Animal Feed Science and Technology 161 (2010) 121–131

Contents lists available at ScienceDirect

Animal Feed Science and Technology

journal homepage: www.elsevier.com/locate/anifeedsci

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

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ARTICLE INFO

Article history:
Received 9 March 2010
Received in revised form 6 August 2010
Accepted 6 August 2010

Keywords:
Faecal output
Ytterbium
Chromic oxide
Dairy cow
Feeding level

ABSTRACT

Daily herbage intake by ruminants at grazing can be estimated from faecal output and herbage digestibility. Chromic oxide has long been used as an external marker to estimate faecal dry matter (DM) output. The aim of this experiment was to determine the performance of ytterbium oxide compared to chromic oxide as an external marker for estimating variations of faecal DM output in dairy cows. Both markers were compared on eight Prim'Holstein lactating dairy cows, of which four were fistulated. Cows were offered a total mixed diet based on maize silage either at 100% or 70% of *ad libitum* DM intake, according to a single 2 × 2 reversal design with 2 experimental periods of 3 weeks. Faecal recovery of markers was determined over the last five days of each period by total faecal collection or by faecal sampling only during milking times. The between-day and the within-day variability of faecal marker concentrations were also investigated, as well as the between-day variability of faecal marker concentrations during the transition between periods 1 and 2. Both markers over-estimated the actual faecal DM output due to an incomplete faecal recovery. The average faecal recovery was slightly greater for Yb than for Cr (0.93 *vs.* 0.89). Faecal recovery of Yb and Cr was unaffected by feeding level irrespective of the faecal collection method. Ytterbium showed similar between-day and within-day variability of faecal concentration as Cr during the experiment (steady-state period), and similar between-day variability of faecal concentration as Cr during the transition period (non steady-state period). We conclude that ytterbium oxide used as a digestive marker seems to have the same accuracy as chromic oxide for estimating daily faecal DM output variations in dairy cows fed a total mixed ration, at low and high feeding level.

1. Introduction

It is not possible to measure directly the daily individual herbage intake by ruminants at pasture. Daily dry matter (DM) intake can be estimated by means of digestion markers (Kotb and Luckey, 1972; Peyraud, 1997; Penning, 2004). Apart from methods based on the combination of internal and an external markers, such as *n*-alkanes (Mayes et al., 1986), the most

Abbreviations: DM, dry matter; FC, faecal collection; FO, faecal output; MPE, mean prediction error; MSPE, mean square prediction error.

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doi:10.1016/j.anifeedsci.2010.08.006

frequently used method involves estimating the faecal output and herbage digestibility separately (Penning, 2004). When total faecal collection is not possible, faecal DM output is generally determined by dilution of an indigestible external marker, *i.e.* whose rate of faecal recovery is expected to be 1.0. Moreover, the faecal marker concentration should vary as little as possible to allow a representative sampling of faeces. Chromic oxide (Cr_2O_3) has been studied and widely used for a long time. Although Cr_2O_3 is known to behave independently of both the liquid and particulate phases of digesta in the gastro-intestinal tract (Faichney, 1975), it is considered as a reference marker in nutrition for the estimation of faecal DM output (Kotb and Luckey, 1972; Le Du and Penning, 1982). Chromium is always given to animals in the trivalent state, which is non-toxic for humans and animals. However, laboratory analysis requires hexavalent chromium for the colorimetric determination of chromium concentration in solution, and hexavalent chromium is highly toxic and carcinogenic (Costa, 1997; Sedman et al., 2006). As individual protection of laboratory workers seems insufficient to prevent these risks for human health, it is now highly recommended not to use chromic oxide in animal nutrition studies. It could thus be relevant to replace chromic oxide by another marker with satisfactory biological properties, but without the major disadvantage of carcinogenicity.

Many other markers have been already used for estimating digestive flows, in particular the rare earth elements, including ytterbium (Faichney, 1975; Mambrini and Peyraud, 1997). Although the transit of markers may or may not be dependent on liquid or solid phases in the digestive tract, continuous perfusion of ytterbium acetate or ytterbium chloride in stall-feeding cows yields the same flow estimates as chromic oxide, particularly for faecal flow (Prigge et al., 1981; Siddons et al., 1985; Brandyberry et al., 1991). At grazing, ytterbium oxide (Yb_2O_3) has already been used to estimate pasture intake of lactating dairy cows (Ribeiro Filho et al., 2005; Pérez-Ramírez et al., 2009) and heifers (Ginane and Petit, 2005), ytterbium oxide being mixed in a pelleted concentrate at a low concentration (5 g/kg) preventing any problem of palatability. However, the variations of faecal recovery and faecal excretion of ytterbium oxide have not been extensively studied.

The objective of this study is to compare the faecal recovery of ytterbium oxide and chromic oxide in dairy cows at two intake levels, as well as compare the variability of faecal excretion of these two markers. Marker excretion is also compared between intact cows and ruminally fistulated cows, to determine whether the way in which markers are administered (orally or through the ruminal fistula) can affect the estimation of faecal excretion and markers recovery.

2. Materials and methods

2.1. Treatments, cows and experimental design

Ytterbium oxide and chromic oxide recoveries were studied in two situations consisting of two feeding levels of a total mixed ration. Feeding levels were 100% and 70% of the voluntary [*i.e. ad libitum*] DM intake, determined over one week prior to the experiment on the same diet, and providing at least 0.10 of refusals daily. The total mixed ration was based on (DM basis) 0.70 maize silage, 0.17 soyabean meal, 0.115 cereal-based concentrate and 0.015 minerals. Table 1 reports the components of the cereal-based concentrate and the chemical composition of the different feeds. The ration was offered in two equal meals daily at 08:30 h and 17:15 h for the 100% treatment, with free access to feeds. The ration was offered in one meal daily at 08:30 h for the 70% treatment, in order to test markers in an extreme feeding situation that can be encountered both indoors and at grazing (Pérez-Ramírez et al., 2009). Water and mineral block were always available.

