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Evaluation of chromium concentration in cattle feces using different acid digestion and spectrophotometric quantification techniques

[*Avaliação da concentração de cromo em fezes de bovinos utilizando diferentes técnicas de digestão ácida e de quantificação espectrofotométrica*]

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ABSTRACT

The objective of this work was to evaluate combinations between acid digestion techniques and spectrophotometric quantification to measure chromium concentration in cattle feces. Digestion techniques were evaluated based on the use of nitric and perchloric acids, sulfuric and perchloric acids, and phosphoric acid. The chromium quantification in the solutions was performed by colorimetry and by atomic absorption spectrophotometry (AAS). When AAS was used, the addition of calcium chloride to the solutions as a releasing agent was also evaluated. Several standard samples containing known chromium contents were produced (0, 2, 4, 6, 8 and 10g of chromium per kg of feces) using cattle feces obtained from three different animals to evaluate the accuracy of the different combinations of techniques. The accuracy was evaluated by adjusting a simple linear regression model of the estimated values on the actual values of chromium content in the standard samples. Regardless of the digestion technique, the chromium content estimates in the standard samples obtained by colorimetry were not accurate ($P<0.05$). Considering the AAS quantification, the digestion techniques based on nitric and perchloric acids and based on sulfuric and perchloric acids provided complete chromium recovery ($P>0.05$). The use of the digestion technique in phosphoric acid provided incomplete recovery of the fecal chromium ($P<0.05$). Subsequently, the digestion techniques in nitric and perchloric acids and digestion in sulfuric and perchloric acids, both evaluated by AAS, were compared using 84 cattle feces samples. The results indicate that these techniques provide similar ($P>0.05$) fecal chromium contents.

Keywords: atomic absorption, colorimetry, external markers, chromic oxide

RESUMO

Objetivou-se avaliar combinações entre técnicas de digestão ácida e quantificação espectrofotométrica para estimar a concentração de cromo em amostras de fezes bovinas. Foram avaliadas técnicas de digestão baseadas na utilização de ácidos nítrico e perclórico, ácidos sulfúrico e perclórico e ácido fosfórico. A quantificação da concentração de cromo nas soluções foi realizada por colorimetria e por espectrofotometria de absorção atômica (EAA). Na quantificação por EAA, foi avaliada a adição de cloreto de cálcio como agente de liberação. Amostras-padrão contendo quantidades conhecidas de cromo foram produzidas (0, 2, 4, 6, 8 e 10g de cromo por kg de fezes) utilizando-se fezes bovinas obtidas de três animais diferentes, para avaliar a acurácia das diferentes técnicas. A acurácia foi avaliada pelo ajustamento de modelo de regressão linear simples dos valores estimados sobre os valores reais de cromo nas amostras-padrão. Independentemente da técnica de digestão ácida, as estimativas da concentração de cromo nas amostras-padrão obtidas por colorimetria não foram acuradas ($P<0,05$). Considerando-se a quantificação de cromo por EAA, as técnicas de digestão baseadas nos ácidos nítrico e perclórico e nos ácidos sulfúrico e perclórico promoveram completa recuperação de cromo ($P>0,05$).

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A utilização da técnica de digestão em ácido fosfórico promoveu recuperação incompleta do cromo fecal (P<0,05). Posteriormente, as técnicas de digestão em ácidos nítrico e perclórico e em ácidos sulfúrico e perclórico, ambas avaliadas por EAA, foram comparadas utilizando-se 84 amostras de fezes bovinas. Os resultados indicam que aquelas combinações de técnicas promovem resultados similares (P>0,05) da concentração fecal de cromo.

Palavras chave: absorção atômica, colorimetria, indicadores externos, óxido crômico

INTRODUCTION

Several chemical elements, either in salt or oxide form, can be used as external markers in digestion assays with ruminant animals. Among these, we can highlight: ytterbium, erbium, europium, cobalt, cadmium, lanthanum, gold, cerium, and chromium. The latter element, noticeably in the chromic oxide form (Cr_2O_3), is the most widely used external marker applied for the quantification of fecal excretion of feedlot or grazing cattle. Such peculiarity is mainly based on the fact that chromic oxide is easily added to the diet, and presents soft working and low cost evaluation methods (Detmann *et al.*, 2004).

Among the ideal characteristics of a marker, the capacity of the marker to be completely recovered in feces (Owens and Hanson, 1992) or any segment of the digestive tract (Valente *et al.*, 2011) can be emphasized. The lack of this characteristic can result in biased estimates of digesta flow or fecal excretion. Although the recovery capacity is theoretically inherent to the marker (Detmann *et al.*, 2007), indirect influences from the methods applied to estimate its concentration may result in apparent recovery deviations (Valente *et al.*, 2011).

Several methods to evaluate the chromium content in fecal samples can be found in literature. Generally, such methods are based on the combination of two different techniques. The first one is used to eliminate the organic matter of the sample and leave the chromium in a chemical form which can be quantified by the second technique, which is based on the use of spectrophotometric quantification.

