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COLLABORATIVE STUDIES ON CAECOTROPHY IN ADULT RABBITS: EFFECT OF FEED INTAKE AND METHODOLOGY[*]

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ABSTRACT

Methodology for measuring the soft faeces (SF) excretion and attempts to establish a relationship between SF and feed intake were treated in the present work. A first study aimed to constitute a collaborative database (n=364 data points) and to analyse the relationship between feed intake and SF excretion in ileo-cannulated adult rabbit (n=284 data obtained on 168 rabbits). Feed intake before (IBC) or during (IDC) SF collection did not differ between the first and second replicates of the collect. IBC and IDC were not affected by the time at which collar was placed (8.00 vs 14.00) for 24 h. However, SF excretion showed greater values when the collar was placed at 8.00 than at 14.00 (22.4 vs 18.6 g DM/d).

A second collaborative experiment aimed to measure SF excretion for rabbits fed the same feed and using the same methodology. SF excretion was similar among laboratories (n=3), but remained affected by a high variability. In the two studies, IDC was more correlated with SF excretion than IBC, but the relationship ($R^2<0.55$) remained too weak to be used for predictive purposes.

INTRODUCTION

Caecotrophy represents a specialised digestive strategy in rabbits that allows the ingestion of protein, enzymes, minerals and vitamins from caecal microbial activity. Soft faeces intake occurs following a marked circadian rhythm opposite to that of feed intake and implies important changes in chemical composition of digesta contents throughout the day (Carabaño and Piquer, 1998). The variation in chemical composition of ileal contents has important implications in the ileal digestibility determination. For this reason, Gidenne (1988) proposed to calculate the ileal digestibility of a diet as a balance between the sum of feed and soft faeces ingested and the ileal flow produced in a day.

Daily soft faeces excretion presents a high individual variability (30% of CV) either within the same experiment or among laboratories using the same methodology and similar diets (Gidenne et al, 1994). Reducing this variability could improve the precision of dietary ileal digestibility determination. Feed intake plays an important role both in the mechanism that regulates soft faeces excretion (Fioramonti and Ruckebush, 1976) and in the quantity of soft

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faeces excreted (Gidenne and Lebas, 1987) in growing rabbits. However, there is not information about the influence of a variation in the feed intake on soft faeces excretion in adult rabbits, particularly for ileo-cannulated rabbits used in ileal digestibility procedures.
The aim of this work was to study the effect of feed intake and methodology (effect of laboratory) on soft faeces excretion in cannulated rabbits in order to reduce ileal digestibility variability.

MATERIAL AND METHODS

Study 1: Collaborative database

The database used in this assay was prepared with the data sets of five laboratories. This implies 364 individual data points where soft faeces excretion (SF), live weight, feed intake during soft faeces collection period (IDC) and feed intake three days before of soft faeces collection period (IBC) were controlled in cannulated rabbits. Data from Lab. 1 were not considered in the statistical analysis because of the lower soft faeces excretion (13.8 *vs* 20.4 g DM / day) respect to the other data sets. The use of conic instead of flat collar for soft faeces collection could explain these differences (Gidenne and Lapanouse, 2000). Furthermore, data from Lab. 2 were discarded because they were obtained using animals with restricted feed distribution that could disturb the rhythms of soft faeces excretion (Fioramonti and Ruckebush, 1976). In consequence, 284 data points were used in the statistical analysis belong to three laboratories data sets. All these data were obtained in 168 adult cannulated animals fed *ad libitum* and wearing a flat collar for preventing soft faeces ingestion. Soft faeces excretion was twice recorded on 111 animals in two independent periods of 24 hours (first and second replicate) one-week delay. On the rest of the rabbits, only one soft faeces collection was recorded. Collar was placed on the animals at 8.00 in Lab. 3 and 5 or at 14.00 in Lab. 4 (137 and 147 data, respectively) for 24 hours. Diets used in each laboratory were different.

