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INTERLABORATORY STUDY ON ILEAL DIGESTIBILITY IN RABBITS: THE EFFECT OF DIGESTA COLLECTION TIME AND A SIMPLIFICATION OF THE PROCEDURE

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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, animals were fed *ad libitum* with the same batch of a diet labelled by the addition of ytterbium attached to fibre particles. The effect of time during the ileal digesta collection period (morning and evening) on ileal digesta composition and ileal digestibility of DM, CP, NDF and ADF was evaluated. Compared with the evening samples, the morning samples had higher content of ytterbium (+5%) and CP (+43%) but lower contents of NDF (-9%) and ADF (-11%). Consequently, ileal digestibility for DM, NDF and ADF resulted in higher figures when calculated from the morning samples as opposed to those from the evening samples. However, CP ileal digestibility results were just the opposite. When compared with the results obtained as a reference value from the 24-hour samples (a pool of collections obtained in the morning, evening and night), the data average from the morning and the evening samples did not differ significantly. Therefore, it is possible to reduce the number of collections and simplify ileal digesta sampling by avoiding night collections.

Key words: ileal digestibility, sampling procedure.

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INTRODUCTION

Ileal digestibility measurements in rabbits are necessary to quantify more precisely the digestion and nutritive value of diets, as well as to study the relationship between the composition of ileal flow and the caecal microbial activity. 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, as well as the rate of passage and faecal digestibility in cannulated and non-cannulated animals (GIDENNE and RUCKEBUSCH, 1989; GIDENNE *et al.*, 1994; CARABAÑO and MERINO, 1996; AMBER, 1997).

However, it would appear the different methodologies used by laboratories to evaluate ileal digestibility complicates the comparison and interpretation of results. Therefore, the EGRAN research group (GIDENNE, 1999) endeavoured to standardise the evaluation process of ileal digestibility in rabbit. In previous studies within the framework of this project, BLAS *et al.* (2000) reported that ytterbium attached to fibre particles was a more adequate marker than was chromium added as Cr_2O_3. Furthermore, it was shown that the frequency of ileal digesta collections (one or two per day) did not affect ileal digesta composition or ileal digestibility.

Usually, the procedure to obtain ileal digesta samples involves collecting at different times in order to gather representative samples of digesta as it passes through the ileum in a 24-hour cycle. In rabbits, changes in ileal digesta composition during the day are mainly due to the circadian pattern of feed and soft faeces intake (CATALA, 1976; GIDENNE and PONCET, 1985; MERINO, 1994). It was the aim of this research to study the changes on ileal digesta composition and ileal digestibility resulting from the time at which digesta collections were made. This was done for the purpose of exploring possible ways of simplifying the ileal digesta sampling by decreasing the number of collections involved.

MATERIAL AND METHODS

Animals, feeding and housing

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, in accordance with the technique described by GIDENNE *et al.* (1988). In the present study, all steps of the surgical procedure were standardised among the laboratories.

Surgery recovery was archieved when feed intake was more than 85% of that which had been recorded in the week previous to surgery, and liveweight was more than 95% of what it was at the moment of cannulation. After recovering from surgery, the animals were used in a digestibility trial consisting in four successive periods.

Table 1: Experimental diet.

Ingredients	(%)
Alfalfa hay	50
Barley	35
Soybean meal (44% CP)	12
Dicalcium phosphate	2.0
Sodium chloride	0.4
Trace minerals/vitamins mixture	0.2
Ytterbium-labelled alfalfa hay	0.4

Analytical composition*	g/kg DM
CP	184
NDF	348
ADF	212
Ytterbium (mg/g DM)	1.47

* Average of analysing in the four involved laboratories.