The first technique is carried out using digestion in acids, which may be preceded by ashing at high temperatures. The acid digestion of samples changes the chromium valence from +3 (sesquioxide) to +6 (dichromate). From this, the element becomes easily quantifiable. In the digestion procedure several acids or acid combinations can be employed, highlighting the digestion in a mixture of nitric and perchloric acids (Kimura and Miller, 1957) in a phosphoric acid solution (Willians *et al.*, 1962), and in a mixture of sulfuric and perchloric acids (Fenton and Fenton, 1979).

The chromium quantification may be performed using atomic absorption spectrophotometry (AAS) or colorimetry. Nevertheless, some authors reported that AAS quantification of chromium could present interferences caused by some elements such as silicon, aluminum, and iron. Considering that type of chemical interference, it would become necessary to use releasing agents to ensure accuracy in the quantification procedures (Williams *et al.*, 1962).

However, the accuracy of the procedures to estimate fecal chromium content depends on the individual accuracy of both acid digestion and spectrophotometric quantification techniques. Therefore, studies involving the simultaneous evaluation of both techniques are demanded.

The objective of this study was to evaluate the combinations of different acid digestion and spectrophotometric quantification techniques on the accuracy of chromium content estimates in cattle feces samples.

MATERIAL AND METHODS

Three acid digestion procedures were evaluated: digestion using nitric and perchloric acids (Kimura and Miller, 1957), digestion using sulfuric and perchloric acids (Fenton and Fenton, 1979), and digestion using phosphoric acid (Williams *et al.*, 1962). The spectrophotometric quantifications were carried out using colorimetric or AAS evaluations. In the quantification based on AAS the addition of calcium chloride ($CaCl_2$) as a releasing agent in the solutions (Williams *et al.*, 1962) was also accomplished.

Several standard samples containing known chromium contents were produced using cattle feces (organic matrix) obtained from three different animals (one growing heifer, one non-lactating dairy cow and one lactating dairy cow) to evaluate the accuracy of the different techniques. The animals were fed with corn silage based diets containing different concentrate levels and none had received chromium in the diet or as an external marker. The fecal samples were collected on the same day, oven-dried (60°C) and processed in a knife mill (1-mm). From each organic matrix, six different standards were produced containing 0, 2, 4, 6, 8 and 10g of chromium per kg of feces, totalizing 18 standard samples. The standard concentrations were produced on an as-is basis to avoid the accumulation of error from the estimation of the total dry matter content (Mertens, 2003). Pure chromic oxide (Cr_2O_3; 99.9% trace metals basis; Sigma-Aldrich 203068) was employed to produce the standards.

All standards (combinations between organic matrix and chromium concentrations) were evaluated in duplicate using every combination of acid digestion and spectrophotometric quantification techniques.

To perform the digestion using nitric and perchloric acids, approximately 250mg of the standards were poured into glass tubes. After that, 5mL of the digestion solution (a mixture of nitric acid and perchloric acids at the ratio of 2:1v/v and containing sodium molybdate at 1g/L) were added. The tubes were then heated at 200°C until the appearance of a yellowish color that indicated the complete digestion of the organic matter and the change of chromium valence from +3 (sexquioxide) to +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 50mL using de-ionized water. Aliquot of the solutions were transferred to polyethylene flasks and kept cooled (4°C).

Two digestion sets were performed according to descriptions above. The first one was performed just as previously described. In the second digestion set, 6.25mL of a calcium chloride solution ($CaCl_2.2\ H_2O$ P.A.; 4g of calcium per liter; Williams *et al.*, 1962) were previously added to the volumetric flasks before the transference. The solutions containing calcium chloride were evaluated by AAS. On the other hand, the solutions produced without adding calcium chloride were evaluated by both AAS and colorimetry.

To perform the colorimetry evaluations a stock solution containing 1000ppm of chromium was produced using potassium dichromate ($K_2Cr_2O_7$; purity 99%; Vetec 270). The stock solution was then diluted to obtain solutions containing 0, 50, 100, 150, and 200ppm of chromium. Those standard solutions were used to generate the standard curve. The colorimetric evaluations were carried out at 440nm in a spectrophotometer UV/Visible BEL Photonics 2000 UV.

In the AAS, standard solutions containing 0, 2, 4, 6, 8, and 10 ppm of chromium were used. Those solutions were produced from a stock solution containing 1000ppm of chromium (Merk 1.09948 Tritisol®). The samples were evaluated in the spectrophotometer GBC Avanta Σ, using a hollow-cathode lamp (357.9nm) and a nitrous oxide-acetylene flame.