The experiment was undertaken with eight Prim'Holstein multiparous and pregnant cows in mid lactation, including four cows fitted with a large ruminal cannula (internal diameter: 123 mm). The mean pre-experimental characteristics of the cows determined during 8 days were as follows: voluntary DM intake 23.0 ± 1.8 kg, days in milk 102 ± 26 days, milk production 36.2 ± 5.3 kg, milk fat concentration 43.0 ± 4.1 g/kg, milk protein concentration 32.0 ± 2.3 g/kg, body weight 656 ± 34 kg. Cows were housed in individual stalls and milked twice daily at 07:30 h and 16:30 h.

Treatments were compared according to a single reversal design with two periods of 21 days. The eight cows were separated into two groups of four (two fistulated cows per group), each group receiving successively both treatments during the two periods. Within each period, days 1–4 were used for transition between feeding levels, days 5–15 for adaptation and days 16–21 for measurements. The experiment took place in February and March 2005 at the INRA experimental farm of Méjusseaume (Lat. 48.11°N, Long. 1.71°W in Brittany, France). Procedures relating to surgery, care and use of cows were in accordance with national legislation on animal care (Certificate No. B35-275-23, Ministry of Agriculture, France).

Table 1
Dry matter (g/kg) and chemical composition (g/kg DM) of feeds.

Variable	Maize silage	Soyabean meal	Cereal-based concentrate[a]
Dry matter	348	898	896
Organic matter	963	931	951
Crude protein	79	488	119
Neutral detergent fibre	408	143	261
Acid detergent fibre	219	79	94
Acid detergent lignin	23	10	16
Starch	315	62	470
Fat	36	20	43

[a] *Components*: 0.2 wheat, 0.2 barley, 0.2 maize, 0.2 beet pulp, 0.15 wheat bran, 0.03 molasses, 0.01 oil, and 0.01 NaCl.

2.2. Measurements

2.2.1. Milk production and DM intake

Milk production was recorded at each milking, and milk fat and protein concentrations were determined on 6 consecutive milkings on week 3 of each period by near infra-red spectrophotometry (Milkoscan, Foss Electric, Hillerød, Denmark). Fresh matter intake was measured individually by weighing the daily fresh amounts of each feed offered and refusals each morning at 08:00 h. Dry matter intake was calculated from fresh weights and the dry matter concentration of concentrates and maize silage, determined once weekly and once daily, respectively, as well as from the DM concentration of refusals measured daily for each cow. Each week, the fresh amount of maize silage to be offered was calculated from the average DM concentration of maize silage determined over the previous week.

2.2.2. Administration of markers

Chromic oxide and ytterbium oxide were mixed in a pelleted concentrate based on 0.32 maize, 0.25 wheat, 0.25 barley, 0.11 soyabean meal, 0.03 rapeseed oil, 0.035 Cr_2O_3 and 0.005 Yb_2O_3 on a fresh matter basis. This pelleted concentrate was prepared in individual doses of 200 ± 0.5 g, given to each cow twice daily at 08:15 h and 17:00 h, just before feeding, either directly into the rumen through the cannula (for the fistulated cows) or offered in a bucket (for the intact cows). Pelleted concentrate was regularly sampled during the preparation of individual doses for DM determination and chemical analysis. Eventual refusals of the pelleted concentrate were weighed and their DM concentration was determined.

2.2.3. Faecal sampling

Faeces were collected and sampled to compare: (1) the recovery of Yb and Cr as a function of the faecal sampling method and the ability of these markers to estimate faecal DM output, (2) the between-day variability of faecal marker concentrations, (3) the within-day variability of faecal marker concentrations, (4) the ability of Yb and Cr to estimate between-day variability of faecal DM output during the transition between periods 1 and 2, where intake level rapidly changed from 100% to 70% or from 70% to 100% *ad libitum* intake.

2.2.3.1. Faecal recovery and between-day variability of faecal marker concentration. The faecal excretion of both markers was estimated from two methods of faeces sampling, total faecal collection and faecal sampling only during milking times (milking faecal collection). The former method involved collecting all the faeces deposited daily in large containers, urine being separated from faeces through a system of harnesses. The later method involved sampling only faeces deposited within a window of 1–2 h around each milking time, in order to 'simulate' rectal sampling. Although less representative than total collection, milking faecal collection is the most generally used sampling method in grazing experiments. Rectal sampling was not possible in this trial due to the use of harnesses for urine collection.

Both methods of faecal sampling were carried out on 5 consecutive days of each period, from day 17 to day 21. For the total faecal collection, each morning, 0.015 of the total fresh weight of faeces was dried in an oven at 80 °C for 72 h. For milking faecal collection, morning and evening 250-g subsamples were collected, then composited per day and dried per cow. Dried faecal samples were either composited per cow and period to determine the recovery of markers or kept daily to determine the between-day variability of faecal marker concentration.

2.2.3.2. Within-day pattern of faecal marker concentration. The daily pattern of faecal marker concentration was studied on day 20 by collecting, weighing and sampling faeces at 06:00 h, 09:00 h, 12:00 h, 15:00 h, 18:00 h and 21:00 h. Faeces collected at 06:00 h represented faecal excretion at night. After drying in an oven (80 °C, 72 h), faecal samples corresponding to the four cows on the same treatment were composited per hour before chemical analyses.

2.2.3.3. Within-transition variability of faecal marker concentration. During the transition period from days 1 to 4 of period 2, feeding levels were 92.5%, 85%, 77.5%, and 70% *ad libitum* for the four cows fed 100% *ad libitum* in period 1, and 77.5%, 85%, 92.5%, and 100% *ad libitum* for the fours cows fed 70% *ad libitum* in period 1. Total faecal collection was carried out on 5 consecutive days, from day 2 to day 6 of the transition, allowing to measure the between-day variability of actual faecal DM output. A 500-g fresh subsample of faeces was sampled each day on each cow, then dried, ground and analysed to estimate the corresponding variations in faecal marker concentrations during the same period.

