0.1266 N $KMnO_4$	19.92
6. Blank due to oxidizing agents in samples and chemicals used (1-5)	0.08

Two blanks are thus established for the analytical determination. One blank of 0.05 ml. of $KMnO_4$ solution is required to give a definite colored end point in the determination when the excess $FeSO_4 \cdot (NH_4)_2SO_4 \cdot 6\ H_2O$ (ferrous ammonium sulfate) is titrated with the $KMnO_4$ solution. The various oxidizing agents in the sample and in the chemicals used in the analysis, as well

as the other inherent errors in the method, require the use of an additional blank expressed as 0.08 ml. of 0.1033 N $FeSO_4 \cdot (NH_4)_2SO_4 \cdot 6\ H_2O$ solution. In routine determinations the blanks for ferrous ammonium sulfate and for permanganate could be disregarded, since one tends to cancel the other.

Calculation (Feed Sample).

1. ml. of 0.1033 N $FeSO_4 \cdot (NH_4)_2SO_4 \cdot 6\ H_2O$ added 39.98
2. ml. of 0.1033 N $FeSO_4 \cdot (NH_4)_2SO_4 \cdot 6\ H_2O$ (blank) 0.08
3. ml. net of 0.1033 N $FeSO_4 \cdot (NH_4)_2SO_4 \cdot 6\ H_2O$ added 39.90
4. ml. of 0.1266 N $KMnO_4$ for back titration 16.55
5. ml. of 0.1266 N $KMnO_4$ for end point 0.05
6. ml. (Net) . of 0.1266 N $KMnO_4$ used for back titration 16.50
7. ml. of $FeSO_4 \cdot (NH_4)_2SO_4 \cdot 6\ H_2O$ $\Rightarrow$ to 16.50 ml. of $KMnO_4$ 20.22
8. ml. of $FeSO_4 \cdot (NH_4)_2SO_4 \cdot 6\ H_2O$ oxidized by dichromate ion (3-7) 19.68
9. Grams of Cr_2O_3 = ml. of ferrous ammonium sulfate $\times$ normality $\times \frac{Cr_2O_3}{6000}$
10. $\%\ Cr_2O_3 = \frac{\text{Step 9}}{\text{weight sample}} \times 100$

RESULTS

Chromic Oxide Analytical Method

From the preliminary experimentation on the various steps of the analytical procedure for the determination of chromic oxide in feeds and feces the following method has been developed and is described step-wise below:

1. Ash 5 gm. of feces or 10 gm. of feed in a 145 ml. porcelain crucible overnight in a furnace at dull red heat. The ash must be free of all organic matter at the end of the heating period.

2. Add 20 gm. Na_2O_2, mix thoroughly, then fuse carefully over a Fisher burner until molten. Increase the heat gradually to prevent spattering. When the sample-peroxide mixture is completely molten it is removed from the flame. The crucible must be covered during the fusion. The fusion temperature is between 1000-1200° C.

3. Cool the molten material, then place the crucible containing the melt in a covered 600 ml. beaker. Transfer the fused material quantitatively with water (using about 200 ml.) to the beaker.

4. Add 30 gm. of C. P. ammonium carbonate and heat to the boiling point. If no insoluble residue is present, this step may be omitted.[1]

5. Cool, add 1:1 H_2SO_4 until neutral, then 40 ml. in excess. This step must be done carefully. When the evolution of CO_2 ceases, the neutral point has been reached.

6. Add 10 ml. of 1:1 syrupy 85% H_3PO_4 to improve the sharpness of the end point. Add an excess of standard ferrous ammonium sulfate (0.1 N

[1]Scott's Standard Methods of Chemical Analysis. 5th Ed., Vol. I, p. 284.

for feed and 0.2 N for fecal samples), about 40 ml. for the sample weights of feed or feces given in Step 1.

7. Back titrate the excess ferrous ammonium sulfate, using standard $KMnO_4$ (0.1 N for feeds and 0.2 N for fecal samples) to a faint pink color resulting from a slight excess of the $KMnO_4$ solution.

8. Make blank determinations of feed and fecal samples containing no Cr_2O_3.

9. Calculate the percentage of Cr_2O_3 from: (ml. of ferrous ammonium sulfate solution) $\times$ normality $\times \frac{Cr_2O_3{}^*}{6000}$ = grams Cr_2O_3 in sample.

