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MEASUREMENT OF ILEAL DIGESTIBILITY IN RABBITS: AN INTER-LABORATORY STUDY TO COMPARE TWO MARKERS AND TWO FREQUENCIES OF DIGESTA COLLECTIONS

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ABSTRACT

In four laboratories, a total of 52 adult rabbits were fitted with a single T glass cannula at terminal ileum. After surgery recovery, all animals were fed *ad libitum* with the same batch of a diet doubly labelled, by addition of Yb attached to fibre particles and Cr as Cr_2O_3. Faecal recovery of both markers and their concentration in ileal digesta and soft faeces were determined to study their suitability to be used in ileal digestibility measurements. The effect of frequency of ileal digesta collections (one or two per day) on ileal digesta composition and ileal digestibility of DM, CP, NDF and ADF was evaluated.

Yb behaved as expected for a marker of the digesta flow. On the contrary, Cr content was lower in hard faeces (201.4 ± 3.8 mg/kg DM) than in ileal digesta (248.6 ± 3.7 mg/kg DM) and it showed the lowest values in soft faeces (136.2 ± 3.7 mg/kg DM), that might result in an overestimation of the ileal digestibility; in addition, its faecal recovery was very low (53.6 ± 1.3 %). These findings support that Cr added to diet as Cr_2O_3 seems inadequate to labelling rabbit diets.

No effects of frequency of ileal digesta collections on ileal digesta composition and ileal digestibility were detected; that allows to reduce the length of ileal digesta sampling period from 6 to 3 days.

Ileal digestibility of DM averaged 38.1% and differences among laboratories tended to be significant (P=0.064), being its residual variability (CV=17.9%) higher than that observed for faecal digestibility of DM (CV=3.5%). Ileal and faecal digestibility of CP, NDF and ADF differed significantly among laboratories, being the residual variability also higher for ileal than for faecal values.

INTRODUCTION

Ileal flow and digestibility measurements in rabbits are necessary to evaluate more precisely the digestion and nutritive value of diets. These studies involve an experimental model based on rabbits fitted with ileal cannula. This model has been validated by comparing feed intake, soft faeces excretion, rate of passage and faecal digestibility in cannulated and non-cannulated animals (Gidenne and Ruckebusch, 1989; Gidenne *et al.*, 1994; Merino, 1994; Amber, 1997).

However, data on ileal digestibility in rabbit literature are normally affected by very large residual variability if compared with that affecting faecal digestibility. Logically, this fact limits the validity of values if the number of involved animals is not large enough, being that

quite complicate because the use of this model implies very hard work. In addition, some differences in methodology to evaluate ileal digestibility might increase the difficulty.
This work presents results of a ring-test involving four laboratories to compare two types of diet labelling (Yb attached to fibre particles or Cr as Cr_2O_3 and two frequencies of ileal digesta collections (one or two per day). This ring-test was managed by the EGRAN research group (Gidenne, 1999), as a part of a common project to standardise procedures to evaluate ileal digestibility in rabbit.

MATERIAL AND METHODS

Animals. In four laboratories, a total of 52 adult rabbits were fitted with a single T glass cannula at ileum, 10-15 cm before the ileo-ceco-colic junction, according to the technique described by Gidenne *et al.* (1988). After surgery recovery (feed intake higher than 85% of that recorded in the week previous to surgery and liveweight higher than 95% of that in the moment of cannulation), the animals were used in a digestibility trial consisting of successive periods of: a) adaptation to diet (9 days); b) faecal digestibility (4 days); c) two periods of ileal digesta sampling differing in the frequency of ileal digesta collection, one per day (Fr 1) or two per day (Fr 2), each period including 6 collections of 30-60' (or until collecting 40 g of fresh matter) covering a 24-hour cycle (i. e. at 1, 5, 9, 13, 18 and 21 h); d) caecotrophy measurement, including 2 collections separated by an interval of 48 h, with rabbits wearing a plastic collar for 24 h (from 8:30 h). Animals were fed *ad libitum* and had a light:dark cycle of 12:12 h, with light period from 7:30 h.

Diet. The same batch of a diet based on alfalfa hay, barley and soybean meal was used in all laboratories. This diet was doubly labelled, by addition of Yb attached to fibre particles of alfalfa hay according Uden *et al.* (1980) and Cr as Cr_2O_3 powder included in the mineral premix. The analytical composition of this diet in each laboratory is presented in Table 1.