2.3. Chemical analyses

Dry matter was determined in an oven (80 °C), with a drying time of 48 h for feeds and 72 h for faeces. All samples were ground through a 0.8-mm screen before chemical analysis. Chromic oxide was determined by the colorimetric method of Mathieson and Davidson (1970) modified for an auto-analyzer (Technicon) by Poncet and Rayssiguier (1980), and then adapted to a multiparameter analyser (KONE Instruments Corporation, Finland). Ytterbium oxide was determined by atomic absorption spectrophotometry with a nitrous oxide/acetylene flame (Varian spectraa-20, Varian France, SA), after calcination and digestion in nitric acid (94.5 g/L) according to Siddons et al. (1985). Ytterbium was determined by a standard addition method to limit inter-element interferences (Marks and Welcher, 1970).

Table 2
Effect of cow type (T, intact *vs.* fistulated) at two feeding levels (FL, 70% *vs.* 100% *ad libitum*) on DM intake, faecal DM output, in vivo diet DM digestibility and on faecal Cr and Yb recoveries, estimated either by total or milking faecal collection, in dairy cows fed a total mixed ration.

Feeding level (FL)	70% *ad libitum*		100% *ad libitum*		s.d.$_{cow}$[a]	s.d.	P value		
Cow type (T)	Intact	Fistul.	Intact	Fistul.			FL	T	FL×T
Total DM intake (kg)	16.8	16.2	23.5	22.4	2.26	0.43	<0.001	0.489	0.304
Faecal DM output (kg)	4.57	4.45	6.91	6.55	0.686	0.252	<0.001	0.514	0.386
In vivo diet DM digestibility	0.728	0.725	0.705	0.707	0.0118	0.0120	0.020	0.911	0.691
Faecal recovery of markers									
Cr (total FC[b])	0.855	0.886	0.927	0.890	0.0453	0.0458	0.161	0.900	0.192
Yb (total FC)	0.912	0.919	0.956	0.939	0.0699	0.0742	0.430	0.879	0.752
Cr (milking FC)	0.880	0.903	0.852	0.849	0.0347	0.0604	0.226	0.592	0.688
Yb (milking FC)	0.938	0.939	0.892	0.895	0.0900	0.0569	0.173	0.967	0.988

[a] s.d.$_{cow}$, standard deviation, the cow type being tested considering the cow within type as the residual term.
[b] FC, faecal collection.

2.4. Calculations

Faecal DM output (FO, kg/day) was calculated for each marker and for each faecal sampling method by dividing the daily amount of dosed marker (D, g) by the corresponding faecal marker concentration (C, g/kg DM) according to the equation $FO = D/C$. Faecal recovery was calculated by dividing the amount of marker recovered in faeces by the amount of dosed marker. The amount of marker recovered in faeces was calculated by multiplying the actual faecal DM output by the faecal marker concentration (total or milking faecal collection).

The between-day variations of faecal Cr and Yb concentrations were compared by calculating the between-day relative variations of the faecal marker concentrations. The latter was calculated by dividing the daily faecal Cr and Yb concentration by the average value on the five sampling days. The same procedure was performed to compare the within-day (6 h) and within-transition (5 days) relative variations of faecal Cr and Yb concentration.

2.5. Statistical analyses

Dry matter intake, milk production, faecal excretion and markers recovery were statistically analysed using average values per cow and period. Animal data were firstly analysed according to a single reversal design, using the GLM procedure of Statistical Analysis System Institute Inc. (SAS, 1987), with the following model:

$$Y_{ijkl} = \mu + \mathrm{TYPE}_i + \mathrm{COW(TYPE)}_j + \mathrm{PER}_k + \mathrm{FL}_l + [\mathrm{TYPE}_k \times \mathrm{FL}_l] + \varepsilon_{ijkl}$$

where μ, TYPE_i, $\mathrm{COW(TYPE)}_j$, PER_k, FL_l, $[\mathrm{TYPE}_k \times \mathrm{FL}_l]$, and ε_{ijkl}, respectively, represent the overall average, the effect of cow type (intact *vs.* fistulated), cow within type, the period, feeding level, cow type by feeding level interaction and the residual error. The effect of cow type was tested using the cow within type as the residual error term.

There was no effect of cow type on total DM intake, faecal DM output, or on *in vivo* diet DM digestibility. Faecal Yb and Cr recoveries estimated by total or milking faecal collection were unaffected by cow type (Table 2). Similarly, there was no interaction of cow type by feeding level. Accordingly, animal data were then analysed without the cow type effect, according to a single reversal design, using the GLM procedure of SAS (1987), with the following model:

$$Y_{ijkl} = \mu + \mathrm{COW}_j + \mathrm{PER}_k + \mathrm{FL}_l + \varepsilon_{ijkl}$$

All data presented in Section 3 are analysed without considering the cow type effect.

The accuracy of each marker and faecal sampling method for estimating the faecal DM output was evaluated in terms of the mean square prediction error (MSPE), the proportional contribution of mean bias, line bias and random variation to the MSPE, and the mean prediction error (MPE), expressed in kg or as a proportion of the mean (Bibby and Toutenburg, 1977).

The between-day and within-day variabilities of faecal marker concentrations were also estimated by calculating the coefficients of variation (s.d./mean) of the faecal marker concentrations within a period ($n = 5$ days) or within a day ($n = 6$ times), respectively.

3. Results

Actual total DM intake and faecal DM output decreased by 6.4 kg (−30%) and 2.22 kg (6.73 *vs.* 4.51, *i.e.* −33%), respectively, when feeding level was reduced from 100% to 70% *ad libitum* (P<0.001, Table 3). Apparent *in vivo* diet DM digestibility increased by 0.020 with decreasing feeding level (P<0.01). Cows showed lower milk production (−5.6 kg), milk fat production (−274 g) and milk protein production (−207 g) at low compared with high feeding level (P<0.01). Milk fat and protein concentrations showed no variation with treatment.