10. $\frac{\text{grams } Cr_2O_3 \text{ in sample}}{\text{sample wt.}} \times 100 = \%\ Cr_2O_3$ in sample.

The Reliability and Precision of the Method

Known amounts of the "purified" chromic oxide were added to samples of feeds and feces and analyzed by the method described above. In addition, analyses were made on accurately weighed samples of "purified" chromic oxide†. The reliability and precision of the chromic oxide method are shown by the results of these determinations which are given in Tables 1, 2, and 3.

In order to ascertain the Cr_2O_3 content of the "purified" chromic oxide, it was subjected to the same analytical procedure as that used for the feed and fecal samples. The results of five such determinations are presented in Table 3. The purity of the material measured in this way was substantially higher (98.74%) than the assay value quoted on the label by the manufacturer (97%). This value may be low since Urone and Anders (1950) cite experimental work showing that the perchloric acid fusion of chromium compounds results in a loss of chromium as chromyl chloride. Since neither an independent, reliable method for the analysis of the "purified" chromic oxide nor a reagent grade 100% pure Cr_2O_3 were obtainable, the analytical value of 98.74% (Table 3) was used in the statistical treatment of the analytical data obtained upon analysis of the feed and fecal samples.

The standard error of the bias for Cr_2O_3 recovered from feeds, feces, and "purified" chromic oxide are given as follows:

$$\text{Standard error of the bias (feeds)} = \sqrt{\frac{0.387}{16} + \frac{0.050}{5}} = 0.18$$

$$\text{Average bias} = 98.74 - 98.72 = 0.02$$

$$\text{"t" value} = \frac{0.02}{0.18} = 0.11$$

*Milliequivalent weight of Cr_2O_3 obtained by dividing the molecular weight of Cr_2O_3 by 6000.

†Chromic oxide, sesqui, "purified", Allied Chemical and Dye Corporation.

TABLE 1.—ANALYSIS OF CHROMIC OXIDE IN FEEDS

Sample No.	10 gram samples of feed plus a weighed amount of Cr_2O_3			
	Mg. of "Purified" Chromic Oxide added	Mg. of Cr_2O_3 Recovered	Difference Mg.	Cr_2O_3 Recovered %
1	49.9	49.3	0.6	98.80
2	50.2	49.9	0.3	99.40
3	58.9	58.1	0.8	98.64
4	51.4	50.5	0.9	98.25
5	50.8	49.8	1.0	98.03
6	50.0	48.5	1.5	97.00
7	52.0	51.5	0.5	99.04
8	57.4	57.1	0.3	99.48
9	50.1	49.9	0.2	99.60
10	55.2	54.7	0.5	99.09
11	54.5	53.8	0.7	98.72
12	52.0	51.3	0.7	98.65
13	50.3	49.6	0.7	98.61
14	50.8	50.1	0.7	98.62
15	57.8	57.2	0.6	98.96
16	51.3	50.6	0.7	98.64

Number of determinations	16
Mean % recovered	98.72
Variance of % recovered	0.387
Standard deviation of % recovered	0.622
Standard error of mean % recovered	±0.15

Ninety-five per cent of the individual determinations of Cr_2O_3 in feeds will fall in the range of 98.72 ± 1.24. The 95% range in means of repeated trials of 16 determinations is about ± 2 standard errors or 98.72 ± 0.30. The range including 2/3 of means in repeated trials of 16 determinations would be 98.72 ± 0.15.

TABLE 2.—ANALYSIS OF CHROMIC OXIDE IN FECES

Sample No.	Mg. of "Purified" Chromic Oxide added	Mg. of Cr_2O_3 Recovered	Difference Mg.	Cr_2O_3 Recovered %
	10 gram samples of feces plus a weighed amount of Cr_2O_3			
1	226.9	223.6	3.3	98.55
2	256.3	252.6	3.7	98.56
3	252.7	249.6	3.1	98.78
4	260.0	258.0	2.0	99.23
5	242.2	236.8	5.4	97.77
6	259.7	255.2	4.5	98.27
7	244.4	238.3	6.1	97.50
8	259.6	256.3	3.3	98.73
9	253.1	252.0	1.1	99.57
10	253.1	250.9	2.2	99.13
11	247.8	244.5	3.3	98.67
12	252.0	249.0	3.0	98.81
13	256.1	251.6	4.5	98.24
14	254.1	250.4	3.7	98.54
	5 gram samples of feces plus a weighed amount of Cr_2O_3			
15	110.5	108.6	1.9	98.28
16	128.0	126.1	1.9	98.52
17	119.9	118.5	1.4	98.83
18	106.2	104.8	1.4	98.68
19	103.5	102.0	1.5	98.55

