

Notas Científicas

Evaluation of acid digestion techniques to estimate chromium contents in cattle feces

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Abstract – The objective of this work was to evaluate the accuracy of digestion techniques using nitric and perchloric acid at the ratios of 2:1, 3:1, and 4:1 v v⁻¹, in one- or two-step digestion, to estimate chromium contents in cattle feces, using sodium molybdate as a catalyst. Fecal standards containing known chromium contents (0, 2, 4, 6, 8, and 10 g kg⁻¹) were produced from feces of five animals. The chromium content in cattle feces is accurately estimated using digestion techniques based on nitric and perchloric acids, at a 3:1 v v⁻¹ ratio, in one-step digestion, with sodium molybdate as a catalyst.

Index terms: chromic oxide, chromium recovery, markers.

Avaliação de técnicas de digestão ácida para estimar teores de cromo em fezes de bovinos

Resumo – O objetivo deste trabalho foi avaliar a acurácia de técnicas de digestão com uso dos ácidos nítrico e perclórico, nas razões 2:1, 3:1 e 4:1 v v⁻¹, em um ou dois passos de digestão, na estimação da concentração de cromo em fezes de bovinos, com uso do molibdato de sódio como catalisador. Foram produzidos padrões fecais com conteúdo conhecido de cromo (0, 2, 4, 6, 8 e 10 g kg⁻¹), a partir das fezes de cinco animais. A concentração de cromo em fezes de bovinos é acuradamente estimada com técnicas de digestão baseadas no uso dos ácidos nítrico e perclórico, na razão 3:1 v v⁻¹, em um passo de digestão, com molibdato de sódio como catalisador.

Termos para indexação: óxido de cromo, recuperação de cromo, indicadores.

Chromic oxide is the most common external marker used in digestion assays with animals. Several techniques are used to estimate fecal chromium concentration and, theoretically, they should converge to the same final result. However, disagreements between techniques in the quantification of fecal chromium have been reported (Saha & Gilbreath, 1991; Souza et al., 2013).

Different aspects of the procedure for chromium quantification in excreta or feces samples have been investigated, including time and temperature of heating, acid combinations, wavelengths, and quantification techniques (Kimura & Miller, 1957; Fenton & Fenton, 1979; Souza et al., 2013). The main goal of those investigations was to obtain the best combination of procedures to ensure the accuracy of the estimates of fecal concentration and, consequently, of fecal excretion or digesta flow.

Some constraints to the complete chromium recovery in feces could be associated with the acid digestion process (Saha & Gilbreath, 1991). Recently, digestion of organic matter with nitric and perchloric acid was confirmed to be better than sulfuric and perchloric or phosphoric acid, and the quantification of chromium using atomic absorption spectrophotometry was found to be most appropriate (Souza et al., 2013). However, the best ratio of nitric to perchloric acid or variations of the procedures based on one- instead of two-step digestion remain to be evaluated.

The objective of this work was to evaluate the accuracy of digestion techniques using nitric and perchloric acid at the ratios of 2:1, 3:1, and 4:1 v v⁻¹, in one- or two-step digestion, to estimate chromium contents in cattle feces, using sodium molybdate as a catalyst.

Seven digestion techniques using nitric (HNO_3 , P.A. 65%; Vetec 191), (Sigma-Aldrich Ltda., São Paulo, SP, Brazil) and perchloric (HClO_4 , P.A. ACS 70%; Vetec 909), (Sigma-Aldrich Ltda., São Paulo, SP, Brazil) acids were evaluated: digestion using the ratios of nitric to perchloric acid of 2:1, 3:1, and 4:1 v v⁻¹ in one or two steps, with sodium molybdate ($\text{Na}_2\text{MoO}_4 \cdot 2\text{H}_2\text{O}$, ACS $\geq 99\%$; Sigma-Aldrich 331058), (Sigma-Aldrich Ltda., São Paulo, SP, Brazil) as a catalyst; and a digestion using nitric and perchloric acid at the ratio of 3:1 v v⁻¹ in one step, without sodium molybdate. The quantifications were carried out through atomic absorption spectrophotometry using the Avanta Σ spectrophotometer (GBC Scientific Equipment, Braeside, Victoria, Australia) with a hollow-cathode lamp (357.9 nm) and a nitrous oxide-acetylene flame – 99.8% acetylene for atomic absorption, and 99.5% nitrous oxide for atomic absorption (White Martins, Rio de Janeiro, RJ, Brazil).