To perform the digestion in sulfuric and perchloric acids, approximately 1g of the standard samples was poured into 25mL erlenmeyer flasks and ashed at 600°C for 4 hours. After cooling at room temperature, 15mL of the solution formed by de-ionized water, sulfuric acid and perchloric acid at the ratio of 0.75:0.75:1 (v/v/v), respectively, were added. That solution also contained 20g/L of sodium molybdate. The erlenmeyer flasks were covered with watch-glasses and kept on a sand-bath at 300°C until developing a yellowish or reddish color. After cooling at room temperature, it was preceded to the quantitative transfer to 100-mL volumetric flasks. The transfer was performed using ash-free quantitative filter paper (Whatman #41). The volume of the solutions was made up to 100mL with de-ionized water. Aliquots from the solutions were poured into polyethylene flasks and kept cooled (4°C).

The calcium chloride addition to the samples and the colorimetric and AAS procedures were performed such as previously described.

To perform the digestion of standard samples in phosphoric acid, approximately 1g of each standard sample was poured into 25-mL erlenmeyer flasks and ashed at 600°C for 4 hours. After cooling at room temperature, 3mL of the digestion solution were added [phosphoric acid 85% (1L) plus a manganese sulphate solution (30mL of a 100g/L solution of $MnSO_4.4H_2O$) and 4mL of potassium bromate solution ($KBrO_3$; 45g/L)]. The erlenmeyer flasks were then covered with watch-glasses and digested on a sand-bath at 300°C until developing a purple color. Then, samples were cooled at room temperature and quantitatively transferred and stored as described before.

The addition of calcium chloride to the samples was carried out as described above. All standard samples, with or without the inclusion of calcium chloride, were evaluated only by AAS as previously described.

The accuracy of techniques was evaluated by adjusting a simple linear regression equation of chromium concentrations estimated by each technique combination (dependent variable) on the actual concentrations of chromium in the standard samples (independent variable). The statistical analysis was conducted under the hypotheses:

$$H_0 : \beta_0 = 0 \text{ vs. } H_a : \beta_0 \neq 0 \quad (1),$$

$$H_0 : \beta_1 = 1 \text{ vs. } H_a : \beta_1 \neq 1 \quad (2).$$

The slope of the adjusted function must be interpreted as the recovery of chromium added in the standard samples. Additionally, the intercept should represent some kind of interference in the medium which could be originated from chemical interference, reagents 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 combination between acid digestion and spectrophotometric quantification that were found accurate were then used to evaluate the chromium concentration in 84 feces samples obtained from feedlot cattle fed with diets based on corn silage, elephant grass silage, or signal grass hay, and containing 0 or 200g of concentrate per kg of dry matter. During the digestibility assay the animals received 10g of Cr_2O_3 per day to evaluate the daily fecal excretion (Sampaio *et al.*, 2011).

The feces samples were oven-dried at 60°C and processed in a knife mill (1-mm). After that, all samples were evaluated in duplicate with regard to chromium content.

The estimates of chromium content obtained by accurate combinations were compared to each other by adjusting a simple linear regression equation, considering both null hypotheses previously presented. The techniques were considered to be similar when both null hypotheses were not rejected.

All statistical procedures were carried out using the PROC REG of SAS (*Statistical Analysis System*; version 9.1) and adopting $\alpha = 0.05$.

RESULTS

Regardless of the digestion technique, the estimates of chromium content in the standard samples obtained by colorimetry were lower than the actual chromium contents (Table 1).

There were no interferences in the medium for any technique combinations. This can be affirmed because none of the intercept estimates were found to be different from zero ($P>0.05$; Table 2). On the other hand, the chromium recovery was found incomplete when colorimetric quantification was used ($P<0.05$; Table 2; Figure 1 and 2).

Considering the AAS quantification, the digestion techniques based on nitric and perchloric acids and based on sulfuric and perchloric acids provided complete recovery of chromium ($P>0.05$; Table 2; Figure 1 and 2). In these circumstances, the addition of calcium as a releasing agent did not influence the accuracy of the estimates (Table 2).

Table 1. Average chromium contents in the standard samples obtained by different combinations of techniques

Combinations			Standards (g chromium/kg sample)					
D[1]	Q[2]	Ca[3]	0	2	4	6	8	10
NP	C	-	0.00±0.03	1.41±0.11	2.82±0.27	4.64±0.15	6.55±0.28	8.56±0.09
NP	AA	-	0.00±0.00	1.24±0.48	3.62±0,02	4.46±1.36	9.01±0.03	10.64±0.24
NP	AA	+	0.00±0.00	1.63±0.16	3.13±0.02	4.72±0.26	8.31±0.34	9.21±0.04
SP	C	-	0.31±0.23	0.85±0.03	1.66±0.07	2.33±0.05	3.43±0.07	3.87±0.15
SP	AA	-	0.00±0.00	2.01±0.06	4.14±0.17	6.24±0.09	9.5±0.15	9.43±0.13
SP	AA	+	0.00±0.00	1.66±0.10	3.53±0.15	5.43±0.12	8.56±0.21	9.07±0.10
P	AA	-	0.00±0.00	1.21±0.20	1.95±0.23	3.54±0.86	6.21±0.26	5.53±0.81
P	AA	+	0.02±0.02	1.71±0.05	4.05±0.03	6.34±0.09	9.99±0.92	12.37±0.36

[1]D, acid digestion technique: NP, nitric and perchloric acids; SP, sulfuric and perchloric acids and P, phosphoric acid. [2]Q, spectrophotometric quantification: C, colorimetry; and AA, atomic absorption. [3]Ca, use of calcium chloride as a releasing agent.

