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NUTRITIVE EVALUATION OF RABBIT DIETS BY DIFFERENT *IN VITRO* DIGESTIBILITY METHODS

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ABSTRACT

Seven experimental diets for rabbits were used to evaluate three different *in vitro* techniques of evaluating their nutritive content, based on the use of multienzyme, caecal or faecal inocula. The prediction equations obtained with the multienzyme and caecal *in vitro* techniques showed higher precision (R^2 = 0.94 and 0.82, respectively) and lower dispersion (SE: 4.36 and 8.16, respectively) than those based on the chemical composition, but the faecal technique gave worse results (R^2 = 0.66 and SE = 11.00). Repeatability was high for the three techniques (S.D.: 0.38 to 1.05), although the multienzyme method was significantly better. Multienzyme and caecal techniques showed adequate precision and repeatability for the prediction of DM digestibility of rabbit diets, especially the multienzyme method.

INTRODUCTION

Feed evaluation is frequently performed in time-consuming and costly experiments based on *in vivo* determinations, requiring animals and relatively large amounts of feed. Different *in vitro* methods have been developed to easy, quick and easy prediction of the nutritive value of rabbit feeds. The *in vitro* methods for the evaluation of feeds have been developed using either contents of the rabbit caecum or different parts of the digestive tract (Aderibigbe *et al.*, 1992; Fernández-Carmona *et al.*, 1993), or blends of enzymes (Ramos and Carabaño, 1996) as inocula for incubations. However, no attempts have previously been made to compare their accuracy and repeatability.

Therefore, the aim of the present work was to provide some information about the precision and repeatability of three different *in vitro* methods based on enzymatic, caecal and faecal inocula for estimating digestibility in rabbits.

MATERIAL AND METHODS

Diets

Seven experimental diets for rabbits described by Fernández-Carmona *et al.* (1996) were used to study the different *in vitro* digestibility techniques evaluated in the present work. Diets were selected in order to obtain a wide range of acid detergent fibre content (87 to 525 g ADF kg^{-1} DM), mainly responsible for low digestibility of rabbit diets.

In vitro techniques

Multienzyme technique. As described by Ramos and Carabaño (1996), 1 gram of 1 mm ground samples were carefully mixed in a flask with 25 ml of a phosphate buffer (0.1M, pH 6.0) and 10 ml of 0.2M HCl solution, and pH was adjusted to pH 2 with HCl or NaOH 1M solutions. Then 1ml of pepsin solution (25mg of pepsine from porcine Merck n 7190 / ml 0.2M HCl) was added and after gentle stirring the flasks were closed and incubated in an oven at 40ºC for 1.5 h. After incubation, pH of each flask was increased up to 6.8 by the addition of 10 ml of a phosphate buffer (0.2M, pH 6.8) and 5 ml of 0.6M NaOH solution. After gentle stirring and a new pH adjustment, 1 ml of pancreatin solution (100 mg of pancreatin from porcine Sigma n 1750 /ml phosphate buffer pH 6.8) was added to each flask, and mixed in. Flasks were closed and incubated in an oven at 40ºC for 3.5 h. After the second incubation, pH weas adjusted to 4.8 with acetic acid, and 0.5 ml of Viscozyme 120L (Novo Nordisk) was added. Flasks were again incubated in an oven at 40ºC for 16 h after gentle stirring.

