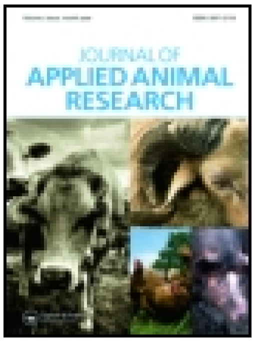

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Evaluation of chromium oxide and titanium dioxide as inert markers for calculating apparent digestibility in sheep

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ABSTRACT
The objective of the present study was to evaluate two markers: chromium oxide (Cr_2O_3) and titanium dioxide (TiO_2). We evaluate the interaction between Cr_2O_3 and TiO_2, and the techniques used to determine it, using atomic absorption spectrophotometry (AAS) and photometry simple (PS). We used six growing sheep distributed in a replicated Latin square 3×3 design, with adjustment for the residual error effect. The TiO_2 and Cr_2O_3–TiO_2 produced values similar to those obtained by total faecal collection (TFC) or the use of Cr_2O_3 alone, determined by AAS and PS. Digestibility of the marker/TFC ratio was similar ($p > .05$) between markers and technique. The use of TiO_2 alone or in combination with Cr_2O_3 seems to be a suitable alternative to TFC and Cr_2O_3 to calculate apparent digestibility of the total digestive tract determined in sheep by PS and AAS.

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Chromium oxide; digestibility; markers; sheep; spectrophotometry; titanium dioxide

1. Introduction

Chromium oxide (Cr_2O_3) has been widely used as an external digestibility marker in digestion trials with ruminants (Amorocho et al. 2009; Delagarde et al. 2010; Al Alami et al. 2014). The recovery of this marker in ruminants' fed forage has revealed variations in faecal recovery (Titgemeyer et al. 2001); on the other hand, the use Cr_2O_3 is restricted in diets for animals because it possesses carcinogenic properties (Peddie et al. 1982; Sedman et al. 2006). Titanium dioxide (TiO_2) is presented as an alternative, but its study in small ruminants is scarce (Titgemeyer et al. 2001; Glindeman et al. 2009). Additionally, the combined use of these markers *in vivo* presented differences in digestibility coefficients but had similar recoveries (Kavanagh et al. 2001; Titgemeyer et al. 2001).

Several analyses for these markers differ for colorimetric and spectroscopy techniques, the latter being the most commonly recommended for the least amount of interference present (AAFCO 2004). However, *in vivo* studies of these two procedures showed no differences (Carciofi et al. 2007). For TiO_2 we do not know whether there are differences between these two methods: colorimetric and spectroscopy.

The aim of this study was to evaluate the use of Cr_2O_3 and TiO_2 as digestibility markers, as well as their interaction $Cr_2O_3 + TiO_2$, as determined by atomic absorption spectrophotometry (AAS) and colorimetric methods, and to compare digestibility coefficients obtained from total faecal collection (TFC) in sheep.

2. Material and methods

The Institutional Committee for Care and Use of Experimental Animals (CICUAE) approved all procedures of the National Autonomous University of Mexico. Six Pelibuey lambs (mean ± SD) (LW 40.7 ± 5.8 kg) were used to assess recovery of markers: Cr_2O_3 (Prince Minerals, Inc. New York, USA, 1308-31-2; 100% Purity), TiO_2 (Fisher Scientific. Pittsburgh, PA, USA, T315-500; >98% Purity) and the combination $Cr_2O_3 + TiO_2$. Food intake as dry matter (DM) was restricted to 2.2% of live weight and there were no feed refusals. The diet (10% CP, 11 MJ ME/kg DM; Table 1) was supplemented with 0.4% of the marker (Cr_2O_3, TiO_2 and the combined mixture) and was given at 10:00 and 22:00 h daily. Every day the food was mixed with each marker before being provided to the animals. The animals were housed in metabolic cages, with a harness to collect faeces individually.

Each experimental period lasted 25 d, and consisted of 20 d for diet adaptation and 5 d for data and sample collection. The sample faeces were collected at 09:00 h, weighed and separated at 10% of total, and mixed to obtain a composite sample for each animal per period and frozen (−20°C) for further analysis.