Study 2

In this study, all the animals were fed with the same diet and the collar was placed on the animals at the same time. Forty adult rabbits were cannulated at terminal ileum in three laboratories (10, 19 and 11 animals for Lab. 1, 2 and 3, respectively). After surgery recuperation, the same diet (18.4% CP and 34.8% NDF on DM, and based on alfalfa hay, barley, and soybean meal) was offered *ad libitum* to the animals for a period of 27 days. On the 28th day, the animals were weighed (3877 ± 52 g) and a collar was placed on the animals at 8.30. The collar was removed 24 hours later. Feed intake and soft faeces excretion were recorded in this period. The same procedure was repeated three days later. Feed intake was also controlled for three days before soft faeces collection period.
Sample of feed and soft faeces were dried at 103º C to determine dry matter content.

Statistical Analysis

Effect of replicates (first and second) on soft faeces production, IDC and IBC was analysed using a *t* test for pair-wise data. General lineal model (GLM) procedure of SAS (1990) was used to study the effect of laboratory and the time at which the collar was placed on the animals to prevent the caecotrophy. The effect of feed intake on soft faeces excretion was analysed using regression procedures (REG) of SAS (1990).

RESULTS AND DISCUSSION

Study 1

The mean values, the range, the standard deviation and the significance of replication for soft faeces excretion and feed intake are shown in table 1.

The prevention of soft faeces ingestion by wearing a collar implies a stress on the animals that, in many cases, leads to a reduction in feed intake during the soft faeces collection period and poor soft faeces excretion. In these situations, we might expect that rabbit would be more used to wear a collar when we replicate the procedure several times. In this study, no significant differences were detected between the first and second replicates neither in soft faeces excretion nor in feed intake. As no influence of replicates was observed, the average value of soft faeces excretion and feed intake for each rabbit was used in the following data analysis.

Table 1. Effect of replication on soft faeces excretion, feed intake during soft faeces collection (IDC) and feed intake before soft faeces collection period (IBC) (study 1)

	Range of values (g DM/day)		Mean (g DM/day)	Standard deviation	*t* probability
Soft faeces					
Replicate 1	46.7	5.7	20.5	8.1	
Replicate2	43.6	6.8	21.0	8.6	
Difference			-0.6	7.8	0.74
IDC					
Replicate 1	196	14	112	39	
Replicate2	225	31	125	40	
Difference			4.3	43	0.39
IBC					
Replicate 1	225	36	128	36	
Replicate2	223	27	129	31	
Difference			0.8	34	0.40

There was not effect of laboratory on both IDC and IBC, however, soft faeces excretion was affected by the laboratory ($P<0.0001$). Soft faeces excretion was 23.1, 18.8 and 18.7 g DM/day, as average for Lab. 3, 4 and 5, respectively. According to Carabaño et al (1988), Fraga et al (1991) and García et al (2000), these differences could be due to differences in the chemical composition of diets in each laboratory. Twenty-three different diets were used in experiments reviewed in this study. Other cause of the laboratory effect could be the differences in the procedures used for soft faeces collection. Two laboratories (Lab. 3 and 5) placed the collar at the beginning of soft faeces period (8.00) and the other (Lab. 4) at the end of soft faeces period previous to that collected (14.00). So, the effect of laboratory could be confounded with the effect of time at the collar is placed (TC). The effect TC on soft faeces excretion, feed intake during soft faeces collection (IDC) and feed intake before soft faeces collection period (IBC) is shown in table 2. Soft faces excretion was 20% greater when the collar was placed at 8.00 than when it was placed at 14.00, whereas the feed intake was not affected.

Table 2. Effect of the time at the collar is placed (TC) on soft faeces excretion, feed intake during soft faeces collection (IDC) and feed intake before soft faeces collection (IBC).

TC	Soft faeces (g DM/day)	IDC (g DM/day)	IBC (g DM/day)
8.00	22.4	119	128
14.00	18.6	114	126
SEM	0.8	4.5	4.0
Probability	0.0001	NS	NS

NS: not significant ($P > 0.05$)

As soft faeces excretion was affected by the TC, the study of the relationship between feed intake and soft faeces excretion was done within each data set. The results are shown in table 3. Feed intake recorded either before or during the soft faeces collection period affected positively ($P < 0.05$) soft faeces excretion. Feed intake before the soft faeces collection period only explained a small proportion of the total variability observed for the soft faeces excretion (6 and 11 % when the collar is placed at 8.00 or 14.00, respectively). However, a greater proportion of this variability was explained by IDC (27 and 52 % when the collar was placed at 8.00 or 14.00, respectively). Biological explanation of these results is unclear, mainly if we take into account that IDC occurs specially after soft faeces collection when the collar is placed at 8.00 (Carabaño and Merino, 1996). It seems that feed intake reflects the existence of uncontrolled factors that could affect both soft faeces excretion and feed intake in the same way when animals are wearing a collar. One of these factors would be the stress produced by the collar. As it is mention above, in these conditions we can observe animals with very low soft faeces excretion and feed intake.