Faecal DM outputs estimated from Cr and Yb were strongly and positively correlated for both faecal collection methods (total: $R^2 = 0.91$; milking: $R^2 = 0.95$) (Fig. 1). Both Cr and Yb over-estimated the actual faecal DM output, whatever the faecal

Table 3
Effect of feeding level on total DM intake, faecal DM output, in vivo diet DM digestibility, milk production, milk composition and live weight in dairy cows fed a total mixed ration.

Variable	Feeding level		s.d.[a]	P value
	70% *ad libitum*	100% *ad libitum*		
Total DM intake (kg/d)	16.5	22.9	0.44	<0.001
Faecal DM output (kg/d)	4.51	6.73	0.251	<0.001
in vivo diet DM digestibility	0.726	0.706	0.0107	0.010
Milk production (kg/d)	28.9	34.5	2.46	0.005
Milk fat production (g/d)	1054	1328	119.4	0.005
Milk protein production (g/d)	893	1100	36.8	<0.001
Milk fat concentration (g/kg)	37.3	39.0	4.30	0.457
Milk protein concentration (g/kg)	30.9	32.1	1.49	0.162
Body weight (kg)	637	661	8.3	0.002

[a] s.d., standard deviation.

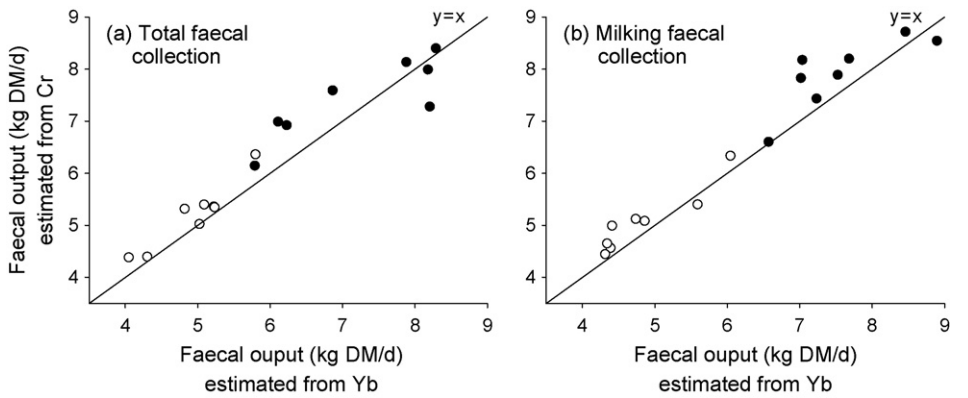

Fig. 1. Relationship between faecal DM output estimated from Cr and Yb in dairy cows fed a total mixed ration (●: 100% *ad libitum*; ○: 70% *ad libitum*), according to faecal sampling method.

collection method (Fig. 2). Total faecal DM output estimated from markers ranged from 4.84 to 5.20 kg for the low feeding level, and from 7.19 to 7.92 kg for the high feeding level (Table 4).

Faecal recovery was slightly higher for Yb than for Cr. When estimated from total faecal collection, the average recovery of Yb and Cr was 0.93 and 0.89, respectively. When estimated from milking faecal collection, the average recovery of Yb

Table 4
Effect of feeding level on faecal Cr and Yb concentrations, on faecal DM output (FO) estimated from Cr and Yb, on the ratio between estimated and measured FO, on the bias between estimated and measured FO and on faecal Cr and Yb recoveries, estimated either by total or milking faecal collection (FC), in dairy cows fed a total mixed ration.

Variable	Feeding level		s.d.[a]	*P* value
	70% *ad libitum*	100% *ad libitum*		
Estimated faecal output (kg DM/d)				
From Cr (total FC[b])	5.20	7.43	0.173	<0.001
From Yb (total FC)	4.94	7.19	0.299	<0.001
From Cr (milking FC)	5.07	7.92	0.217	<0.001
From Yb (milking FC)	4.84	7.55	0.369	<0.001
Faecal recovery of markers				
Cr (total FC)	0.871	0.908	0.0504	0.185
Yb (total FC)	0.915	0.948	0.0684	0.392
Cr (milking FC)	0.892	0.850	0.0561	0.187
Yb (milking FC)	0.939	0.894	0.0519	0.132
Bias estimated-measured FO (g DM/d)				
From Cr (total FC)	0.69	0.70	0.342	0.955
From Yb (total FC)	0.43	0.46	0.438	0.902
From Cr (milking FC)	0.57	1.19	0.339	0.011
From Yb (milking FC)	0.33	0.82	0.291	0.015

[a] s.d., standard deviation.
[b] FC, faecal collection.