Feed and faeces samples were analysed for DM in a forced air oven (Lindbergh Blue M) at 50°C, 48 h; ash concentration at 660°C, 3 h; organic matter (OM) was determined by difference (AOAC, 1990); neutral detergent fiber (NDF) in feed samples was analysed according to Van Soest et al. (1991) with alpha amylase and uncorrected for ash, and N content

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Table 1. Ingredients and chemical composition (g/kg DM) in the diet using Cr_2O_3, TiO_2 and their interaction used in growing lambs.

Ingredients (g/kg DM)	Cr_2O_3	TiO_2	$Cr_2O_3 + TiO_2$
Sorghum grain	720.0	720.0	720.0
Alfalfa hay	61.8	61.8	61.8
Oat hay	123.5	123.5	123.5
Molasses	54.6	54.6	54.6
Urea	10.0	10.0	10.0
Calcium, 38%	11.5	11.5	11.5
Phosphate 18/20	2.0	2.0	2.0
Magnesium oxide	2.1	2.1	2.1
Na Bicarbonate	5.0	5.0	5.0
NaCl	5.0	5.0	5.0
Minerals mix[a]	0.5	0.5	0.5
Cr_2O_3	4.0	ND[b]	4.0
TiO_2[b]	ND	4.0	4.0
Chemical composition			
DM	917	921	919
OM	871	870	871
NDF	166	163	164
N	15	15	15

[a]Minerals mix: Ca 15.0%, P 8.0%, Mg 0.5%, K 0.035%, S 0.2%, Fe 450 mg/kg, Zn 1900 mg/kg, Mn 1800 mg/kg, Se 15 mg/kg, I 30 mg/kg, Co 25 mg/kg.
[b]ND, no data; the marker is not part of the diet.

by the Kjeldahl method (AOAC 1990); chromium oxide suspension was performed according to Hill and Anderson (1958), and titanium dioxide according to Titgemeyer et al. (2001). Both were analysed through a photometry simple (PS) (Thermo Fisher model Scientyfic Genesys 10 Vis) with a wavelength of 430 nm for Cr_2O_3 and 410 nm for TiO_2; an AAS (Perkin Elmer 3110) was used to determine Cr_2O_3 and TiO_2 with a Perkin Elmer lamp for Cr (part#303-6021 Serial H235571) and Ti (part#303-6075 Serial H167419) for each marker, respectively. The calibration curve was performed with standard solutions for Cr_2O_3 PS with 0.05, 0.01, 0.15 and 0.5 mg/ml ($y_{mg/ml} = 0.481x_{(OD)} - 0.006$; $R^2 = 0.99$), and 20, 50 and 100 ppm for AAS ($y_{ppm} = 206.19x_{(ABS)} - 3.7113$; $R^2 = 0.99$) (standard for AAS from J.T. Baker 1000 µg/ml CAS 6449-04 for Cr), as for TiO_2 PS with 0.005, 0.01, 0.075, 0.15 and 0.2 mg/ml ($y_{mg/ml} = 0.3226x_{(OD)} - 0.008$; $R^2 = 0.98$) and for AAS 10, 20 and 40 ppm ($y_{ppm} = 0.175.6x_{(ABS)} + 1.5$; $R^2 = 0.99$) (standard for AAS from J.T. Baker 1000 µg/ml CAS 6472-04 for Ti), where ABS is the absorption wavelength value and OD is the optical density value. The combinations of $Cr_2O_3 + TiO_2$ differ in that the two markers are mixed, but we only suspended and analysed one of the compounds.

The proportion of DM and OM degraded was calculated using the following equation:

$$\text{Digestibility}\left(\frac{g}{g}\right) = \frac{(\text{intake } x - \text{excretion } x)}{\text{intake } x}, \quad (1)$$

where intake and excretion are expressed in g/d, and x represents DM and OM content, respectively.

The recovery of the markers was determined using the following equation:

$$\text{Marker recovery (\%)} = \frac{\text{total faecal excretion (DM, g/d) with marker}}{\text{total faecal excretion (DM, g/d) using TFC (g/d)}} \times 100. \quad (2)$$

The faecal excretions of chromium and titanium dioxide were estimated by multiplying the total DM excretion by the marker content in the representative faecal sample, according to the following equation:

$$\text{MFE} = \text{FM} \times [\text{M}]_{(RS)}, \quad (3)$$

where MFE is the faecal excretion of marker (kg/d), FM is the faecal mass obtained by total collection (g/d) and $M_{(RS)}$ is the marker content in the representative faecal sample (g/kg).

