Proceedings 5th World Rabbit Congress, 25-30 July 1992, Corvallis – USA, 938-946.

AN IN VITRO METHOD FOR ESTIMATING DIGESTIBILITY IN RABBITS

Ramos, M.A.*; Carabaño, R.* and Boisen, S.**

*Departamento de Produccion Animal. E.T.S. Ingenieros Agronomos.
Universidad Politecnica. 28040 Madrid. Spain.
**National Institute of Animal Science. Foulum P.O. Box 39.
DK-8830 Tjele. Denmark.

Abstract

An "in vitro" method for feed evaluation in rabbits, using a multienzyme system was developed.

Relationships between "in vitro" and "in vivo" dry matter (DM), crude protein (CP) and crude fibre (CF) digestibilities of 21 diets were analysed.

An accurate correlation was found for dry matter digestibility (r^2 = 0.88, rsd = 1.47) whereas the correlation for crude protein and crude fibre was not so good (r^2 = 0.54, rsd = 2.01 and r^2 = 0.62, rsd = 4.54, respectively). In all cases, the inclusion of crude fibre as an independent variable in a multiple regression equation improved the accuracy.

The regression equations obtained were compared with prediction equations based on chemical parameters of the diet as proposed by several authors. The former gave better predictions.

In conclusion the results suggest that the "in vitro" method could be an alternative to predict the nutritive value of feeds for rabbits. Nevertheless because of the limited number of diets used for this tudy, the accuracy and the usefulness of this method has to be confirmed.

Proceedings 5th World Rabbit Congress, 25-30 July 1992, Corvallis – USA, 938-946.

AN IN VITRO METHOD FOR ESTIMATING DIGESTIBILITY IN RABBITS

Ramos, M.A.*; Carabaño, R.* and Boisen, S.**

*Departamento de Produccion Animal. E.T.S. Ingenieros Agronomos.
Universidad Politecnica. 28040 Madrid. Spain.
**National Institute of Animal Science. Foulum P.O. Box 39.
DK-8830 Tjele.Denmark.

Introduction

The best way to determine the nutritive value of foods is to analyze the chemical composition and the digestibility coefficients of the nutrients of the diet.

However, "in vivo" determinations of digestibility are expensive, time consuming and require relatively large amounts of feed which makes them not very useful in routine analysis at the animal feed industry. Therefore it is of great importance to develop new methods to estimate in an easy, quick and less costly way this nutritive value as alternatives to "in vivo" trials.

"In vitro" digestibility methods to simulate the physiological process of"in vivo" digestion have been tested in pigs showing good results (Van der Meer and Perez, 1990; Boisen, 1991; Boisen and Fernandez,1991). In rabbits there are several equations to predict faecal digestibility of nutrients based on chemical parameters (Battaglini and Grandi,1984; de Blas et al,1984; Maertens et al,1988; Ortiz and de Blas,1989). However these equations are not always very predictive. So, the purpose of the present paper is to describe and evaluate an "in vitro" method (based on three consecutive incubations with commercial enzyme preparations of pepsin, pancreatin and microbial fibre-degrading enzymes) to predict apparent faecal digestibility of rabbit diets.

The present paper will show faecal digestibility values and relate these to "in vitro" values from identical materials. The relationship between both values will give an idea of the validity of the technique for prediction of the nutritive value of rabbit feeds.

Materials and Methods

Diets

To carry out this research, 21 compound rabbit diets were used. Ten commercial diets were provided by Nanta, S.A.; Pascual, S.A.; Coren, S.C.L. and Piensos Rosell,S.A. The other ones were experimental diets with known "in vivo" faecal digestibility data obtained from the Department of Animal Production of Madrid and Department of Animal Science of Valencia.

The digestibility trials for the commercial diets were done in Madrid.

Table 1 shows the average and variation range in chemical composition and digestibility of 21 diets.

Raw materials were similar in all diets. The main fibre sources were lucerne and straw, also wheat bran was a component of most diets. Soybean meal and sunfower meal were the main protein sources and wheat an barley were included as energy sources. Commercial diets had added fat at levels lower than 2%.

Table nº 1 Chemical composition and digestibility of 21 diets. Mean value and variation range

	Average	Minimum	Maximum
Chemical composition (% DM)			
Crude protein	18,0	15,8	20,0
Crude fibre	15,9	12,0	19,2
Digestibility (%)			
Dry matter	61,5	55,2	72,0
Crude protein	74,1	68,4	78,8
Crude fibre (1)	18,4	7,5	25,6

(1). These figures were obtained with 13 diets

The "in vitro" technique

A multienzyme system simulating digestion in the whole gastro-intestinal tract was carried out in the laboratory following the method of Boisen (1991) for pigs at the Institute of Animal Science, Foulum, Denmark.

It consists of three incubation steps. Steps 1 and 2 try to reproduce digestion in stomach and small intestine whereas the 3rd step is a simulation of the microbial digestion at the caecum.