Table 1.- Analytical composition of diet (g/kg DM).

	Lab 1	Lab 2	Lab 3	Lab 4
CP	193.3	171.8	177.7	191.4
NDF	321.2	336.5	355.2	379.4
ADF	208.5	210.0	199.7	230.7
Yb (mg/kg DM)	1.644	1.496	1.455	1.300
Cr (mg/kg DM)	137.9	159.8	158.5	130.0

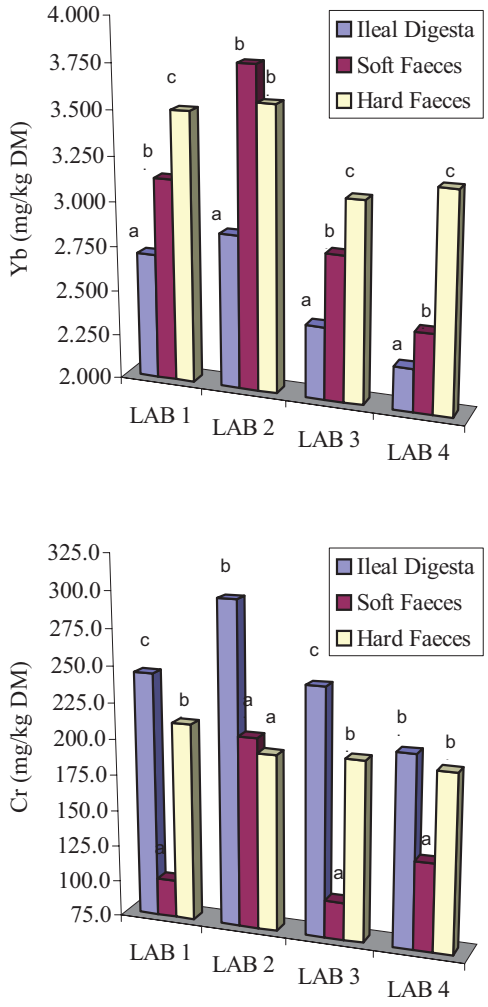
Chemical analyses. Chemical analyses were carried out on diet, hard faeces (oven dried), soft faeces (freeze-dried) and ileal digesta (freeze-dried). The methods of the AOAC (1984) were followed for DM and CP. NDF and ADF were analysed according to Van Soest *et al.* (1991). Yb and Cr were analysed by atomic absorption spectrometry after ashing and acid extraction with HNO_3 (1.5 N).

Calculations and statistics. Ileal flow of nutrients (IF, g/day) was calculated as $IF=(F*M_F+SF*M_{SF})*A_{ID}/M_{ID}$, where F is the feed intake during the faecal digestibility period (g DM/day), SF is the soft faeces excretion (g DM/day), M_F, M_{SF} and M_{ID}, are the marker content in feed, soft faeces and ileal digesta respectively (mg/kg DM), and A_{ID} is the content

of the concerned nutrient in ileal digesta (g/g DM). Ileal digestibility (ID, %) was expressed as ID=(I_F+I_{SF}-IF)*100/ I_F, where I_F is the intake as feed and I_{SF} is the intake as soft faeces.
Effect of frequency of ileal collections (Fr 1, Fr 2) on ileal digesta content of each marker was analysed using a *t* test for pair-wise data. General linear model (GLM) procedure of SAS (1990) was used to study: a) the effects of sample (ileal digesta, soft faeces, hard faeces) and laboratory (Lab 1, Lab 2, Lab 3, Lab 4) on the content of each marker; b) the effects of frequency and laboratory on ileal composition and digestibility; c) the effect of laboratory on soft faeces composition and faecal digestibility.

RESULTS AND DISCUSSION

Figure 1.- Content of Yb and Cr in ileal digesta, soft faeces and hard faeces in the different laboratories.