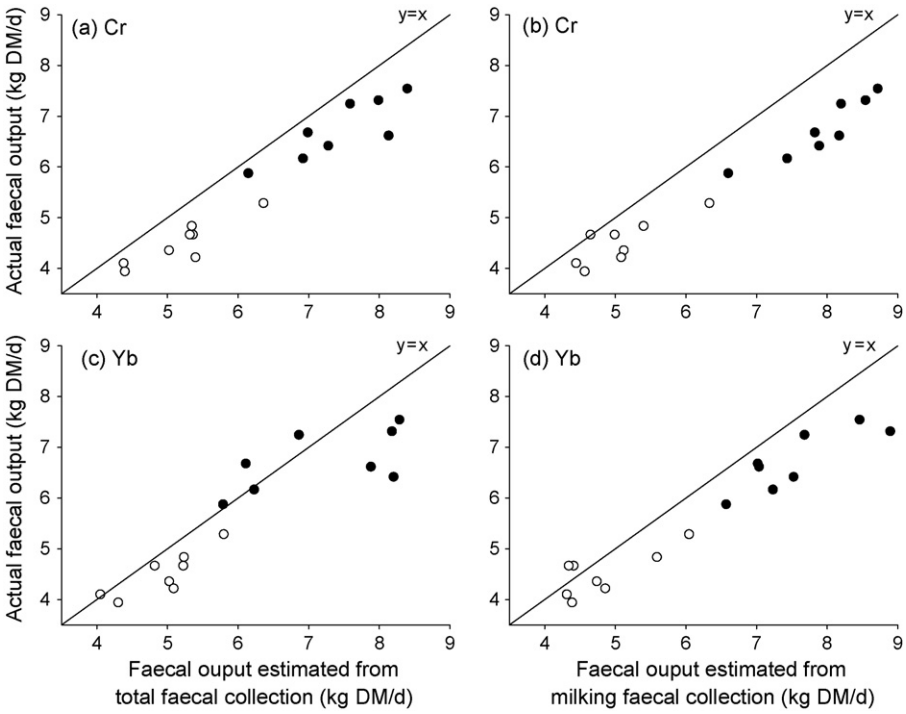

Fig. 2. Relationship between measured faecal DM output and faecal DM output estimated by total or milking faecal collection with Cr or Yb in dairy cows fed a total mixed ration (●: 100% *ad libitum*; ○: 70% *ad libitum*).

and Cr was 0.92 and 0.87, respectively. Faecal recovery of Cr and Yb remained unaffected by feeding level whatever the faecal collection method (Table 4). For total faecal collection, the bias between estimated and actual faecal DM output was unaffected by feeding level, for both Cr and Yb. Consequently, the difference in faecal DM output between feeding levels estimated with Yb (2.25 kg DM) and Cr (2.23 kg DM) was similar to the actual value (2.22 kg DM). For milking faecal collection, this bias was higher at high than at low feeding level, for both Cr and Yb. Consequently, the effect of feeding level on faecal DM output was over-estimated by both markers when compared to the actual value (2.71 and 2.85 kg DM for Yb and Cr, respectively).

Both markers were able to predict faecal DM output with a mean prediction error ranging from 0.13 to 0.17 (Table 5). With total faecal collection, the overall accuracy was similar for both markers. With milking faecal collection, the accuracy seemed slightly better for Yb than for Cr. Faecal DM output estimated from Cr and Yb were positively correlated with actual faecal DM output, with R^2 ranging from 0.82 to 0.96 (Table 5 and Fig. 2). Most of the MSPE could be explained by the mean bias for both markers (range 0.37–0.81). The proportion of MSPE explained by the line bias was low for both markers (range 0.02–0.22).

Daily faecal Yb and Cr concentrations were strongly and positively correlated for both total (R^2 = 0.94) and milking (R^2 = 0.94) faecal collection (Fig. 3). Considering the data within cow and period, the between-day relative variation of faecal marker concentration ranged from 0.8 to 1.2 for both markers and both faecal collection methods (Fig. 4). The between-day relative variation of faecal Yb and Cr concentrations was positively correlated, particularly for total faecal collection

Table 5
Statistical evaluation of the accuracy of Cr and Yb in estimating faecal DM output (FO, kg) in dairy cows fed a total mixed ration according to faecal collection method.

	Mean estimated FO	Mean bias[a]	R^2	MSPE[b]	Proportion of MSPE			MPE[c]	
					Bias	Line	Random	kg DM	Proportion mean
Total faecal collection									
Cr	6.31	0.69	0.93	0.595	0.81	0.02	0.17	0.77	0.14
Yb	6.07	0.45	0.82	0.542	0.37	0.14	0.49	0.74	0.13
Milking faecal collection									
Cr	6.50	0.88	0.96	0.951	0.81	0.13	0.06	0.97	0.17
Yb	6.19	0.57	0.93	0.549	0.60	0.22	0.18	0.74	0.13

[a] Bias, estimated minus actual value.
[b] MSPE, mean square prediction error.
[c] MPE, mean prediction error.

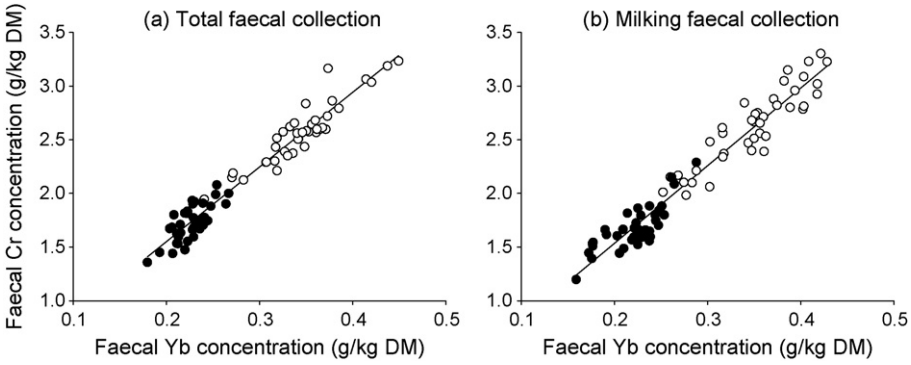

Fig. 3. Relationship between daily faecal Cr and Yb concentrations in dairy cows fed a total mixed ration (●: 100% *ad libitum*; ○: 70% *ad libitum*), according to faecal sampling method.