From the assumptions of Equation (3), the faecal recovery of the markers was calculated as the ratio of faecal excretion to the intake of marker, described as:

$$\text{FR} = \left[\frac{\text{MFE}}{D}\right] \times 100, \quad (4)$$

where FR is the faecal recovery of marker (%), MFE is the faecal excretion of marker (g/d) and D is the daily dose of external marker (g/d).

The faecal excretion was also determined by using the faecal content of markers according to the following equation:

$$\text{FE}_{RS} = \frac{D}{[\text{M}]_{RS}}, \quad (5)$$

where FE_{RS} is the faecal excretion estimated by using the marker content in the faecal representative sample (kg/d), D is the daily dose of external marker (g/d), and $[M]_{RS}$ is the marker content in the representative faecal sample (g/kg) (Lippke 2002). The values of total tract apparent digestibility in each treatment were subjected to analysis of variance using a design of two replicated Latin squares with adjustment for residual error, including three treatments in six animals. Treatment sequences within each Latin square were organized to balance the effects of carryover such that each treatment followed every other treatment one time within each square using the GLM procedure SAS (1999), following the model:

$$Y_{jkl} = \_\mu + P_j + A_k + T_l + \varepsilon_{ijk}, \quad (6)$$

where Y_{jkl} is the dependent variable, $\_\mu$ is the overall mean, P_j is the effect of period j, A_k is the effect of animal k, T_l is the effect of treatment l, and ε_{jkl} is the residual error.

Furthermore, in order to evaluate the technical values of total tract apparent digestibility and the digestibility of the markers/TFC ratio (DM and OM), a factorial arrangement 4×2 with six replicates for each one (Kuehl 2000) was performed using the statistical program Statistical Package for Social Sciences (SPSS, version 15.0, November 2006),

$$Y_{jk} = \_\mu + \text{M}_j + T_k + \text{M} \times T_{jk} + \varepsilon_{jk}, \quad (7)$$

where Y_{jk} is the dependent variable, $\_\mu$ is the overall mean, M_j is the effect of marker treatment j, T_k is the effect of determination technique k (PS vs. AAS), $M \times T_{jk}$ is the interaction jk, and ε_{jk} is the residual error. Differences between the means of the least squares were considered significant at $p < .05$, and differences were considered to indicate a trend towards significance at $.05 > p < .10$.

3. Results and discussion

Diets (g/kg DM) averaged 919 g/kg DM; OM, 871 g/kg DM; NDF, 165 g/kg DM, and N, 15 g/kg DM, consisting of the same amount of ingredients for each marker (Table 1).

The recovery percentage (Table 2) was similar between markers ($p > .05$), and the techniques used do not affect the TiO_2 determination (this may be due to the inclusion of the Cr_2O_3 level used in the present study). Therefore, it can be assumed that chromic oxide and titanium dioxide achieve the necessary requirement for an ideal marker, because faecal recovery of both markers seems to be unaffected by different feeding conditions. From this, assuming that there is total faecal recovery and there are no diet effects on recovery, both external markers can be presumed to be similar to each other. Thus, titanium dioxide can be assuredly used as a substitute for chromic oxide, which is more desirable because it has not been reported as a carcinogenic precursor.

Although the recovery of Cr_2O_3 in the presence of TiO_2 is the highest numerically (Table 2), no statistical difference was found ($p = .388$) from those markers analysed by PS and AAS. It remains unclear as to how the presence of Ti could improve the assessment of Cr recovery. The values differ from Titgmeyer et al. (2001), who recovered 112% of Cr_2O_3+ TiO_2, analysed by AAS using the technique proposed by Williams et al. (1962). In another study, Kavanagh et al. (2001) showed 96% recovery in pig diets adding Cr_2O_3; however, Jagger et al. (1992) obtained recoveries of 74% and 80% for diets supplemented with 1 g and 5 g Cr_2O_3/kg respectively, not in combination with TiO_2, using the colorimetric method of Fenton and Fenton (1979). Using the same technique, Carciofi et al. (2007) recovered 106 ± 0.044 and 101 ± 0.045% of Cr_2O_3 with AAS and PS, respectively. The recovery percentages differ from those of Jagger et al. (1992), who reported recoveries of uncombined Cr_2O_3 with TiO_2 of 98.3% and 96.9%, respectively, for additions of 1 g and 5 g/kg of marker; moreover, Kavanagh et al. (2001) found 92.3% recoveries with 1 g/kg when combined with Cr_2O_3; Titgmeyer et al. (2001) found recoveries of 95% adding 5 g TiO_2/kg DM. Hafez et al. (1988) obtained 99% recovery in concentrate-based diets and corn silage forage diets.