Frequency of collection had no effect on marker content of ileal digesta, for both Yb and Cr. Thus, the difference between collections with Fr 1 and Fr 2 was 0.003 ± 0.038 mg/kg DM (P=0.95) for Yb content and 3.0 ± 4.1 mg/kg DM (P=0.46) for Cr content. The same pattern was also observed when analysing separately the data of each particular laboratory. For this reason, the mean value of two frequencies was used to compare the content of markers in ileal digesta, soft faeces and hard faeces. Figure 1 shows the content of Yb and Cr in ileal digesta, soft faeces and hard faeces. Yb level changed as expected for a marker, being its level clearly

higher in hard faeces than in ileal digesta in all laboratories and showing an intermediate value in soft faeces, except in Lab 2. On the contrary, variations in Cr level, lower in hard faeces than in ileal digesta (except in Lab 4) and showing the lowest values in soft faeces (except in Lab 2), were quite surprising for a marker and might result in an overestimation of the ileal digestibility. These differences between both markers are corroborated when calculating their faecal recovery; thus, Table 2 shows an incomplete faecal recovery of Yb (87.5 ± 1.3 %) but a very low value in the case of Cr (53.6 ± 1.3 %).

Table 2.- Faecal recovery of markers (%, LSM and SE).

	Lab 1		Lab 2		Lab 3		Lab 4	
Yb	84.2 [a]	2.5	88.1 [ab]	3.0	82.9 [a]	2.0	95.0 [b]	2.5
Cr	60.8 [b]	2.5	46.1 [a]	2.7	48.9 [a]	2.0	58.7 [b]	2.7

[a, b] Values in the same row with different superscript differ with $p<0.05$

These findings seem support the use of Yb linked to fibre particles as marker in studies on ileal digestibility in rabbit. However, more work is necessary to know the possible effects of the size of fibre particles and its level of inclusion in the diet. On the contrary, Cr added to diet without attaching to fibre, as Cr_2O_3, seems inadequate to this aim, in contrast to trials performed in pigs and poultry. As an hypothesis, the high density and very little size of particles of Cr_2O_3 (fine powder) could cause a distinctive transit of this marker through the ileo-caeco-colic junction, caecum and proximal colon: taking into account its low faecal recovery, this marker perhaps could be accumulated in the distal part of caecum. Consequently, only Yb was in fact used as marker in the current study.

Table 3 shows no effects of frequency of ileal collections on ileal digesta composition and ileal digestibility. Similar results had been reported by Merino (1994) using Cr attached to fibre particles. Thus, it would be advisable to perform two collections per day, in order to reduce the length of ileal digesta sampling period from 6 to 3 days. The values differed significantly between laboratories. No significant interactions between frequency and laboratory were detected.

Table 3.- Statistical significance of the effects of frequency of ileal collections (Fr), laboratory (Lab) and their interaction (Fr*Lab) on ileal digesta composition and ileal digestibility when using Yb as marker.

	P		
	Fr	Lab	Fr*Lab
Ileal digesta composition			
Yb	0.718	< 0.001	0.295
CP	0.866	0.029	0.837
NDF	0.101	< 0.001	0.612
ADF	0.622	< 0.001	0.906
Ileal digestibility			
DM	0.780	0.064	0.375
CP	0.516	0.027	0.643
NDF	0.781	< 0.001	0.883
ADF	0.910	< 0.001	0.766

Table 4.- Composition of ileal digesta and soft faeces (g/kg DM, LSM and SE).

	Lab 1		Lab 2		Lab 3		Lab 4	
Ileal digesta								
Yb (mg/kg DM)	2.700 [c]	0.042	2.811 [c]	0.053	2.423 [b]	0.035	2.239 [a]	0.042
CP	149.6 [b]	3.4	136.5 [a]	3.8	142.0 [ab]	2.8	150.0 [b]	3.6
NDF	410.9 [a]	4.3	413.7 [a]	4.7	462.1 [c]	3.4	435.2 [b]	4.5
ADF	272.7 [a]	2.5	273.1 [a]	2.8	291.8 [b]	1.9	329.4 [c]	2.6
Soft faeces								
Yb (mg/kg DM)	3.128 [c]	0.077	3.776 [d]	0.084	2.822 [b]	0.064	2.443 [a]	0.077
CP	317.1 [b]	5.6	273.4 [a]	6.1	334.7 [c]	4.4	311.6 [b]	5.8
NDF	435.2 [ab]	8.3	431.9 [a]	9.1	455.1 [b]	6.6	426.9 [a]	8.3
ADF	249.0 [b]	5.7	291.7 [c]	6.3	231.6 [a]	4.6	287.8 [c]	6.0

[a, b, c] Values in the same row with different superscript differ with $p<0.05$

The effect of laboratory on ileal digesta and soft faeces composition is presented in Table 4. The differences between laboratories in the Yb content seem to be related with the differences observed in the diet (see Table 1). Daily soft faeces excretion averaged 27.7 g DM and varied between laboratories ($P<0.001$), from 19.7 ± 2.4 g DM in Lab 1 to 32.4 ± 1.9 g DM in Lab 3.

