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Effects of Cr_2O_3 labelling dose, and of faeces sampling schedule, on faecal Cr concentration and on digestibility estimation in cattle fed high-concentrate diets ☆

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ABSTRACT

Chromic oxide (Cr_2O_3) is used as digestibility marker with ruminants, although there are concerns about diurnal excretion patterns, irregular excretion over days and optimal concentration in the feed. This study aimed to assess the between- and within-day variability of faecal Cr concentration ([Cr]) in intensively fed cattle given concentrates spiked with 2000 or 4000 mg/kg Cr_2O_3. Repercussions on digestibility estimations were also studied. Sixteen calves were given concentrates labelled with 4000 mg/kg (period 1-Ph1) or 2000 mg/kg (period 2-Ph2) Cr_2O_3. After four days of marker consumption, spot samples were taken from the rectum at 9:00 h and 17:00 h, during four (Ph1) or five (Ph2) consecutive days. Measured Cr in concentrate varied less in Ph1 than in Ph2 (2820 ± 30.1 vs 1690 ± 46.3 mg/kg dry matter). Increasing sampling days from 1 to 4 in Ph1, and from 1 to 5 in Ph2, decreased the coefficient of variation of estimated dry matter digestibility from 5.17% to 3.37% in Ph1, and from 6.87% to 3.58% in Ph2, respectively. In conclusion, four days of adaptation to Cr_2O_3-labelled feed allows a steady [Cr] in intensively reared cattle. Labelling with 4000 mg/kg Cr_2O_3 guarantees the homogeneity of the marker in the diet, and a low time-dependent variability of [Cr].

1. Introduction

Chromic oxide has long been the most commonly used external digestibility marker in site and extent of digestion trials with ruminants (Titgemeyer, 1997; Myers et al., 2006; Borucki Castro et al., 2008; Amorocho et al., 2009; Delagarde et al., 2010). Most put forward reasons are the low cost of the readily available commercial form, and relative simplicity of the analytical procedures (Carvalho et al., 2007). However, several problems associated with Cr_2O_3 have been reported, including concerns over potential carcinogenic properties and the health hazard when Cr_2O_3 is inhaled (Titgemeyer et al., 2001). In addition, researchers have reported diurnal excretion patterns (Hopper et al., 1978; Prigge et al., 1981), low recovery rates (Titgemeyer, 1997) and irregular excretion over the days (Ribeiro Filho et al., 2008).

Chromium is always given to animals in the trivalent state, which is non-toxic for humans and animals (Delagarde et al., 2010). Although laboratory analysis requires hexavalent chromium (highly toxic and carcinogenic) (Costa, 1997;

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Sedman et al., 2006) for the determination of chromium concentration in solution, individual protection of laboratory workers and appropriate disposal of waste as dangerous residues should minimise the risks for human health and environment. On the other hand, a number of reasons may explain the incomplete apparent recovery of Cr_2O_3, although type of sample (e.g., faeces, or rumen or duodenal contents) has a dramatic influence (Vicente et al., 2004). For this reason, standards should always be made using similar material to that analysed, especially when samples are very heterogeneous (Vicente et al., 2004).

Titgemeyer (1997), among other authors, stated that problems with diurnal variation in the faecal pattern of Cr excretion can be overcome if enough samples are collected throughout the day to provide an average sample in which the marker concentration is representative of that over the entire day. Most studies, however, have been carried out with forage-fed animals (Prigge et al., 1981; Brandyberry et al., 1991), with no information appearing available for individuals fed high-concentrate diets.

When Cr_2O_3 is mixed with the feed, a compromise about concentration must be attained. Low amounts will not be correctly mixed with the whole diet, whereas high amounts can be potentially toxic for rumen microbes (Salem et al., 2011).

The aim of the present study was to assess the between- and within-days variability of faecal Cr concentration when Cr_2O_3 was used at two concentrations (2000 and 4000 mg/kg) in the diet of intensively reared calves. The implications on digestibility estimations were also studied.

2. Material and methods

This work was part of a study focussed on the effects of cereal type (barley or maize) and processing (ground or crushed) on animal performance and rumen fermentation characteristics. This gave an opportunity to check whether the variability between diets, and hence rumen environments, would have an influence on the time variation in faecal Cr concentration.