Table 2. Markers recovery percentage in faeces, using Cr_2O_3, TiO_2 and Cr_2O_3+TiO_2, estimated by PS and AAS.

Markers[a]	CrPS[b]	CrAAS[c]	TiPS[d]	TiAAS[e]
Cr_2O_3	140.8	138.3	ND[h]	ND
TiO_2	ND	ND	148.7	121.1
$Cr_2O_3 + TiO_2$	148.2	162.9	143.5	128.4
SEM[f]	8.0	6.5	8.3	10.8
p value[g]	.388			

[a]Markers, 0.4% Cr_2O_3, 0.4% TiO_2, 0.4% + 0.4% TiO_2 Cr_2O_3.
[b]Cr-PS: Cr_2O_3 determined by PS.
[c]Cr-AAS: Cr_2O_3 determined by AAS.
[d]Ti-PS: TiO_2 determined by PS.
[e]Ti-AAS: TiO_2 determined by atomic absorption spectrophotometry.
[f]SEM, standard error of the mean.
[g]*p* value: value of general significance.
[h]ND, no data; the marker is not part of the mixture.

Different methodologies for determining TiO_2 vary according to the use of substance for the wet samples. Leone (1973) recommended 10 ml of concentrated H_2SO_4, but Jagger et al. (1992) and Short et al. (1996) modified this technique, using twice as much H_2SO_4. Titgmeyer et al. (2001) modified this technique using 7.4 M H_2SO_4 and 10 ml of 30% H_2O_2. However, Myers et al. (2004) changed the use of the ashing procedure prior to the suspension, by the wet suspension of the sample with concentrated H_2SO_4. The wavelength used was different in each technique, varying from 400 nm by Short et al. (1996) and Kavanagh et al. (2001), to 408 nm by Jagger et al. (1992) and Leone (1973) and 410 nm by Titgmeyer et al. (2001) and Myers et al. (2004). The wavelength used in each technique may affect the sensitivity obtained for TiO_2. Myers et al. (2004) founded better recoveries when using 409 vs. 410 nm; in the present study TiO_2 was determined at 410 nm.

DM and OM intake were similar among treatments (Table 3), due to the feed intake being restricted to 2.2% of live weight; DM digestibility (g/d) was higher ($p < .05$) for the combination $TiO_2 + CrO_3$ than for either CrO_3 or TiO_2 alone. The OM digestibility showed a rising trend between markers ($p = .087$), being higher for the combination $TiO_2 + CrO_3$. The DM and OM digestibility (g/g) and the digestibility marker/TFC ratio were similar between markers ($p > .05$). DM digestibility (g/d) comparing PS vs. AAS showed a rising trend ($p = .062$), being higher (1.8%) for AAS than PS, and similar ($p > .05$) when data

Table 3. Intake (g DM/d), digestibility (g/g) and digestibility marker/TFC[1] ratio, obtained by Cr_2O_3, TiO_2 or combined determined by PS and AAS.

	Markers (M)[2]				Technique (T)			*p* value		
Item	Cr_2O_3	TiO_2	$Cr_2O_3 + TiO_2^3$	$TiO_2 + Cr_2O_3^4$	PS	AAS	SEM[5]	M	T	M × T
Intake, g/d										
DM	909	909	909	909						
OM	863	860	862	862						
Digestibility, g/d										
DM	746[a]	751[a]	752[ab]	774[b]	749	763	3.8	.041	.062	.625
OM	727	731	729	748	729	739	3.3	.087	.115	.569
Digestibility, g/g										
DM	0.83	0.81	0.84	0.83	0.83	0.84	0.004	.070	.258	.264
OM	0.85	0.83	0.86	0.85	0.85	0.85	0.004	.101	.290	.243
Digestibility, marker/TFC ratio										
DM	1.00	0.99	1.02	1.02	1.01	1.01	0.007	.367	.927	.271
OM	1.00	0.99	1.02	1.02	1.00	1.01	0.006	.360	.913	.204