First, the animals were allowed to adapt to the diet for nine days. This was followed by four days of faecal digestibility and an ileal digesta sampling, in two consecutive periods, each one involving 6 collections; these collections did not exceed 60 min (or until 40 g of fresh matter was collected), spanning a 24-hour cycle (i. e. at 1, 5, 9, 13, 18 and 21 h), with 12-28 hour intervals between two consecutive collections. Finally, caecotrophy measurement was carried out, including two 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 feed intake was recorded throughout the four periods. The same batch of an experimental feed elaborated in one laboratory was used in all four laboratories. The diet consisted mainly of alfalfa hay, barley and soybean meal. It was labelled by the addition of ytterbium, which was attached to fibre particles of alfalfa hay in accordance with UDEN *et al.* (1980); the ingredients and the analytical composition of the diet is presented in Table 1. During the experiment rabbits, which were housed individually in metabolism cages, had a light:dark cycle of 12:12 h, with a light period at 7:30 h.

Ileal digesta samples

From the ileal digesta collections mentioned above, three different samples of ileal digesta per rabbit and period were obtained. These consisted of 24-hour samples, by pooling half-collections performed at 1, 5, 9, 13, 18 and 21 h; morning samples, by pooling half-collections performed at 9 and 13 h; and evening samples, by pooling half-collections performed at 18 and 21 h.

Chemical analyses

Chemical analyses were carried out on diet, faeces (oven-dried), soft faeces (freeze-dried) and ileal digesta (freeze-dried). The EGRAN methods (2001), which derive from AOAC (1984), were followed for DM and CP. NDF and ADF were analysed sequentially in accordance with VAN SOEST *et al.* (1991). Ytterbium was analysed by atomic absorption spectrometry after ashing and acid extraction with 1.5 *M* HNO_3 (GARCÍA *et al.*, 1999).

Calculations and statistics

The ileal flow of nutrients (IF, g/day) was calculated as $IF=(F\times M_F+SF\times M_{SF})\times A_{ID}/M_{ID}$, 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 concentration 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 $ID=(I_F+I_{SF}-IF)\times 100/I_F$, where I_F is the intake as feed and I_{SF} is the intake as soft faeces.

Feed intake during the faecal digestibility period, faecal digestibility, soft faeces excretion and soft faeces composition were analysed with the effect "laboratory" as a block. Ileal digesta composition and ileal digestibility were analysed with time of the ileal digesta collections (morning sample, evening sample) as main factor, laboratory as a block, and rabbits nested to laboratory as a factor of repeated measures. Statistical analyses were performed using the general linear model (GLM) procedure of Statistical Analysis System (SAS, 1997).

Morning and evening samples were not independent of 24-hour samples obtained in the same period, due to the method used to constitute ileal digesta samples. Thus no comparison could be performed between them. However, Student's t-tests for paired data were used to compare values for 24-hour samples obtained in a period with those calculated by averaging data from morning and evening samples obtained in the other period, weighed according to their weight.

RESULTS AND DISCUSSION

Feed intake during the faecal digestibility period, faecal digestibility, soft faeces excretion and soft faeces composition are presented in Table 2. The estimated contribution of soft faeces to the total intake of nutrients and the marker was 13%, 21%, 16%, 16% and 24% for DM, CP, NDF, ADF and ytterbium respectively.

Table 2: Feed intake, faecal digestibility and soft faeces production.

	Mean	RSD*
Feed intake (g DM/day)	178	26
Faecal digestibility (%)		
DM	61.4	2.1
CP	65.1	2.8
NDF	30.8	3.8
ADF	23.6	4.3
Soft faeces excretion (g DM/day)	27.7	8.4
Soft faeces composition (g/kg DM)		
CP	314	19
NDF	440	29
ADF	259	20
Ytterbium (mg/kg DM)	2.99	0.27

* Residual standard deviation, degrees of freedom of error = 49.