Table 3. Relationship between soft faeces excretion and feed intake before (IBC) or during (IDC) soft faeces collection (study 1).

TC	Equation	Intercept	a1 (IDC)	a2 (IBC)	R^2	RSD	N
8.00	1	$Y = 6.6 \pm 3.1$ P =0.04	0.13 ± 0.025 P=0.0001		0.27	6.3	67
	2	$Y = 16.4 \pm 3.5$ P =0.0001		0.05 ± 0.026 P=0.0001	0.06	7.9	70
14.00	3	$Y = 4.2 \pm 2.0$ P =0.04	0.12 ± 0.016 P=0.0001		0.52	4.5	52
	4	$Y = 10.2 \pm 2.8$ P =0.0007		0.07 ± 0.022 P=0.004	0.11	6.4	76

Study 2

In the previous study many factors affecting soft faeces excretion and feed intake were confounded; thus, a second collaborative experiment was designed to study the effect of laboratory on these variables and their relationship. In this assay, all the animals were fed with the same diet and the collar was placed on the animals at the same time. The effect of laboratory on soft faeces excretion and feed intake before and during the soft faeces collection is shown in table 4. Figures that appear in the table 4 correspond to the average value of the two replicates of these variables measured in the same animal. Laboratory did not affect either soft faeces excretion or feed intake. Soft faeces excretion was greater than that recorded in previous study (30.3 vs 22.4 g DM/day). These differences could be due to differences in the diets used in both studies. In this sense, Carabaño and Merino (1996), using a diet with similar chemical and raw material composition, observed similar soft faeces excretion (29 g DM/day).

Table 4. Effect of laboratory on soft faeces excretion (SF), feed intake during soft faeces collection (IDC) and feed intake before soft faeces collection (IBC).

Laboratory	SF (g DM/day)	IDC (g DM/day)	IBC (g DM/day)
1	26.4	159	161
2	32.4	151	151
3	29.7	136	169
SEM	2.4	9	11
Probability	NS	NS	NS

NS: not significant (P > 0.05)

The relationship between soft faeces excretion and feed intake is shown in table 5. As was observed in the study 1, feed intake recorded either before or during the soft faeces collection period affected positively (P<0.01) soft faeces excretion. Also, IDC explained a greater proportion of the variability observed than when we use IBC as independent variable (50 vs 20%, respectively). However, the correlation between these variables was greater than that observed in the study 1. This effect could be due to the elimination of one important cause of variation as it is the diet. When we compared the regression equations obtained in the study 1 and 2 (equations 1 and 2 vs 5 and 6) we can observed a lack of consistence in the slopes. These slopes were greater in the study 2 than in the study 1 (0.18 and 0.09 vs 0.13 and 0.05, respectively). This implies the existence of interactions with other factors that avoid the use of these equations with predictive proposes in other experimental conditions.

Table 5. Prediction equation of soft faeces excretion (study 2).

Equation	Intercept	a1 (IDC)	a2 (IBC)	R^2	RSD	N
5	Y = 3.3 ± 4.6 P =0.47	0.18 ± 0.03 P=0.0001		0.50	6.3	40
6	Y = 15.2 ± 5.2 P =0.005		0.09 ± 0.03 P=0.004	0.20	7.9	40

In conclusion, the current method to collect soft faeces, on rabbits wearing a collar, gives reliable results among laboratories, but affected by a high variability. Feed intake is only one of the factors that influence caecotrophy and could not be used alone to predict the soft faeces excretion. As the caecotrophy is a complex behaviour, several factors are able to affect it, such as the time to start the collection or the individual biorhythm.

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