Table 5 shows the effect of laboratory on faecal and ileal digestibility. Faecal digestibility of DM averaged 61.4% (CV=3.5%) and, as expected, was quite similar in all laboratories ($P=0.089$). Differences in ileal digestibility of DM were higher but not statistically significant because its residual variability was much higher (CV=17.9%). On the other hand, using data from literature, Amber (1997) reported a linear regression equation relating ileal digestibility of OM (y) and faecal digestibility of OM (x): $y=-57.3+1.56x$ ($r^2=0.75$, RSD=6.1, n=12); if assuming the validity of this equation for DM, the average value of ileal digestibility obtained in the present work (38.1%) is very near to the expected value (38.5%).

Table 5.- Faecal and ileal digestibility (%, LSM and SE).

	Lab 1		Lab 2		Lab 3		Lab 4	
Faecal digestibility								
DM	60.42	0.62	62.78	0.68	61.22	0.49	61.62	0.62
CP	63.02 [a]	0.81	67.60 [c]	0.89	64.54 [ab]	0.65	66.07 [bc]	0.81
NDF	24.5 [a]	1.1	31.7 [b]	1.2	32.8 [b]	0.9	33.3 [b]	1.1
ADF	21.8 [a]	1.2	24.9 [ab]	1.4	22.1 [a]	1.0	26.7 [b]	1.2
Ileal digestibility								
DM	37.0	1.4	41.7	1.7	36.5	1.1	39.2	1.4
CP	61.6 [a]	1.6	64.5 [ab]	2.1	68.2 [b]	1.4	66.5 [ab]	1.7
NDF	20.1 [a]	2.1	30.0 [b]	2.6	16.9 [a]	1.7	30.7 [b]	2.2
ADF	16.3 [c]	2.3	25.8 [d]	2.8	1.5 [a]	1.9	11.5 [b]	2.4

[a, b, c, d] Values in the same row with different superscript differ with $p<0.05$

Some differences in faecal and ileal digestibility of CP were detected between laboratories. The average values were respectively 65.1% (CV=4.3%) and 66.0% (CV=11.6%). In other

studies (Gidenne and Ruckebusch, 1989; Gidenne, 1992; Merino and Carabaño, 1992; Merino, 1994; Amber, 1997), ileal digestibility of CP averaged 90% of its faecal value. High ileal digestibility of CP may be related with recycling of microbial protein by caecotrophy, as it is well established that caecotrophy contributes strongly to protein digestion (Carabaño and Fraga, 1989). In fact, the highest value is observed in Lab 3 where both soft faeces excretion and its CP content show the highest figures.

Both faecal and ileal digestibility of fibrous fractions varied between laboratories. For NDF, the average values were respectively 30.8% (CV=12.2%) and 23.0% (CV=42.5%); for ADF, the figures were respectively 23.6% (CV=18.3%) and 11.2% (CV=105.5%). The high pre-caecal digestibility of fibre generally observed in the current study, together with its large residual variability, reveals methodological limitations affecting the accuracy of measurements on ileal flow of DM and, probably some problems in fibre analyses of ileal digesta (Weltzien and Aherne, 1987). However, we cannot exclude that fibre could be partly degraded before caecum, because of a recycling through caecotrophy of some bacterial fibrolytic enzymes, such that found in stomach by Marounek *et al.* (1995).

In conclusion, Yb linked to fibre particles is a suitable marker in studies on ileal digestibility in rabbit (Cr added to diet as Cr_2O_3 seems inadequate) and it is advisable to perform two collections of ileal digesta per day instead of one per day in order to reduce the length of ileal digesta sampling period. More work is necessary to reduce intra and inter-laboratory variability and meet a standard method for ileal digestibility measurements allowing more progress in rabbit nutrition.

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