To evaluate the accuracy of the different techniques, several fecal standards containing known chromium contents were produced using cattle feces (organic matrix) obtained from five different animals: one calf, one growing heifer, one bull, one non-lactating dairy cow, and one lactating dairy cow. The animals were fed with corn silage-based diets containing different forage:concentrate ratios and none of them received chromium, neither as mineral supplement nor as an external marker. The fecal samples were collected on the same day, oven-dried at 60°C, and processed in a 1 mm knife mill. From each organic matrix, six different standards were produced, containing 0, 2, 4, 6, 8, and 10 g chromium per kg of feces, totalizing 30 fecal standards. The standard concentrations were produced on as-is basis in order to avoid the accumulation of error from the estimation of the total dry matter content. Pure chromic oxide (Cr_2O_3 , 99.9% trace metals basis; Sigma-Aldrich 203068), (Sigma-Aldrich Ltda., São Paulo, SP, Brazil) was used to produce the standards.

To perform the one-step digestion procedures, approximately 250 mg of the fecal standards were poured into glass tubes. After that, 5 mL of the digestion solution were added, that is, a mixture of nitric and perchloric acid at the ratio of 2:1, 3:1, or 4:1 v v⁻¹. When applicable, sodium molybdate was added at 1 g L⁻¹ digestion solution. The tubes were then heated at 200°C until the appearance of a yellowish/orangish color and a brownish smoke stopped being released, indicating the complete digestion of the organic matter and the

change of chromium valence from Cr^{+3} (sesquioxide) to Cr^{+6} (dichromate). The tubes were allowed to cool at room temperature. After that, the digested samples were quantitatively transferred to 50 mL volumetric flasks. The transfer was done using ash-free quantitative filter paper (Whatman #41). The volume of the solutions was made up to 50 mL using deionized water. Aliquots of the solutions were transferred to polyethylene flasks and kept cooled at 4°C.

The same amount of sample was used to perform the two-step digestion procedures. After pouring the samples into the tubes, nitric acid was added at 3.3, 3.7, and 4.0 mL per tube, which correspond to the amount of acid for the 2:1, 3:1, and 4:1 ratios, respectively. Sodium molybdate was added to the nitric acid in order to provide 1 g L⁻¹ final digestion solution. The use of sodium molybdate serves as a catalyst in acid digestion to convert Cr^{+3} to Cr^{+6} , the quantifiable form of chromium (Kimura & Miller, 1957). The tubes were then heated at 200°C until the acid was half gone. After cooling at room temperature, perchloric acid was added at 1.7, 1.3, and 1.0 mL per tube, corresponding to the 2:1, 3:1 and 4:1 ratios, respectively. After that, the tubes were heated again at 200°C. Digestion end point, quantitative transference, and sample storage followed the same procedures described for one-step digestion.

To perform atomic absorption spectrophotometry procedures, standard solutions containing 0, 2, 4, 6, 8, and 10 ppm chromium were used to produce the standard curve. Those solutions were produced from a stock solution containing 1,000 ppm chromium, 109948 Tritisol, (Merck Millipore, Barueri, SP, Brazil).

The accuracy of the techniques was evaluated by adjusting a simple linear regression equation of chromium concentrations estimated by each technique (dependent variable) on the actual concentrations of chromium in the fecal standards (independent variable). The statistical analysis was carried out under the hypotheses: $H_0 : \beta_0 = 0$ vs. $H_a : \beta_0 \neq 0$, and $H_0 : \beta_1 = 1$ vs. $H_a : \beta_1 \neq 1$.

The slope of the adjusted model must be interpreted as the recovery of chromium added to the fecal standards. In addition, the intercept represents any interference in the medium, which could be originated from chemical interference, reagent impurity, as well as incomplete digestion. Accordingly, the estimated concentrations of chromium were considered to be

accurate when both null hypotheses were not rejected. The different organic matrixes were included as a random effect during the adjustment of the models and also for estimating the standard errors of the parameter estimates.

The acid digestion technique that was most accurate and presented the greatest chromium recovery was then compared with the digestion technique adopted by the Brazilian Institute of Science and Technology in Animal Science (Instituto Nacional de Ciência e Tecnologia de Ciência Animal, INCT-CA), method M-005/1 (Detmann et al., 2012), by evaluating the chromium concentration in 104 feces samples obtained from grazing calves (n=20) and lactating dairy cows (n=84), which received 10–20 g Cr₂O₃ per day. The comparison between estimates obtained using both techniques was performed by adjusting a simple linear regression equation, considering both null hypotheses previously presented. The effect of animal category (calves or cows) was included as a random effect in the model.

Statistical analyses were performed using the MIXED procedure of the SAS software, version 9.2 ($\alpha = 0.05$) (SAS Institute, Cary, NC, USA).

No medium interferences were observed for any technique combinations, since the contents obtained for the 0 g kg⁻¹ standard were very close or equal to zero and none of the intercept estimates were found to be different from zero (Table 1). Chromium recovery was complete when the following ratios from nitric to perchloric acid were used: 3:1 v v⁻¹, with sodium molybdate and one-step digestion; and 4:1 v v⁻¹, regardless of the number of digestion steps. However, chromium recovery was incomplete when other techniques were used.

