

EUROPEAN REFERENCE METHOD FOR *IN VIVO* DETERMINATION OF DIET DIGESTIBILITY IN RABBITS

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SUMMARY : This paper describes a standardized method for *in vivo* determination of total digestibility in rabbits. This method was working out within the European Group on Rabbit Nutrition (EGRAN) which involves six laboratories from five countries (Belgium, France, Italy, Portugal and Spain). The aim of the group was to propose a method at once reliable, quick and simple. This new method can be

regarded as a baseline for calibrating individual laboratory procedures. The method is based on 7 to 8 weeks old rabbits caged individually and fed *ad libitum*. The pre-experimental period lasts 7 days and the collection period only 4 days. For either feed and faeces, standardized conditions of sampling and drying are described.

RÉSUMÉ : Méthode européenne de référence pour la détermination *in vivo* de la digestibilité des aliments destinés au lapin.

Ce texte a pour objet de décrire une méthode standardisée de mesure de la digestibilité totale des aliments chez le lapin. Cette méthode a été élaborée en commun au sein du Groupe Européen de Nutrition Cunicole (EGRAN) qui rassemble six laboratoires appartenant à cinq pays (Belgique, Espagne, France, Italie et Portugal). Cette nouvelle méthode a été

conçue pour être à la fois précise, rapide, et reproductible. Facilement applicable à l'ensemble des laboratoires, elle peut désormais servir de référence dans le secteur cunicole. Cette méthode utilise des lapins de 7 à 8 semaines logés en cages individuelles et nourris à volonté. La durée de la période pré-expérimentale est de 7 jours et celle de la période de récolte de 4 jours seulement. Les conditions standardisées d'échantillonnage et de séchage sont décrites pour l'aliment et les faeces.

INTRODUCTION

At present, no official method is available for *in vivo* determination of digestibility in rabbits. The procedures used for digestibility assays are usually inconsistent : size of experiment, age of animals, length of adaptation and collection period, process of sampling and determination of dry matter (DM) of feeds and faeces.

Accordingly, several European laboratories have decided to adopt a common reference method in order to calibrate their own data and to make comparisons easier. This method was working out within the European Group on Rabbit Nutrition (EGRAN) which gathers six laboratories from five countries (Belgium, France, Italy, Portugal and Spain). The aim of the group was to propose a method at once reliable, quick and simple. In this way, two collaborative studies were undertaken successively to assess the reproducibility of

the common method and to compare the digestibility values obtained with domestic procedures. The full results of these ring-tests involving respectively four french laboratories and six european teams are reported in separate papers (PEREZ *et al.*, 1994; PEREZ *et al.*, 1995). They allow to propose this new procedure as a reference method for digestibility measurements in rabbits.

DESCRIPTION OF THE REFERENCE METHOD

1. ANIMALS

1.1 Number of replicates :

It is highly recommended to use a minimum of 8 rabbits per diet at the beginning of the experiment; 10 rabbits per diet being considered as the optimum. At the end of the experiment 7 rabbits minimum have to be considered. In case of evident health or behavior problems (e.g., diarrhoea or other diseases, low feed intake), one rabbit (among an initial group of eight) or two rabbits (among ten) can be excluded during the experiment. The exclusion cannot be based on the results of digestibility and must be explain.

1.2 Age :

At the beginning of the adaptation period, the age of the animals should be homogeneous and contained between 42 and 56 days. Weaning must occur 7 days minimum prior to the start of this period. When rabbits come from an external rabbitry, 4 days minimum must occur before starting the adaptation to the feeds.

1.3 Breed :

All the rabbits must be of the same genotype. It is recommended to use a commercial strain or breed, when the influence of the genotype is not the subject of the study.

1.4 Sex :

Not controlled or balanced.

1.5 Weight :

Homogeneous within and among groups (coefficient of variation < 10%).

1.6 Litter :

If possible (e.g., rabbits from an experimental rabbitry), the greatest number of litters should be used. Within-litter rabbits are chosen by taking into account the mean live weight of the litter (rabbits of extrem weight are excluded). Litters have to be distributed homogeneously over the treatments.

1.7 Housing :

Rabbits are individually kept in digestibility cages at the beginning of the adaptation period. The minimum cage surface recommended is 0.06 m² (e.g., width 0.20, length 0.30 m). Feeders must avoid feed waste and contamination of feed by faeces or urine. Automatic drinkers and cage floor on stainless steel or galvanized wire (mesh 1.3 cm minimum) are recommended. Faeces collection system should be designed to permit a quick escape of urine and to avoid direct contamination of faeces.