Number of determinations	19
Mean % recovered	98.59
Variance of % recovered	0.223
Standard deviation of % recovered	0.472
Standard error of Mean % recovered	± 0.11

Ninety-five per cent of the individual determinations of Cr_2O_3 in feces will fall in the range of 98.59 ± 0.944. The 95% range in means of repeated trials of 19 determinations is about ± 2 standard errors or 98.59 ± 0.22. The range including 2/3 of means in repeated trials of 19 determinations would be 98.59 ± 0.11.

TABLE 3.—ANALYSIS OF SAMPLES OF "PURIFIED" CHROMIC OXIDE

Sample No.	Mg. of "Purified" Chromic Oxide added	Mg. of Cr_2O_3 Recovered	Difference Mg.	Cr_2O_3 Recovered %
1	132.2	130.7	1.5	98.87
2	133.2	131.3	1.9	98.57
3	118.5	117.0	1.5	98.73
4	124.6	123.4	1.2	99.04
5	131.4	129.4	2.0	98.48

Number of determinations	5
Mean % recovered	98.74
Variance of % recovered	0.05
Standard deviation of % recovered	0.224
Standard error of mean % recovered	± 0.10

Ninety-five per cent of the individual determinations of "purified" Cr_2O_3 will fall in the range of 98.74 ± 0.448. The 95% range in means of repeated trials of 5 determinations is about ± 2 standard errors or 98.74 ± 0.20. The range including 2/3 of means in repeated trials of 5 determinations would be 98.74 ± 0.10.

P, from "Student's" t table, is about 0.9 for this value of t; this is a high probability that no bias exists, that is, that the method is accurate for feed samples.

$$\text{Standard error of the bias (feces)} = \sqrt{\frac{0.223}{19} + \frac{0.050}{5}} = 0.15$$

$$\text{Average bias} = 98.74 - 98.59 = 0.15$$

$$\text{"t" value} = \frac{0.15}{0.15} = 1$$

P is about 0.33, indicating that a difference this large would be expected in 1/3 of repeated trials of this size, even though no real bias actually existed, because of random error of determinations in the analysis of fecal samples. Even if the bias of 0.15 were real, its magnitude in the per cent Cr_2O_3 recovery is quite small.

The value of 98.74% (Table 3) may be taken as the actual quantity of Cr_2O_3 in the "purified" chromic oxide. This value serves as a basis for evaluating the data obtained from the analyses of feed and fecal samples. If this figure is the actual percentage composition of the "purified" chromic oxide, then the statistical analysis above would indicate the true accuracy of the analytical procedure. Regardless of whether 98.74% Cr_2O_3 represents the true value or not, the statistical analysis of the data (Tables 1, 2, and 3) demonstrates at least the precision and reliability of the method and its applicability in digestibility studies.

SUMMARY AND CONCLUSIONS

The main purpose of this investigation was to develop an accurate and precise analytical method for the determination of chromic oxide in feeds and feces applicable in digestibility studies with farm animals using the chromic oxide ratio technique.

A discussion has been given, covering in a rather general manner, the use of reference substances in digestibility studies.

In preliminary experiments it was found necessary to overcome several major difficulties in the determination of chromic oxide. A presentation of these difficulties and the methods whereby they were solved has been made.

A reliable, precise, and accurate quantitative method for the determination of chromic oxide (Cr_2O_3) incorporating the results of this investigation has been described.

Known quantities of "purified" chromic oxide were added to samples of feeds and feces and analyses were made of the Cr_2O_3 content. Analyses were

also made of samples of "purified" chromic oxide for Cr_2O_3 content. The statistical analysis of the results of these chemical analyses (Tables 1, 2, and 3) indicate the precision and accuracy of the "chromic oxide" method developed in this laboratory.

The accuracy and precision of the "chromic oxide" method and the high recovery of added Cr_2O_3 from feeds and feces (Tables 1 and 2) demonstrate the applicability of this method to digestibility studies with farm animals in which the chromic oxide ratio technique is employed.

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