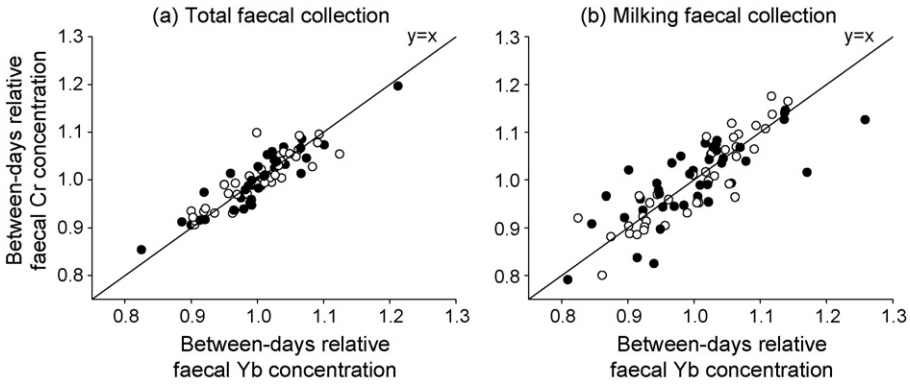

Fig. 4. Between-day relative variation of faecal Yb and Cr concentrations at two feeding levels (●: 100% *ad libitum*; ○: 70% *ad libitum*), according to faecal sampling method. Faeces are collected over five consecutive days. Faecal marker concentrations are expressed as a proportion of the mean of five values for each cow during each period ($n = 80$).

($R^2 = 0.80$) but less for milking faecal collection, where there was higher dispersion of the data ($R^2 = 0.68$). The between-day coefficient of variation of faecal marker concentration showed no difference between markers and feeding levels, but was on average higher for milking (0.088) than for total (0.058) faecal collection (Table 6).

The within-day relative variation of faecal marker concentrations ranged from 0.86 to 1.13 for Cr and from 0.88 to 1.12 for Yb, and were positively correlated ($R^2 = 0.68$, Fig. 5). The range of within-day relative variation of Yb and Cr faecal concentrations was unaffected by feeding level.

During the five days of the transition period between feeding levels, the relative variation of actual faecal DM output ranged from 0.74 to 1.26. The same range was observed for the relative variation of faecal DM output estimated from Yb (0.76–1.26) and Cr (0.76–1.26) (Fig. 6). There was a strong positive correlation between the within-transition relative variations of faecal Yb and Cr concentrations ($R^2 = 0.88$). When the feeding level was changed from 100% to 70% *ad libitum*, both Cr and Yb were able to predict – without any time lag – the strong daily decrease in actual faecal DM output (Fig. 7a). On the contrary, when the feeding level was changed from 70% to 100% *ad libitum*, both Cr and Yb were unable to predict rapidly the strong daily increase in faecal DM output (Fig. 7b).

Table 6
Mean within-day ($n = 6$) and between-day ($n = 5$) coefficient of variation (CV) of faecal Cr and Yb concentrations in dairy cows fed a total mixed ration at 70% or 100% *ad libitum*.

Marker	Within-day CV		Between-day CV	
	70% *ad libitum*	100% *ad libitum*	70% *ad libitum*	100% *ad libitum*
Total faecal collection				
Cr	0.070	0.076	0.055	0.058
Yb	0.087	0.092	0.061	0.059
Milking faecal collection				
Cr	–	–	0.096	0.084
Yb	-	–	0.084	0.086

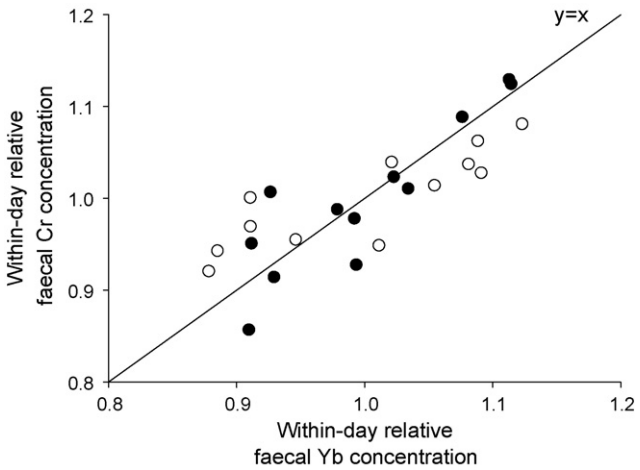

Fig. 5. Within-day relative variation of faecal Yb and Cr concentrations at two feeding levels (●: 100% *ad libitum*; ○: 70% *ad libitum*). Faeces are collected six times daily at 06:00 h, 09:00 h, 12:00 h, 15:00 h, 18:00 h and 21:00 h. Faecal marker concentrations are expressed as a proportion of the mean of six values for each feeding level and each period ($n = 24$).

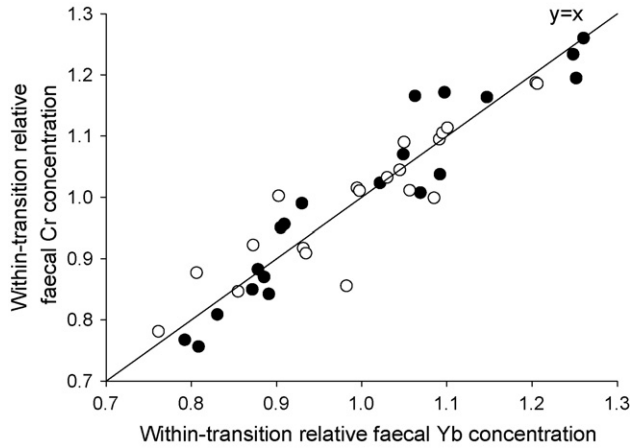

Fig. 6. Between-day relative variation of faecal Yb and Cr concentrations during the 5-day transition period between feeding levels. During the 5-day transition period, feeding level changes from 100% to 70% *ad libitum* (four cows, ●) or from 70% to 100% *ad libitum* (four cows, ○). Faecal marker concentrations are expressed as a proportion of the mean of the five values.