Table 3 shows the effects of time at the ileal digesta collection on the ileal digesta composition and ileal digestibility. Compared with the evening samples, the morning samples had a higher content of ytterbium (+5%) and CP (+43%) but lower contents of NDF (-9%) and ADF (-11%). Consequently, ileal digestibility for DM, NDF and ADF, when calculated from the morning samples, resulted in higher figures than those calculated from the evening samples. However, CP ileal digestibility results were just the opposite. Moreover, it was observed that all these variables differed significantly among laboratories. Figure 1 illustrates noteworthy interactions involved between the time of the ileal digesta collections and the laboratory. The differences in the CP content of ileal digesta and the ileal digestibility of CP between morning and evening samples were higher in Lab 3 than in the other laboratories, while the opposite was observed in the NDF content of ileal digesta.

Differences in the composition of the morning and evening samples coincide with those reported by other authors, and could be explained as a consequence of the soft faeces intake in early morning. However, variations in endogenous nitrogen at ileal level can also be hypothesized (MERINO, 1994), especially if one considers that endogenous nitrogen accounts for 60-80% of the total nitrogen in ileal digesta (GARCÍA *et al.*, 2001). In comparison with diet, soft faeces contain more ytterbium (+106%) and CP (+69%), which might result in higher values at the ileal level after soft faeces ingestion. MERINO (1994) reported no differences in the marker content of samples taken every three hours from cannulated adult rabbits in a circadian circle; however, the changes in the CP content of ileal digesta observed in the present work followed closely the pattern described in the Merino's work, with maximum values at 9-12 h (18.7% on DM basis) and minimum at 18-21 h (11.9% on DM basis). GIDENNE and PONCET (1985) found a higher marker content trend in the ileal digesta of fattened rabbits when slaughtered at 9-12 h rather than at 18-21 h, whereas

Table 3: Effect of time of ileal digesta collections on ileal digesta composition and ileal digestibility.

	Morning	Evening	RSD*	*P*
Ileal digesta composition (g/kg DM)				
CP	174	122	22	< 0.001
NDF	413	456	30	< 0.001
ADF	277	312	23	< 0.001
Ytterbium (mg/kg DM)	2.62	2.50	0.24	< 0.001
Ileal digestibility (%)				
DM	40.7	36.8	7.1	< 0.001
CP	55.2	73.9	9.7	< 0.001
NDF	30.0	16.0	11.1	< 0.001
ADF	20.6	3.5	12.9	< 0.001

* Residual standard deviation, degrees of freedom of error = 149.

CP content was clearly higher at 9-12 h (31.5% on DM basis) than at 18-21 h (22.2% on DM basis). Likewise, CATALA (1976) observed that the CP content of ileal digesta of adult rabbits was much higher when slaughtered at 9 h (30.3% on DM basis) than at 17 h (18.6% on DM basis). The increase in the content of NDF and ADF in the soft faeces with respect to those in the diet (+26% and +23% respectively) was less significant than it was in the CP content. Thus, the highest content of CP in the ileal digesta collected in morning could dilute the fibrous fractions. Similarly, MERINO (1994) observed minimum contents of crude fiber in ileal samples obtained at 9-12 h (20.8% on DM basis) and maximum values in those obtained at 18-21 h (24.4% on DM basis). Logically, changes in composition of ileal digesta between the morning and the evening samples were reflected in the corresponding ileal digestibility values.

Table 4: Effect of sampling procedure on ileal digesta composition and ileal digestibility.

	24H samples of 1st period minus ME samples of 2nd period		24H samples of 2nd period minus ME samples of 1st period	
	Difference	*P*-value†	Difference	*P*-value†
Ileal digesta composition (g/kg DM)				
CP	- 1.4	0.62	- 3.1	0.30
NDF	- 7.2	0.09	- 0.8	0.85
ADF	- 4.1	0.25	- 0.8	0.82
Ytterbium (mg/kg DM)	- 0.022	0.59	0.039	0.30
Ileal digestibility (%)				
DM	- 0.6	0.68	0.7	0.57
CP	0.4	0.82	1.4	0.39
NDF	1.0	0.63	1.6	0.41
ADF	2.5	0.25	2.1	0.40

24H samples: by pooling 6 collections corresponding to every four hours on a 24-hour cycle
ME samples: by averaging data from morning and evening samples, † Degrees of freedom = 51.