Caecal and faecal techniques. Caecal and faecal inocula, and the artificial saliva solution were prepared as described Fernández-Carmona *et al.* (1993). Twelve New Zealand White × Californian growing rabbits, given the same commercial diet and showing a normal weight gain and food intake, were randomly selected. Their faeces were collected daily for five days and then they were slaughtered. Two hundred grammes of caecal or faecal content were diluted with 320 ml of artificial saliva solution (8 g of $NaHCO_3$, 4 g of K_2HPO_4, 0.5 g of $(NH_4)_2HPO_4$, 1.5 g of NaCl and 0.5 g of $MgSO_4 \cdot 7H_20$ per litre of distilled water) under a stream of CO_2 gas. Caecal and faecal contents were filtered and macerated at 40ºC under a constant stream of CO_2 gas, for 0.5 and 1 h. for caecal and faecal solutions respectively. After maceration, caecal and faecal solutions were centrifuged at 3500 rpm for 5 minutes, and 1680 ml of artificial saliva solution were added to the supernatant, obtaining the caecal and faecal inocula, which were maintained at 40ºC under a constant stream of CO_2. In each dried and pre-weighed filter crucible digestion glass (volume 100 ml and filter porosity n 2) 1 g of 1-mm ground sample was added to 50 ml of caecal or faecal inoculum. Digestion glasses were closed under a constant stream of CO_2, and incubated in an orbital bath at 40ºC for 36 h. under constant stirring at 40 fluctuations per minute.

After incubation, undigested residue was collected by filtration, and washed with distilled water 5 times and with ethanol and acetone (50 ml) once. DM of undigested residue was determined following the method of the Association of Official Analytical Chemists (1984). Three replicates were carried out for each sample in order to determine the repeatability of methods.

Statistical analysis

Data were analysed by variance analysis, using a mixed procedure (PROC MIXED) of SAS (Statistical Analysis System Institute, 1996) and according to a repeated measures design that takes into account the variation between diets and covariation within them. The model included fixed effects of the method (3 levels) and the replicate (3 levels). Covariance structures of mixed procedure were objectively compared using the most severe criteria (Schwarz Bayesian criterion), as suggested by Littell *et al.* (1998).

RESULTS AND DISCUSSION

As can be seen in Table 1, the apparent digestibility coefficient of DM can be well estimated from the chemical composition of the diets, especially from their ADF and CF content. The equations for the diets used in the present experiment are:

$$dDM = 93.44 - 1.793\ CF(\%DM) \quad (R^2 = 0.78 \quad SE = 8.693)$$
$$dDM = 86.41 - 1.01\ ADF(\%DM) \quad (R^2 = 0.80 \quad SE = 8.343)$$

where the coefficient of determination and SE values are not very different from those deduced in other works (De Blas *et al.*, 1992; Villamide and Fraga, 1998), and especially the work of Fernández-Carmona *et al.* (1996) with all 23 diets.

Table 1. Prediction of dry matter digestibility from chemical composition and *in vitro* digestibility of diets.

Correlation matrix

			In vitro digestibility		
	CF	ADF	multienzyme	caecal	faecal
dDM	0.884	0.894	0.970	0.903	0.771
Repeatability of in vitro method[a]			0.348	0.644	0.853

Linear regression equations[b]

				In vitro digestibility				
	Intercept	CF	ADF	multienzyme	caecal	faecal	R^2	SE
y = dDM(%)	93.444	-1.793					0.781	8.693
	86.405		-1.014				0.799	8.343
	2.692			1.010			0.942	4.462
	7.884				1.089		0.815	8.161
	5.693					1.194	0.663	11.002

[a] Repeatability as standard deviation of laboratory.
[b] CF and ADF in % on DM basis, *in vitro* and *in vivo* digestibility in %.

However, the prediction equations obtained with the multienzyme and caecal *in vitro* techniques showed higher precision (R^2 = 0.94 and 0.81, respectively) and lower SE (4.46 and 8.16, respectively) than CF and ADF based equations, although faecal technique gave worse results (R^2 = 0.66 and SE = 11.00). The differences between them were mainly due to the inadequate prediction of beet pulp with the caecal and faecal methods, giving higher dDM for the beet pulp than those expected from their caecal and faecal *in vitro* digestibility.

Table 1 also shows the repeatability of the different *in vitro* techniques evaluated. The multienzyme technique showed a better repeatability than caecal and faecal techniques, but repeatability values obtained for all methods were good (S.D.: 0.35 to 0.85).

In conclusion, multienzyme and caecal techniques showed adequate precision and repeatability for DM digestibility prediction, especially the multienzyme method. However, values obtained in the present work showed a more disappointing accuracy and repeatibility for methods based on the use of faecal inocula.

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