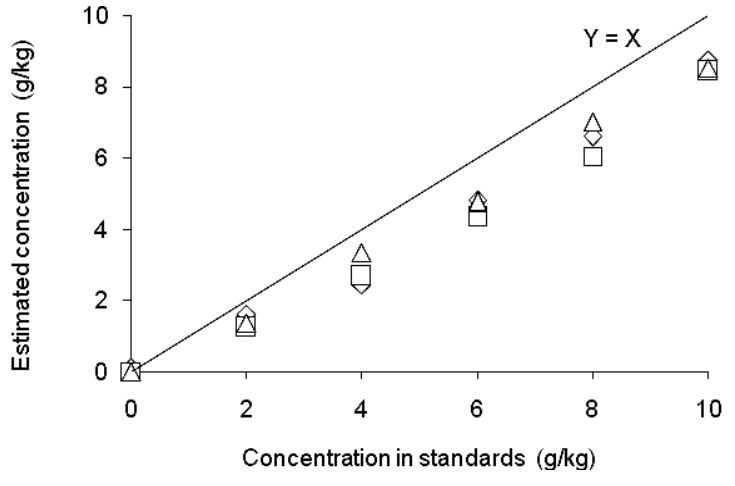
The use of a digestion technique based on phosphoric acid provided incomplete recovery of the fecal chromium (P<0.05; Table 2; Figure 3). In these conditions, the addition of calcium chloride increased the chromium recovery, but still provided inaccurate estimates (P<0.05; Table 2; Figure 3). The evaluation of the estimates indicated that the use of calcium chloride provided an approximately complete chromium recovery of the fecal chromium up to 6g/kg. However, the recovery became higher than 1.0g/g when the samples containing 8 and 10g/kg were evaluated (Table 1; Figure 3).

Table 2. Estimates of linear regression parameters for the chromium concentration in the standards obtained by different technique combinations

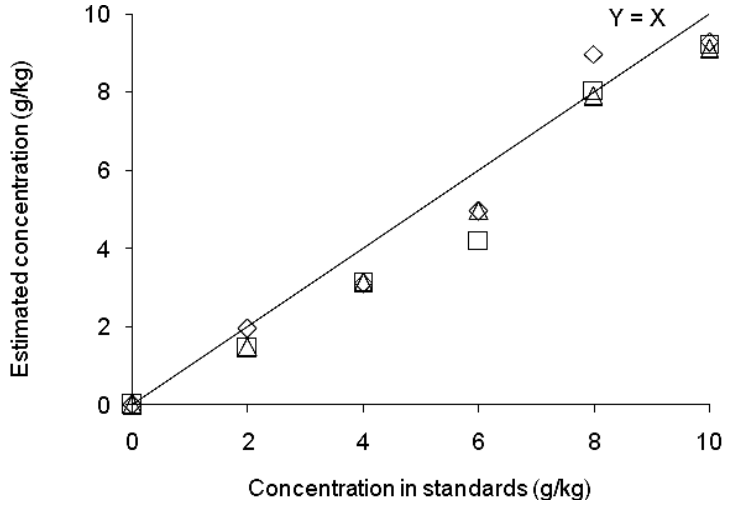
Combinations			Regression Parameter				P-Value	
D[1]	Q[2]	Ca[3]	Intercept	Slope	s_{XY}	r^2	H_0: $\beta_0 = 0$	H_0: $\beta_1 = 1$
NP	C	-	-0.278±0.156	0.853±0.026	0.37	0.986	0.093	<0.001
NP	AA	-	-0.692±0.511	1.103±0.084	1.22	0.914	0.195	0.241
NP	AA	+	-0.318±0.265	0.966±0.044	0.63	0.968	0.248	0.444
SP	C	-	0.203±0.096	0.374±0.016	0.23	0.972	0.051	<0.001
SP	AA	-	0.091±0.284	1.025±0.047	0.68	0.968	0.754	0.598
SP	AA	+	-0.141±0.219	0.970±0.036	0.53	0.978	0.529	0.427
P	AA	-	-0.089±0.419	0.633±0.069	1.00	0.839	0.835	<0.001
P	AA	+	-0.603±0.329	1.269±0.054	0.79	0.971	0.086	0.001

[1]D, acid digestion technique; NP, nitric and perchloric acids; SP, sulfuric and perchloric acids and P, phosphoric acid.[2]Q, spectrophotometric quantification; C, colorimetry; and AA, atomic absorption.[3]Ca, use of calcium chloride as a releasing agent.