1.8 Environmental conditions :

Ideal controlled temperature is between 18 and 22°C; extrem temperature outside 15-25°C should be avoid. Relative humidity should be contained between 65 and 85%. Air ammonia concentration should not exceed 10 ppm.

2. ADAPTATION PERIOD

2.1 Length :

At least 7 days.

2.2 Feeding :

The experimental diet is always offered *ad libitum* and feed intake is measured during this period. If the experimental design includes comparisons between different diets, none of the rabbits should receive one of the experimental diet before the adaptation period.

3. COLLECTION PERIOD

3.1 Length :

4 days.

3.2 Feeding :

The experimental diet is given *ad libitum* (feeders filled for at least 4 days). Feed intake is recorded individually on the whole 4 days period (e.g., initial weight of the feeder on monday at 9:00 a.m., final weight on friday at 9:00 a.m.). Pellets found outside the feeder are stored at -18°C. Their dry matter (DM) content is determined (24 h, 103°C) at the end of the collection period in order to correct the DM intake. Caecotrophy is not prevented.

3.3. Collection of faeces :

Total faecal excretion is collected every day at the same time in the morning. The first collection occurs on the 2nd day morning and the last on the 5th morning (e.g., tuesday morning and friday morning). During the collection of faeces, attention should be given to avoid the inclusion of rabbit's lost hair. Total faecal output (hard faeces and soft faeces if any) is

collected in the same individual bag and stored at a temperature of -18°C or less.

4. FEED

4.1 Initial sampling :

At the beginning of the collection period a sample (initial sample) of around 1000 g for each experimental diet is made progressively during the feeders filling up.

4.2 Determination of feed dry matter content for digestibility calculation :

The DM content of the feed is determined at 103°C for 24 h with 4 samples of 50 g of pellets from the initial sample. It is recommended to do this determination on the first day of the collection period. Dried samples are weighed the most quickly after leaving the oven (precision minimum required: $\pm 0,01$ g). DM intake is calculated by using the mean of DM content of the 4 samples (coefficient of variation $< 4\%$).

4.3 Preparation of feed samples for chemical analysis :

At least 100 g of pellets are taken from the initial sample and ground with centrifuge or hammer-mill (sieve of 1 mm). For analytical purposes, it is necessary to perform a second evaluation of DM simultaneously with the determination of the feed constituents, to take account of the effect of storage and grinding on DM content. This new DM is determined as described above (24 h, 103°C) on a 2-5 g sample (precision of the scale: $\pm 0,1$ mg). Duplicate analyses are suggested.

5. FAECES

5.1 Determination of the total excretion of DM :

Total frozen individual faecal excretion is placed in the oven by using large plates (e.g., 25 x 30 cm). DM excreted is assessed into two successive steps : a first drying of the whole faeces at 80°C for 24 h followed by a final drying of around half part of the faeces at 103°C for 24 h. The first drying extracts the major part of the water from the faeces without deterioration of their chemical constituents. The final drying allows to eliminate the residual water. After each step of drying, plates are weighed as soon as possible (precision minimum required: $\pm 0,01$ g). Total DM excreted is calculated as follows :

$$\text{Total DM excreted} = (W1-T) \times (W3-T) / (W2-T)$$

T = weight of the empty plate

W1 = weight of the plate + total faeces dried at 80°C
 W2 = weight of the plate + remaining faeces (\approx one half of the total previously dried at 80°C)
 W3 = weight of the plate + remaining faeces dried at 103°C .

If residual pellets are observed in the dried faeces (after the 80°C drying), total weight (faeces + pellets) is measured. After manual separation, pellets are weighed and this value is subtracted from the total weight of faeces (and from the DM intake). The remaining hair should also be removed.

5.2 Preparation of faeces samples for chemical analysis :

Analyses are carried out on the part of faeces dried only at 80°C . After taking off residual pellets, faeces are ground with the same procedure used for the feed. A second determination of DM (24 h, 103°C) is also performed on the ground dried faeces simultaneously with the other analyses. Duplicate analyses are suggested.

6. DIGESTIBILITY

For each rabbit, the apparent dry matter digestibility of the diet is ultimately calculated according to the classical formula :

$$\text{DM digestibility (\%)} = 100 \times \frac{\text{DM Intake} - \text{DM excreted}}{\text{DM intake}}$$

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