Note: Means with the different letter in the same row are significantly (p < 0.05) different.
[1]TFC: total faecal collection.
[2]Markers, 0.4% de Cr_2O_3, 0.4% TiO_2, 0.4% Cr_2O_3 + 0.4% TiO_2.
[3]Estimated digestion based on chromium.
[4]Estimated digestion based on titanium.
[5]SEM, standard error of mean.

are expressed in OM; the DM and OM digestibility and the digestibility marker/TFC ratio was similar between techniques (PS and AAS; $p > .05$). Across treatments, DM digestion averaged 74.35%; this agrees closely with values reported previously (74.1%, Almaraz et al. 2010 and 75.25%, Cabral Filho et al. 2013) with lambs fed a sorghum-based diet, even though the chemical composition of the diets was different.

This results are similar to Carciofi et al. (2007), who obtained no differences in digestibility using Cr_2O_3 compared with TFC determined by PS or AAS, while Titgmeyer et al. (2001) overestimate the digestibility using Cr_2O_3 and underestimate with TiO_2 when compared with TFC. Kavangh et al. (2001) obtained similar values for TFC and Cr_2O_3, while the values obtained with TiO_2 were lower compared with TFC and Cr_2O_3.

The observed difference between DM digestibility (g/d) by ASS vs. PS may be due to variations in the estimation of digestibility caused by a lack of certainty in the analysis of the marker ($TiO_2 + CrO_3$), as the use of the wet sample proposed by Hill and Anderson (1958) includes $HClO_4$, which could saturate the solutions with potassium perchlorate and cause errors in the readings when it is mixed with TiO_2. Kavanagh et al. (2001) and Titgmeyer et al. (2001) found TFC values similar to those using Cr_2O_3 mixed with TiO_2, determined by the technique proposed by Williams et al. (1962).

The results of the present study regarding titanium dioxide agree with Ferreira et al. (2009), Batista Sampaio et al. (2011), and Glindeman et al. (2009), who reported similar results for the marker/TFC ratio obtained with titanium dioxide. Additionally, Marcondes et al. (2008) and Ferreira et al. (2009) observed that titanium dioxide could be accurately used for estimating the individual intake of concentrate in group feeding when it was associated with other markers.

4. Conclusions

These experiments show that the use of TiO_2 or TiO_2 with Cr_2O_3 is a reliable marker for calculating TFC and for determining the apparent digestibility of the total digestive tract in growing lambs, as determined by AAS and colorimetric methods.

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Disclosure statement

No potential conflict of interest was reported by the authors.

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References

Al Alami A, Gimeno A, de Vega A, Fondevila M, Castrillo C. 2014. Effects of Cr2O3 labelling dose, and of faeces sampling schedule, on faecal Cr concentration and on digestibility estimation in cattle fed high-concentrate diets. Livest Sci. 168:53–59.

Almaraz I, Segundo González S, Pinos-Rodríguez JM, Miranda A. 2010. Effects of exogenous fibrolytic enzymes on in sacco and in vitro degradation of diets and on growth performance of lambs. Ital J Anim Sci. 9(2):6–10.

Amorocho AK, Jenkins TC, Staples CR, 2009. Evaluation of catfish oil as a feedstuff for lactating Holstein cows. J Dairy Sci. 92:5178–5188.

Association of American Feed Control Officials. 2004. Dog and cat nutrient profiles. Oxford (IN): Official Publication of the Association of American Feed Control Officials Incorporated.

Association of Official Analytical Chemists. 1990. Official methods of analysis. 15th ed. Arlington (VA): Association of Official Analytical Chemists.

Batista Sampaio C, Detmann E, Pereira Valiente TN, Augusto de Souza M, Valadares Filho SC, Fonseca Paulino M. 2011. Evaluation of fecal recovering and long term bias of internal and external markers in a digestion assay with cattle. Revista Brasilerira de Zootecnia. 40:174–182.

Cabral Filho SLS, Abdalla AL, Bueno ICS, Gobbo SP, Olivera AAM. 2013. Effect of sorghum tannins in sheep fed with high-concentrate diets. Arq Bras Med Vet Zootec. 65:1759–1766.

Carciofi CA, Souza VR, Domingues OL, Antonio BM, Valerio AG, Sousa BR, Martins CE, Prada F. 2007. Chromic oxide as a digestibility marker for dogs: a comparison of methods of analysis. Anim Feed Sci Tech. 134:273–282.