From these results, the fecal samples obtained from the digestion trial were evaluated using the digestion techniques based on nitric and perchloric acids and based on sulfuric and perchloric acids, both considering the quantification by AAS (Table 2). Calcium chloride was not used because it did not improve the accuracy of the results (Table 2) and its omission makes the analytical procedures simpler. Considering this, it was verified that both technique combinations provided similar results (P>0.05) and were strongly correlated (r = 0.970; P<0.05; Figure 4).



(a)



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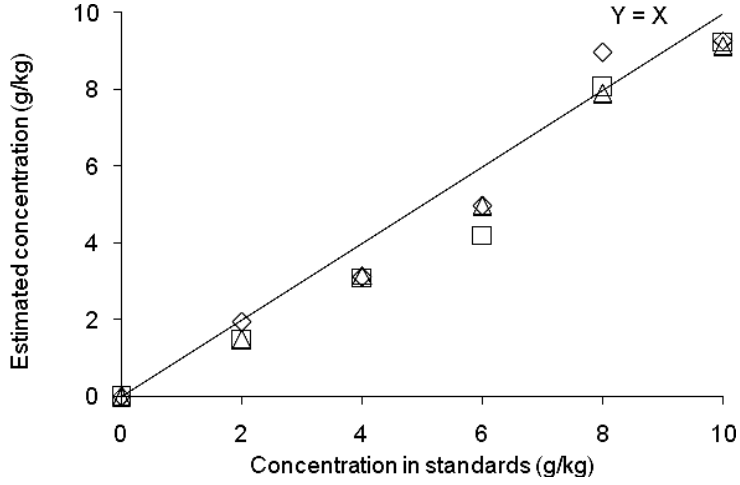
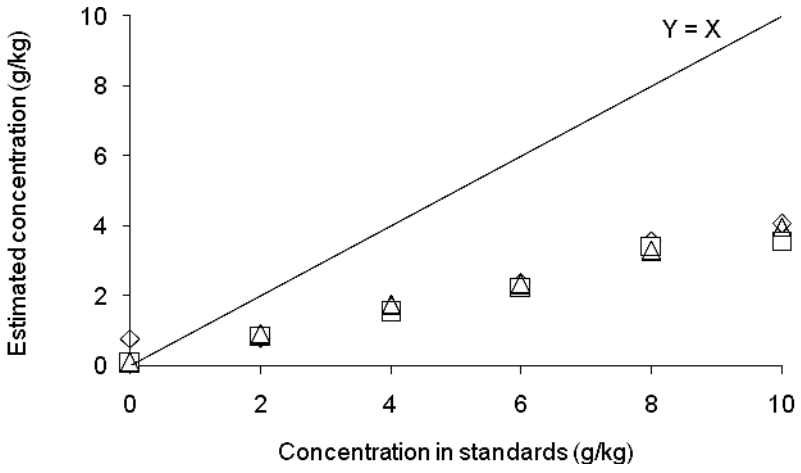
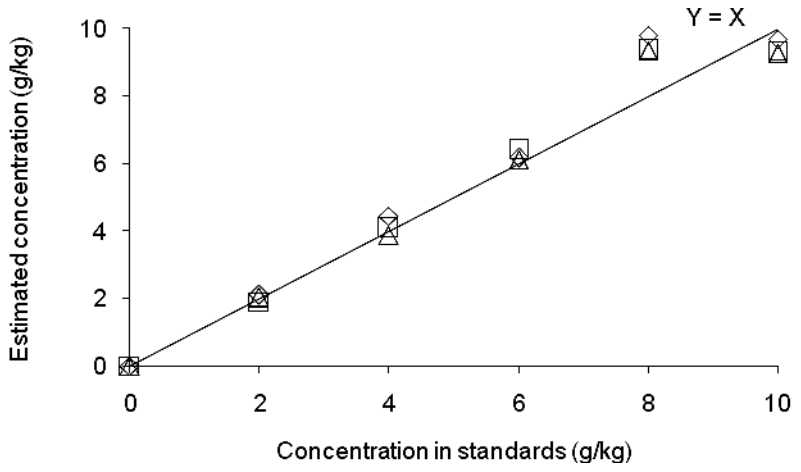


(c)

Figure 1. Relationship between chromium concentrations estimated using the digestion technique in nitric and perchloric acids and the actual concentrations of chromium in standards (a, colorimetry; b, AAS without calcium chloride; c, AAS with calcium chloride; ◇, animal 1; □, animal 2; △, animal 3).