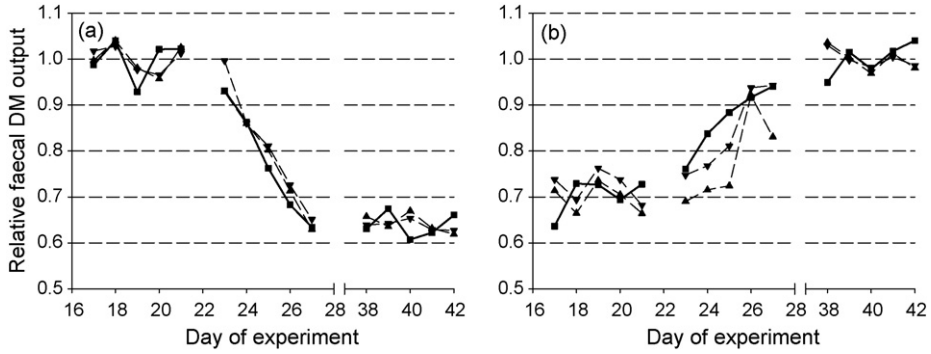

Fig. 7. Between-day relative variation of measured faecal DM output (■), faecal DM output estimated from Cr (▼), and faecal DM output estimated from Yb (▲), during period 1 (days 17–21), transition period (days 23–27), and period 2 (days 38–42). (a and b) Average values for the four cows from high to low feeding level and for the four cows from low to high feeding level, respectively. All daily values are expressed as a proportion of the 5-day mean value measured during the high feeding level period.

4. Discussion

The main objective of the present study was to determine the potential of ytterbium oxide as an external marker for predicting the variability of faecal DM output in dairy cows, in order to replace chromium oxide in cattle nutrition studies. From the constancy of faecal recovery as a function of feeding level, faecal sampling method and type of cow, as well as the between-day and the within-day variability of both faecal marker concentrations, we clearly demonstrated that ytterbium oxide has the same accuracy as chromic oxide for estimating the variability of faecal DM output.

4.1. Faecal recovery of markers

In the present study, the actual faecal DM output was over-estimated by 5–10% because of the incomplete recovery of Cr_2O_3 and Yb_2O_3 (0.89 and 0.93, respectively). This average Cr recovery is within the bracket of lowest values reported by Le Du and Penning (1982). Reviewing 55 indoor studies, these authors reported an average recovery of Cr of 0.96, with a standard deviation of 0.05, suggesting than 0.30 of the cited faecal Cr recoveries were lower than or equal to 0.91. Faecal recovery of chromic oxide close to or lower than 0.90 has been previously reported by several authors (Raleigh et al., 1980; Moran et al., 1987; Moshtaghi Nia and Wittenberg, 2002).

To our knowledge, there are no reported data concerning the faecal recovery of orally administered ytterbium oxide in ruminants. When offering $YbCl_3$-labelled forage to sheep, Hatfield et al. (1990) reported high Yb faecal recovery with values close to 1.1. The faecal Yb recovery appears close to 1 when the marker is continuously infused as chloride or acetate (Siddons et al., 1985; Peyraud, 1987; Brandyberry et al., 1991). As observed in the present study, Peyraud (1987) found slightly higher recovery for Yb (as chloride) than for Cr (as oxide).

Even assuming some digestive absorption of the markers, many authors have discussed a number of reasons that could explain the incomplete apparent recovery of external markers: loss of marker after administration due to total or partial regurgitation, loss of markers into the ruminal fluid through the canula in fistulated animals, incomplete faecal collection, and biases during analytical laboratory procedures (Kotb and Luckey, 1972; Owens and Hanson, 1992; Peyraud, 1997; Penning, 2004). In our study, the difference (0.04) between recoveries of Cr and Yb cannot be attributed to the method of marker administration or the faecal collection method, since these two procedures remained strictly similar between the two markers. A possible reason could be related to the analytical procedures and calculations. As the faecal recovery is calculated from the ratio between the amounts of excreted and dosed marker, a small analytical error on either or both of these parameters may rapidly affect this ratio and the final estimation of recovery. Since Yb was determined by standard addition method with atomic absorption spectrometry, possible biases due to the large difference in ash concentration between concentrate and faeces are minimized. Such a procedure was not necessary for the colorimetric determination of Cr in concentrate and faeces, and this could lead to a slight bias in estimating the absolute value of Cr recovery. However, these analytical considerations do not prevent from comparing the relative variation of faecal concentration and recovery of the two markers.

The faecal recovery of Yb, like that of Cr, was not affected by a strong decrease of feeding level, which is an essential quality for use in nutrition studies, allowing unbiased relative comparisons between feeding levels. Although of primary importance in nutrition studies, there are few reports of the influence of feeding level on the recovery of external markers. As seen in our trial, Raleigh et al. (1980) reported on steers no variation of Cr recovery between feeding levels of 80% and 100% *ad libitum*. Moran et al. (1987) reported a lower Cr recovery in dry cows eating 0.61 of the intake level of lactating cows, relating this result not only to feeding level as such but also to changes in gut motility and ruminal activity.

The method of marker administration, orally for intact cows or ruminally for fistulated cows, did not affect the recovery of either marker. This result would indicate that the dilution of markers in the ruminal contents and the transit of markers in the digestive tract were similar in both fistulated and intact cows, and that losses of markers via the cannula were negligible. Raleigh et al. (1980) also reported similar Cr recovery between intact and ruminally fistulated steers. When fistulated cows are used in nutritional studies, we can thus recommend introducing the markers via the cannula, which facilitates control of the amount of marker administered, avoiding between-day variations in the amount of dosed marker due to eventual regurgitation or refusals when the marker is mixed in a pelleted concentrate.

4.2. Within-day variability of faecal marker concentration and representativity of milking faecal collection

The within-day variations of faecal Yb concentration were similar to those observed with Cr, demonstrating that Yb has the same limits as Cr regarding its irregular transit in the digestive tract. This result is in agreement with the few studies comparing Cr (oxide) and Yb (chloride) (Prigge et al., 1981; Brandyberry et al., 1991). These comparisons reveal similar daily patterns of faecal Cr and Yb concentrations, either when markers are administered once daily through a gelatine capsule (Prigge et al., 1981) or when continuously infused into the rumen (Brandyberry et al., 1991). The within-day relative variation observed (±0.10–0.15) for faecal Cr and Yb concentrations is similar to that reported by these authors (±0.20 for Prigge et al., 1981, ±0.10 for Brandyberry et al., 1991). Many authors have reported similar values of about 0.10–0.20 for Cr (Raleigh et al., 1980; Bartiaux-Thill and François, 1980; Moran et al., 1987; Malossini et al., 1996).