Delagarde R, Perez-Ramirez E, Peyraud JL. 2010. Ytterbium oxide has the same accuracy as chromic oxide for estimating variations of faecal dry matter output in dairy cows fed a total mixed ration at two feeding levels. Anim Feed Sci Technol. 161:121–131.

Fenton TW, Fenton M. 1979. An improved procedure for the determination of chromic oxide in feed and feces. Can J Anim Sci. 59:631–634.

Ferreira MA, Valadares Filho SC, Silva LFC, Nascimiento FB, Detman E, Diniz Vaaladares EF. 2009. Avaliação de indicadores em estudos com ruminantes: estimativa de consumos de concentrado e de silagem de milho por vacas em lactação. Revista Brasileira de Zootecnia. 38:1574–1580. (In Portuguese).

Glindeman T, Tas BM, Wang C, Alvers S, Susenbeth A. 2009. Evaluation of titanium dioxide as an inert marker for estimating faecal excretion in grazing sheep. Anim Feed Sci Tech. 152:186–197.

Hafez S, Junge W, Kalm E. 1988. Estimation of digestibility with an indicator method in dairy cows compared to Hohenheim lining value test. Arch Anim Nutr. 38:929–945. (In German).

Hill FW, Anderson DL. 1958. Comparison of metabolizable energy and productive energy determinations with growing chicks. J Nutr. 64:587–603.

Jagger S, Wiseman J, Cole DJA, Craigon J. 1992. Evaluation of inert markers for the determination of ileal and faecal apparent digestibility values in the pig. Brit J Nutr. 68:729–739.

Kavanagh S, Lynch PB, Mara FO, Caffrey PJ. 2001. A comparison of total collection and marker technique for the measurement of apparent digestibility of diets for growing pigs. Anim Feed Sci Tech. 89:49–58.

Kuehl RO. 2000. Design of experiments: statistical principles of research design and analysis. 2nd ed. Pacific Grove (CA): Duxbury Press; p. 473.

Leone JL. 1973. Collaborative study of the quantitative determination of titanium dioxide in cheese. Assoc Offic Anal Chem. 56:535–537.

Lippke H. 2002. Estimation of forage intake by ruminants on pasture. Crop Science. 42:869–872.

Marcondes MI, Valadares Filho SC, Paulino PVR, Detmann E, Paulino MF, Diniz LL, Santos TR. 2008. Consumo e desempenho de animais alimentados individualmente ou em grupo e características de carcaça de animais Nelore de três classes sexuais. Revista Brasileira de Zootecnia. 37:2243–2250. (In Portuguese).

Myers WD, Ludden PA, Nayigihugu V, Hess BW. 2004. Technical note: a procedure for the preparation and quantitative analysis of samples for titanium dioxide. J Anim Sci. 82:179–183.

Peddie J, Dewar WA, Gilbert AB, Waddington D. 1982. The use of titanium dioxide for determining apparent digestibility in mature domestic fowls (*Gallus domesticus*). J Agric Sci. 99:233–236.

SAS Institute Inc. 1999. SAS/STAT user's guide: version 8. Cary (NC): SAS Institute Inc.

Sedman RM, Beaumont J, McDonald TA, Reynolds S, Krowech G, Howd R. 2006. Review of the evidence regarding the carcinogenicity of hexavalent chromium in drinking water. J Environ Sci Heal C – Environ Carcinog Ecotoxicol Rev. 24:155–182.

Short FJ, Gorton P, Wiseman J, Boorman KN. 1996. Determination of titanium dioxide added as an inert marker in chicken digestibility studies. Anim Feed Sci Techn. 59:215–221.

Statistical package for Social Science. 2006. SPSS Base 15.0 user's guide. Chicago (IL): SPSS Inc.

Titgemeyer EC, Armendariz CK, Bindel DJ, Greenwood RH, Loest CA. 2001. Evaluation of titanium dioxide as a digestibility marker in cattle. J Anim Sci. 79:1059–1063.

Van Soest PJ, Robertson JB, Lewis BA. 1991. Methods for dietary fiber, neutral detergent fiber, and nonstarch polysaccharides in relation to animal nutrition. J Dairy Sci. 74:3583–3597.

Williams CH, David DJ, Lismaa O. 1962. The determination of chromic oxide in faeces samples by atomic absorption spectrophotometry. J Agric Sci. 59:381–385.