(a)



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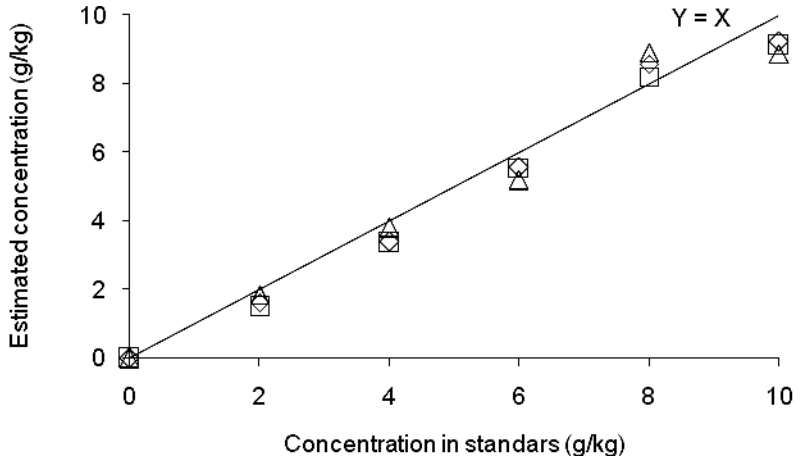

(c)

Figure 2. Relationship between chromium concentrations estimated using the digestion technique in sulfuric and perchloric acids and the actual concentrations of chromium in standards (a, colorimetry; b, AAS without calcium chloride; c, AAS with calcium chloride; ◇, animal 1; □, animal 2; △, animal 3).

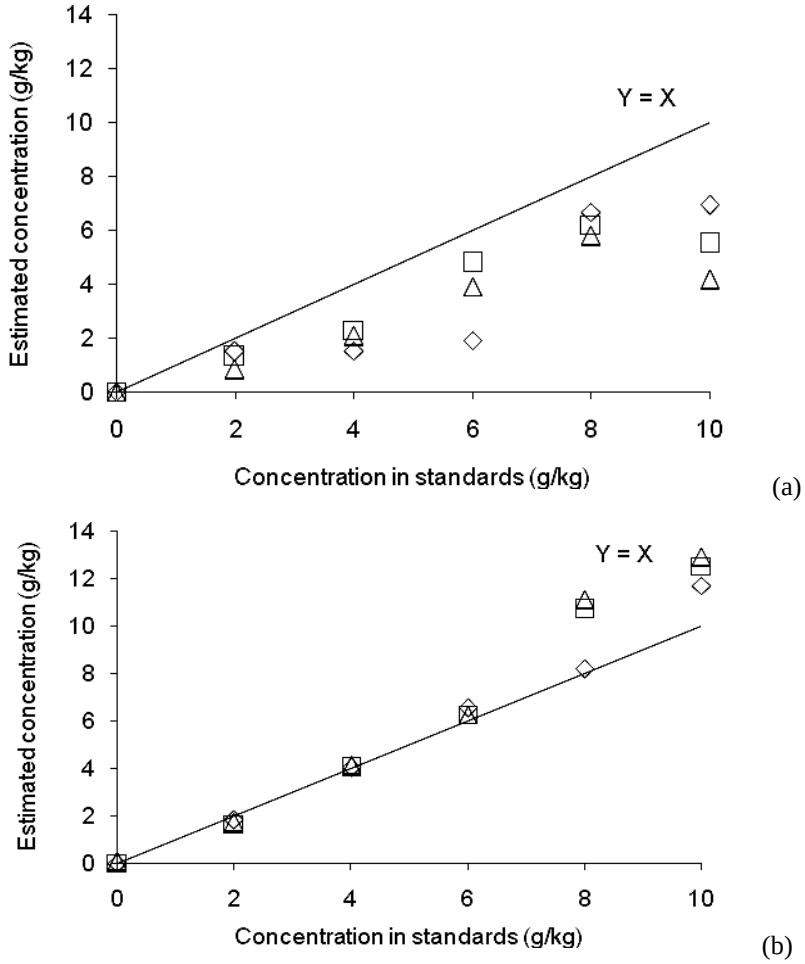

Figure 3. Relationship between the chromium concentration estimated using the digestion technique in phosphoric acid and the real concentrations of chromium in standards (a, AAS without calcium chloride; b, AAS with calcium chloride; ◇, animal 1; □, animal 2; △, animal 3).

DISCUSSION

The lack of accuracy of the chromium contents quantified by colorimetry seems to be due to the lack of sensibility of the technique to detect the compounds containing chromium in the medium (Table 2; Figure 1 and 2).

Conversely, Rodrigues *et al.* (2010) evaluated the chromium content in feces of sheep using colorimetry quantification and obtained accurate results. Those authors used the method suggested by Graner (1972), who indicated the use of the 1.5-diphenylcarbazide which reacts with the chromium oxide and produces a red/purple compound that presents high absorptivity. Such modification can increase the sensibility of chromium detection by colorimetry.

Bremer Neto *et al.* (2005**)** redefined the Graner (1972) method, specifically for fecal chromium evaluation. Those authors did not verify differences between the chromium content estimated by colorimetry using the 1.5-diphenylcarbazide and the chromium content estimated by AAS.

In this study, the 1.5-diphenylcarbazide was not used because the original colorimetric methods here evaluated (Kimura and Miller, 1957; Fenton and Fenton, 1979) do not recommend the use of this substance in the chromium content evaluation.