It is noteworthy that, compared to feeding twice daily *ad libitum*, feeding the cows only once daily at 70% *ad libitum* did not increase the variability of the within-day excretion pattern of the two markers. This result indicates that, at least with

twice daily administration, the feeding pattern has little overall effect on the faecal marker dilution in digesta. Faecal output variations appear to be satisfactorily estimated from Cr or Yb in a wide range of feeding strategies. This also implies that faecal grab sampling could remain relatively representative of total faecal collection under disturbed feeding strategies when marker is administered twice daily. On the contrary, once-daily marker administration has long been known to increase the within-day variability of faecal marker concentrations, decreasing the ability of grab sampling to accurately represent the daily excretion of markers (Prigge et al., 1981). In the present study, with low feeding level and once-daily feeding, milking faecal sampling was found to be just as accurate as total faecal collection for estimating faecal output, both for Yb and Cr.

4.3. Between-day variability of faecal marker concentration

Ytterbium oxide clearly displayed similar overall behaviour as chromium oxide in the digestive tract, as supported by their similar between-day CV of faecal markers concentration, irrespective of feeding level and faecal sampling method. The strong positive correlation between their relative between-day faecal concentrations is also in good agreement with the observed within-day pattern. This could not be an analytical artefact, since laboratory procedures are different between Cr and Yb. Despite of the difference in their molecular weight, the two markers seem to exhibit a similar transit in the digestive tract, even if they are not really associated with either the flux of liquid or solid phases of digesta (Faichney, 1975). The dilution of both markers in a pelleted concentrate probably contributed to this similar transit behaviour. Transitory or partial retention of markers in some parts of the digestive tract, particularly in the rumen, is sometimes suggested to explain the variability of faecal excretion or discrepancies between marker excretion (Chamberlain and Thomas, 1983, Titgemeyer, 1997). From our study, it is clear that any eventual retention of markers in the rumen would be insufficient to affect the faecal excretion of Yb compared to Cr.

No studies were reported on Cr and Yb comparing the between-day variability of faecal concentrations of these markers. In our study, the between-day coefficients of variation of the faecal Cr concentration (0.05–0.06 with total collection, 0.08–0.10 with milking-time collection) are in the range reported by Bartiaux-Thill and François (1980) and Mélix et al. (1987).

4.4. Faecal marker excretion during non-steady state transition between feeding levels

Various authors have concluded that it is inaccurate to use markers to estimate faecal output under non-steady state conditions (Raleigh et al., 1980; Penning, 2004). However, there have been no previous trials on the ability of markers to predict the variation of faecal output under such conditions. In the present study, we test the ability of Yb and Cr to predict the strong and rapid variations of faecal DM output due to a 0.08 daily reduction or increase in feeding level over 4 days.

The very good ability of both Cr and Yb to predict – without any time lag – the strong decrease of faecal DM output shows that the daily increase in faecal marker concentration is directly proportional to the daily decrease of faecal DM output. This result does not support the hypothesis of transitory retention of either Cr or Yb in the digestive tract; retention sometimes being proposed to account for the low recovery or high variability of faecal marker excretion (Chamberlain and Thomas, 1983; Moran et al., 1987; Titgemeyer, 1997). One practical consequence of our results is that, by using either Cr or Yb as external markers, it should be possible to obtain an accurate estimate of the decline of pasture intake or at least faecal output in rotational grazing systems from the first to the last day in each paddock (Wade et al., 1989). However, it should be pointed out that the 0.08 between-day variability of feeding level and faecal output that we imposed is close to the random between-day variability of faecal marker concentration observed during the steady-state period (0.05–0.10). Therefore, it appears entirely out of the question to estimate the between-day variation of faecal output from individual cows based on daily variations in feeding level. Only average daily variations of faecal marker concentrations at the herd level can allow us to estimate daily variations of faecal output. Further investigations are needed to check these preliminary results.

Neither Cr nor Yb was able to predict accurately the daily increase in faecal DM output when feeding level was increased by 0.08 daily over 4 days. This result suggests that the concentration of faecal markers remains higher than expected during the first days of the transition, and that the dilution of both markers in digesta requires a certain time lag. It could be hypothesized that both markers tend to transit more rapidly than digesta, which also supports the absence of any retention of markers in the digestive tract.

5. Conclusion

In dairy cows fed a total mixed ration at 70% or 100% of their *ad libitum* DM intake, the average faecal recovery of ytterbium oxide was slightly higher than with chromic oxide, but faecal recovery was less than 1 for both markers.Faecal Yb and Cr recoveries were unaffected by feeding level, both with total and with milking faecal collection. Ytterbium oxide and chromic oxide showed similar between-day and within-day variabilities of faecal concentration during the steady-state periods of the experiment. Both ytterbium oxide and chromic oxide showed the same accuracy in estimating the increase or decrease of faecal DM output during a non steady-state transition period of five days between feeding levels. We can conclude that ytterbium oxide seems to have the appropriate characteristics to be used as a digestive marker in cattle nutrition studies at grazing. However, an incomplete faecal recovery of 0.90–0.95 should be considered to not over-estimate faecal output.

Acknowledgments

The CONACYT is gratefully acknowledged for financing the PhD studies of E. Pérez-Ramírez. Many thanks are due to P. Lamberton, A. Cozien, D. Chevrel, M. Texier, J.L. Harel and B. Gréhal (Méjusseaume experimental farm, INRA, UMR1080 Production du Lait) for measurements, milking, feeding and care of the cows throughout the experiment, as well as A. Brasseur, I. Jicquel and T. Le Mouel (INRA, UMR1080 Production du Lait, Saint-Gilles) for laboratory chemical analyses. M.S.N. Carpenter post-edited the English style.

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