When compared the results obtained from 24-hour samples, the results achieved by averaging data from the morning and the evening samples did not differ significantly (Table 4). Consequently, it appeared possible to simplify ileal digesta sampling by avoiding night collections, without modification in the value of the ileal flow of nutrients.

Ileal digestibility presented higher residual variability than faecal digestibility, CV being 18% *vs* 4%, 12% *vs* 4%, 43% *vs* 12% and 106% *vs* 18% for DM, CP, NDF and ADF respectively, probably because the respective authentic contributions of feed and soft faeces to the intake of nutrients and the marker were not precisely estimated. In this work, feed and soft faeces intake during the ileal sampling periods were estimated respectively as feed intake during the previous faecal digestibility assay and soft faeces excretion during the later caecotrophy measurements. Furthermore, no significant reduction in residual variability of ileal digestibility was induced when calculating from feed intake during the ileal sampling periods. In addition, the calculated contribution of the soft faeces to the total intake of nutrients and the marker varied widely among the rabbits, with coefficients of variation of

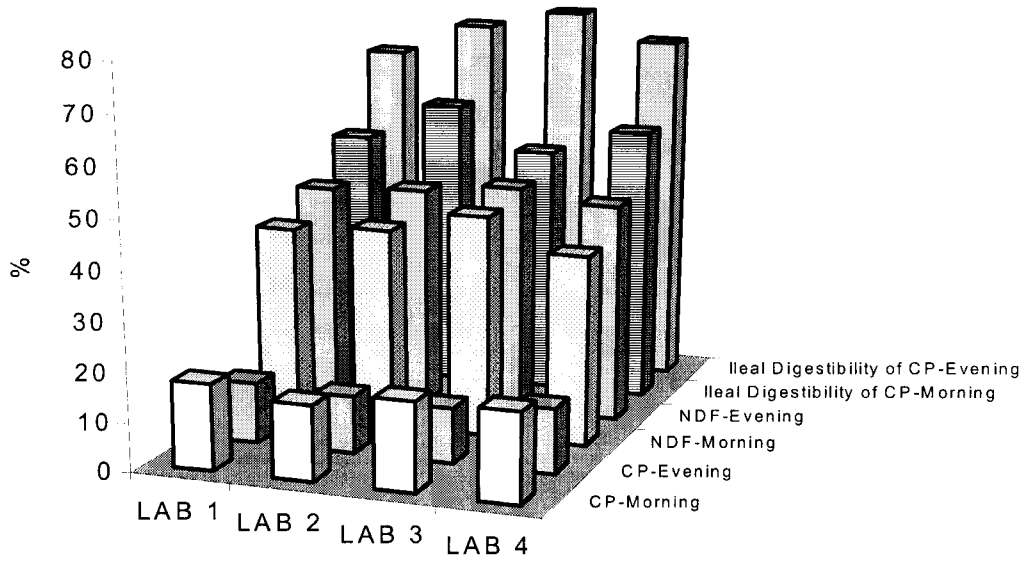

Figure 1: Interactions between time at the ileal digesta collections and laboratory on ileal digesta composition (on DM basis) and ileal digestibility.

26-29%, as GIDENNE *et al.* (1994) and CARABAÑO *et al.* (2000) also reported. A wide daily variation in feed intake and/or soft faeces excretion of cannulated adult rabbits fed *ad libitum* could be hypothesized.

The differences detected among laboratories may be due to laboratory effects on the analyses of feed, soft faeces and ileal digesta. In a ring-test, PEREZ *et al.* (1995) reported laboratory effects on the results of analyses provided by the laboratories participating in the current work.

In conclusion, the present study has indicated that it is possible to simplify the ileal digestibility procedures without affecting the validity of the measures. However, further work is needed in order to reduce intra and inter-laboratory variability and establish a standardised animal and analytical methodology for ileal digestibility measurements.

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