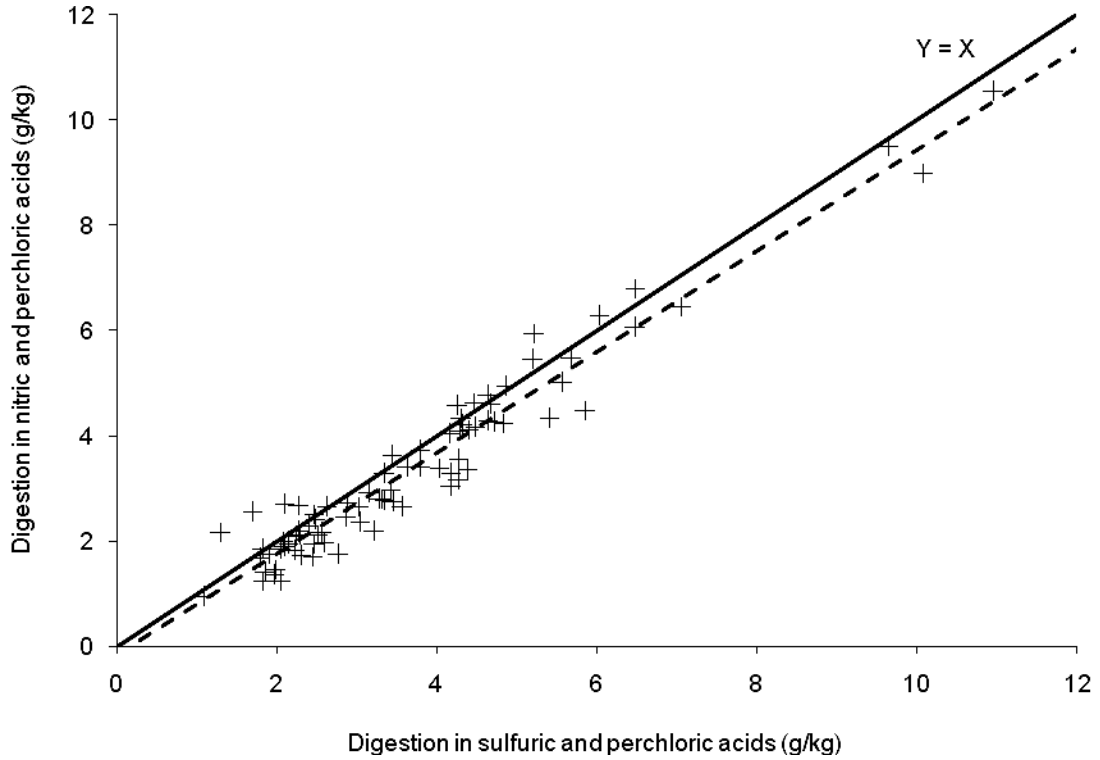

Figure 4. Relationship between the fecal chromium concentrations obtained with the digestion technique in nitric and perchloric acids and in sulfuric and perchloric acids, both associated with AAS without calcium addition ($\hat{Y} = -0.1673 + 0.9602 \times X$; $r^2 = 0.941$; n = 84).

The addition of calcium as a releasing agent in AAS quantification was suggested by Williams *et al.* (1962), who used the phosphoric acid digestion. According to these authors, several compounds or elements, as silicates, iron, aluminum, and others, can form refractory compounds with chromium during the burning of the solution in the spectrophotometer. Such compounds would be not readily dissociated at the flame temperatures, which could decrease the accuracy of the chromium quantification. These interferences could be suppressed by the calcium chloride addition to the test solutions. The calcium would bind with the interfering ions and these ions could not form refractory compounds with the chromium, leaving it free to be quantified.

The digestion techniques based on nitric and perchloric acids and based on sulfuric and perchloric acids provided accurate results regardless of calcium chloride addition in the solutions. In other words, under these digestion conditions the addition of calcium was not able to improve the accuracy of chromium content using ASS, which indicates that in the presence of these acids there are no chemical interferences, such as mentioned above (Table 2; Figure 1 and 2).

On the other hand, the digestion technique in phosphoric acid (Williams *et al.*, 1962) did not propitiate accurate chromium content even considering the lower bias when calcium chloride was added (Table 2; Figure 3). A hypothesis for apparent improvement caused by calcium is that interference in the chromium reading by AAS can be due to the acid type used in the sample digestion and not by the presence of specific ions such as silicates. After the acid digestion, some anions such as phosphates (originated form phosphoric acid) can be present in solutions and they could cause interferences in the nitrous oxide-acetylene flame, and this could affect the chromium concentration obtained by AAS (Sahuquillo *et al.*, 1995). The main interferences in the nitrous oxide-acetylene flame

described in literature refer to the acid matrix and cations (Rubio *et al.*, 1991).

Therefore, the lack of accuracy obtained with digestion in phosphoric acid could be attributed to the interferences of this acid on the formation of elemental chromium in the nitrous oxide-acetylene flame, whereas the other techniques evaluated by AAS, using nitric and perchloric acids and sulfuric and perchloric acids, presents accurate results, regardless of the addition of calcium to the solutions.