2.1. Potential toxicity of Cr for rumen microbes

Previously to the essay itself, the potential toxicity of Cr for rumen microbes at different concentrations was assessed in vitro. For this purpose, 0.64 g of a compound feed (0.15 barley grain, 0.45 maize, 0.002 gluten feed, 0.144 soybean meal, 0.044 wheat bran, 0.105 sunflower meal, 0.049 sugar beet pulp, 0.02 palm oil, 0.013 calcium carbonate, 0.008 sepiolite, 0.008 dicalcium phosphate, 0.003 sodium chloride and 0.004 vitamin–mineral premix (AGROAL S.A.)) plus 0.16 g of barley straw (both ground through a 1 mm sieve) were incubated with 16 ml rumen liquid (collected from three steers fed on an 0.8:0.2 concentrate: barley straw diet) and 64 ml of a buffer solution (Theodorou et al., 1994; Mould et al., 2005). The concentrate was spiked with Cr_2O_3 amounts equivalent to 0, 3000, 6000 or 9000 mg/kg of the total substrate incubated (0.8 g), and four replicates were used for each Cr_2O_3 concentration. Gas production (volume) was recorded at 2, 4, 6, 8, 12, 16, 24 36 and 48 h of incubation using a pressure transducer.

2.2. Main experiment

For the experiment itself 16, 3-month old Holstein–Friesian male calves (119 ± 2.1 kg body weight-LW) were housed individually and randomly allocated to one of four diets consisting in barley straw (882 g/kg organic matter -OM–(dry matter-DM-basis), 29 g/kg crude protein, 735 g/kg neutral detergent fibre, 523 g/kg acid detergent fibre and 104 g/kg acid detergent lignin) and a concentrate (Table 1) including 0.6 cereals (barley and maize in proportions 75:25 -HB- or 25:75 -HM-), presented crushed (CR) or ground to pass a 3.5 mm pore size mesh (GR). Both straw and concentrate were offered *ad libitum* at 9:00 h. The essay was designed following a 2 (barley or maize as main cereals) $\times$ 2 (ground or crushed) factorial arrangement, with four animals per treatment. Separate intake of both straw and concentrate was measured daily along the whole experiment. At two different phases of growth of the steers (210 ± 3.9 kg -Ph1- and 342 ± 3.7 kg -Ph2- mean body weight) animals were given the concentrate spiked with 4000 mg/kg (in Ph1) and 2000 mg/kg (in Ph2) Cr_2O_3 (as fed). To this purpose, 1.2 (Ph1) or 0.8 (Ph2) kg of Cr_2O_3 were thoroughly mixed with 40 kg of each concentrate and then with another 260 (Ph1) or 360 (Ph2) kg. Four samples of each labelled concentrate were collected on four different days along the marker consumption period before distribution.

After four days of marker consumption, spot samples (50–100 g) were taken directly from the rectum at 9:00 h (just after feed distribution) and 17:00 h, during four (Ph1) or five (Ph2) consecutive days. Samples of labelled concentrates, daily concentrate refusals and faeces were dried at 60 °C for 48 h and ground to pass through a 1 mm sieve for Cr analysis. This was performed following the technique described by de Vega and Poppi (1997) and by inductively coupled plasma atomic emission spectroscopy. Calibration standards were made using either blank concentrates or blank faeces, as appropriate.

2.3. Chemical analysis

Organic matter in feeds was obtained by ashing at 550 °C for 8 h (AOAC, 2005), and total *N* following the Kjeldahl method using Cu as a catalyst and a 2300 Kjeltec Analyser Unit (Foss Tecator). Neutral detergent fibre (NDF) in feeds was measured with an ANKOM^{200} Fibre Analyser as described by Mertens (2002). Acid detergent fibre (ADF) and acid detergent lignin (ADL) in feeds were measured as described by AOAC (2005) (official method 973.18) and Robertson and Van Soest (1981) for ADF and ADL, respectively. Both NDF and ADF were expressed as ash-free residues. Starch content in concentrates was determined polarimetrically at the Agro-Environmental Laboratory of Zaragoza, according to the method and regulations provided by the European Commission (2009).