The use of the pre-digestion step with nitric acid was unnecessary because none of the digestion techniques were improved by two-step digestion. That pattern must be highlighted because the pre-digestion step makes the technique more labor and time consuming and also more susceptible to errors due to a greater number of analytical steps and sample manipulation. Therefore, a one-step digestion procedure is recommended.

Considering that one-step digestion should be used to decrease labor in analytical procedures, both 3:1 v v⁻¹ and 4:1 v v⁻¹ ratios produced accurate results (Table 1). However, the 3:1 v v⁻¹ nitric to perchloric ratio, with sodium molybdate in one-step digestion, presented the recovery closest to totality (Table 1) and the lowest mean prediction error compared to the 4:1 v v⁻¹ ratio (-0.195 vs. -0.282 g kg⁻¹, respectively). From this, it can be concluded that the 3:1 v v⁻¹ nitric to perchloric ratio with sodium molybdate in one-step digestion should be recommended.

In the present study, the digestion technique based on the 3:1 v v⁻¹ ratio without sodium molybdate provided incomplete recovery of the fecal chromium (Table 1), which was not observed for the same technique when sodium molybdate was employed. Therefore, sodium molybdate must be used to perform chromium analysis correctly.

From these results, the fecal samples obtained from the digestion trial were compared using the digestion techniques based on the 2:1 and 3:1 v v⁻¹ nitric and perchloric acid ratios with sodium molybdate in one-step digestion. The first one corresponds to the official method adopted by INCT-CA (Detmann et al., 2012). Considering this, it was verified that both technique combinations provided similar and

Table 1. Means and standard errors for chromium contents in the standard samples and estimates of linear regression parameters for chromium concentration in fecal standards obtained using different digestion techniques.

Combinations			Standards (g chromium per kg of sample)						Regression parameters		s _{xy}	r	p-value	
R ⁽¹⁾	N ⁽²⁾	Mo ⁽³⁾	0	2	4	6	8	10	Intercept	Slope			Ho: $\beta_0=0$	Ho: $\beta_1=1$
2:1	1	+	0.01±0.00	1.98±0.21	3.77±0.39	5.94±0.34	7.72±0.49	8.93±0.34	0.15±0.25	0.91±0.04	0.69	0.98	0.571	0.029
2:1	2	+	0.01±0.00	1.81±0.05	3.94±0.54	5.59±0.11	6.56±0.36	8.61±0.38	0.21±0.24	0.84±0.04	0.67	0.98	0.414	<0.001
3:1	1	+	0.00±0.00	1.96±0.26	3.40±0.62	5.80±0.44	7.60±0.47	10.35±0.82	-0.20±0.42	1.00±0.07	1.30	0.94	0.665	0.955
3:1	1	-	0.01±0.00	1.71±0.05	3.45±0.08	5.11±0.15	7.37±0.29	8.04±0.38	0.08±0.18	0.84±0.03	0.51	0.98	0.460	<0.001
3:1	2	+	0.01±0.00	1.57±0.13	3.65±0.35	6.33±0.53	6.40±0.29	8.37±0.93	0.18±0.37	0.84±0.06	1.12	0.94	0.657	0.013
4:1	1	+	0.00±0.00	1.67±0.24	4.06±0.29	5.87±0.27	7.60±0.62	9.10±0.53	0.07±0.28	0.93±0.04	0.79	0.98	0.813	0.108
4:1	2	+	0.01±0.00	1.82±0.12	3.94±0.44	5.26±0.10	7.44±0.30	9.51±0.36	-0.02±0.19	0.94±0.03	0.59	0.99	0.910	0.060

⁽¹⁾R, nitric to perchloric acid ratio. ⁽²⁾N, number of digestion steps. ⁽³⁾Mo, sodium molybdate as a catalyst.

strongly correlated results [$\hat{Y} = -0.1527 + 1.0367x$; $r = 0.996$; $n = 104$; p -value ($\beta_0 = 0$): 0.497; p -value ($\beta_1 = 1$): 0.115], although only the 3:1 v v⁻¹ ratio was found accurate (Table 1). Besides showing accurate results, the 3:1 v v⁻¹ ratio with sodium molybdate in one-step digestion is also advantageous over 2:1 v v⁻¹ due to lower cost of nitric acid when compared to perchloric acid.

Chromium contents in cattle feces are accurately estimated through atomic absorption spectrophotometry, using digestion techniques based on nitric and perchloric acids, at the ratio of 3:1 v v⁻¹, in one-step digestion with sodium molybdate as a catalyst.

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