On the other hand, the lack of accuracy of the phosphoric acid digestion seems to also be caused by its low efficiency to quantify high contents of chromium in fecal samples. When calcium chloride was added, accurate results were obtained with chromium contents up to 6g/kg. There was overestimation of chromium with higher contents (Table 1; Figure 3). In the study of Williams *et al.* (1962), the chromium concentrations evaluated were not higher than 4g/kg. In other words, the satisfactory results obtained by those authors can be due to the use of chromium concentrations lower than 6g/kg feces. Thus, the accuracy of the Williams *et al.* (1962) method would be assured if, and only if, low chromium content samples are evaluated. It seems to be a limitation of this method because several factors can influence fecal chromium contents (e.g., daily dose of chromium, size of the animal, feed intake) and some kind of bias could occur only in some samples (and not in all samples) and the overall results of the experiment would be distorted.

The digestion procedures based on nitric and perchloric acids and on sulfuric and perchloric acids, both associated with AAS quantification, presented accurate results (Table 2) and were found similar each other (Figure 4). Considering this, the choice of a particular method should be based on secondary characteristics, such as analytical costs and labor. The digestion based on sulfuric and perchloric acids is more time and labor consuming compared to digestion using nitric and perchloric acids because it demands an ashing step (Fenton and Fenton, 1979). Thus, considering the secondary characteristics, the digestion procedure based on nitric and percloric acids and using AAS seems to be a more realistic method to quantify chromium content in cattle feces.

CONCLUSIONS

The chromium content in cattle feces are accurately evaluated using digestion procedures based on in nitric and perchloric acids or based on sulfuric and perchloric acids, both associated with quantification by atomic absorption spectrophotometry.

REFERENCES

BREMER NETO, H.; GRANER, C.A.F.; PEZZATO, L.E. *et al.* Determinação de rotina do crômio em fezes, como marcador biológico, pelo método espectrofotométrico ajustado da 1,5-difenilcarbazida. *Cienc. Rural*, v.35, p.691-697, 2005.

DETMANN, E.; VALADARES FILHO, S.C.; PAULINO, M.F. *et al.* Avaliação da técnica dos indicadores na estimação do consumo por ruminantes em pastejo. *Cad. Tec. Vet. Zootec.*, v.46, p.40-57, 2004.

DETMANN, E.; SOUZA, A.L.; GARCIA, R. *et al.* Avaliação do vício de "tempo longo" de indicadores internos em ensaio de digestão com ruminantes. *Arq. Bras. Med. Vet. Zootec.*, v.59, p.182-188, 2007.

FENTON, T.W.; FENTON, M. An improved procedure for the determination of chromic oxide in feed and feces. *Can. J. Anim. Sci.*, v.59, p.631-634, 1979.

GRANER, C.A.F. *Determinação do crômio pelo método colorimétrico da s-difenilcarbazida*. 1972. 112f. Tese (Doutorado em Ciências) – Faculdade de Ciências Médicas e Biológicas, Universidade Estadual Paulista "Júlio de Mesquita", Botucatu.

KIMURA, F.T.; MILLER, V.L. Improved determination of chromic oxide in cow feed and feces. *J. Agric. Food Chemist.*, v.5, p.216-216, 1957.

MERTENS, D.R. Challenges in measuring insoluble dietary fiber. *J. Anim. Sci.*, v.81, p.3233-3249, 2003

OWENS, F.N.; HANSON, C.F. External and internal markers for appraising site and extent of digestion in ruminants. *J. Dairy Sci.*, v.75, p.2605-2617, 1992.

RODRIGUES, P.H.M.; GOMES, R.C.; SIQUEIRA, R.F. *et al.* Acurácia, precisão e robustez das estimativas da digestibilidade aparente da matéria seca determinada com o uso de indicadores em ovinos. *Rev. Bras. Zootec.*, v.39, p.1118-1126, 2010.

RUBIO, R.; SAHUQUILLO, A.; RAURET, G. Determination of chromium in environmental and biological samples by atomic absorption spectroscopy: a review. *Anal. Chem.*, v.47, p.99-128, 1991.

SAHUQUILLO, A.; RUBIO, R.; RAURET, G. *et al.* Determination of total chromium in sediments by FAAS. *Anal. Chem.*, v.352, p.572-576, 1995.

SAMPAIO, C.B.; DETMANN, E.; VALENTE, T.N.P. *et al.* Evaluation of fecal recovering and long term bias of internal and external markers in a digestion assay with cattle. *Rev. Bras. Zootec.*, v.40, p.174-182, 2011.

VALENTE, T.N.P.; DETMANN, E.; VALADARES FILHO, S.C. *et al. In situ* estimation of indigestible compounds contents in cattle feed and feces using bags made from different textiles. *Rev. Bras. Zootec.*, v.40, p.666-675, 2011.

WILLIAMS, C.H.; DAVID, D.J.; IISMAA, O. The determination of chromic oxide in faeces samples by atomic absorption spectrophotometry. *J. Agric. Sci.*, v.59, p.381-385, 1962.