Step 1

A series of 20 samples with about 1 g of finely ground material (ground to pass a screen with a pore size of 1 mm) were weighed to an accuracy of ± 0.1 mg in 100 ml conical flasks. In each of the series a blank was included. A small magnetic rod and 25 ml of phosphate buffer (0.1 M, pH 6.0) were added to each flask and sample and buffer were mixed carefully by gentle magnetic stirring. 10 ml 0.2 M HCl were added to the mixture and pH was adjusted to pH 2 with a 1 M HCl or a 1 M NaOH solution. Then 1 ml of a freshly prepared pepsin solution, containing 25 mg pepsin (porcine, 2000 FIP-U/g, Merck n. 7190) was added. In order to prevent bacterial growth, especially during the second incubation step, 0.5 ml of a chloramphenicol solution (0.5 g/100 ml ethanol) was added. The flasks

were then closed with a rubber stopper and the samples incubated with gentle magnetic stirring in a thermostatic controlled heating chamber at 40 °C for 1.5 h.

Step 2

To the mixture 10 ml of a phosphate buffer (0.2 M, pH 6.8) and 5 ml of a 0.6 M NaOH solution were added. The pH was then adjusted to pH = 6.8 with a 1 M HCl or a 1 M NaOH solution.

Then 1 ml of freshly prepared pancreatin solution containing 100 mg porcine pancreatin (porcine, grade VI, sigma n. P-1750) was added to each flask. The preparation of the pancreatin solution requires thorough stirring and a 3000 r.p.m., 30 minutes centrifugation before it is ready to be used.

After closing with a rubber stopper, the flasks were again incubated with gentle magnetic stirring in a heating chamber at 40 °C for 3.5 h.

Step 3

After adjustment to pH = 4.8 with acetic acid, two different procedures were used. One of them (proc. a) includes 0.5 ml of an enzimatic cocktail (Viscozyme), whereas the other one (proc. b) includes a mixture of 0.5 ml Viscozyme + 0.5 ml Celluclast + 0.1 ml Novozyme.

All flasks were then closed with a rubber stopper and placed back in the heating chamber at 40 °C with gentle stirring overnight.

All enzymes were from Novo, Bagsvaerd, DK. Viscozyme is a multienzyme complex containing a wide range of carbohydrases including cellulase, hemicellulase, arabinase, xylanase, ß-glucanase and pectinase. The degradation from this enzyme cocktail corresponds to the potentially fermentable fibre. Celluclast has a high cellulase activity. Novozyme is a cellobiase.

Procedure b was tested in order to offset the fibre degradability of Viscozyme on its own.

After incubation, the rubber stopper was removed and the undigested residues were then collected in a filtration unit (Fibertec system, Tekator) by transfering to dried and preweighed glassfilter crucibles containing 0.4 gr of celyte as a filteraid. Filtration includes several rinses with distilled water plus washing with ethanol and acetone. The residue was dried at 80 °C overnight and undigested DM was measured.

To determine the digestibility of CP, celyte and undigested material were wrapped into a piece of nitrogen-free paper, and undigested CP was measured by the Kjeldahl method.

Digestibility of CF was calculated after the determination of CF in the residue in a Fibertec System, Tekator.

In vitro digestibility of DM, CP and CF were calculated from DM, CP and CF in the sample and in the undigested residue after correction for DM, CP and CF

in the blank. All calculations were on a dry matter basis.

Statistical analysis

The results obtained "in vitro" were related to the obtained "in vivo" for DM, CP and CF digestibility using the GLM and REG procedures of S.A.S. program (1985).

Results and Discussion

Relationships between "in vitro" and "in vivo" digestibility of dry matter (DM), crude protein (CP) and crude fibre (CF) for the two treatments (I and II) are shown in table 2.

Dry matter "in vitro" digestibility (DMv) showed an accurate correlation with "in vivo" data (DMd) in both treatments.

When regression equations were compared, no significant differences were found. However, the correlation and the precision were slightly better when only Viscozyme was used in the third step (r^2 = 0.88 vs 0.83; rsd = 1.47 vs 1.74). Furthermore, the use of only one enzyme cocktail simplifies the methodology so we have chosen equation (1) to predict DMd. This equation is shown in figure 1.

A stepwise procedure including DMv, CF,ADF and CP as independent variables was carried out to study if the chemical composition of the diet could improve the accuracy of this equation. DMv was included in the first step and only CF (% DM) was included together with DMv in the second step.

The following equation was obtained:

DMd (%) = 7.54 + 0.88 DMv (%) - 0.3 CF (% DM) (6)
r^2 = 0.90
rsd = 1.36

The inclusion of CF in the equation (6) improved ($P< 0.0602$) the correlation and the precision with respect to equation (1). However, CF only explains 2% of the variation after DMv data have already been taken into account.

On the other hand, several authors (Battaglini and Grandi, 1984; De Blas et al, 1984; Maertens et al, 1988; Ortiz and De Blas, 1989) have proposed different equations to predict energy digestibility or digestible energy based on the chemical composition of the diets. Dietary fibre content (ADF or CF) have been the most correlated figures. Since energy digestibility is highly correlated with DM digestibility, we tried to compare the "in vitro" prediction (equation (1)) with a prediction equation based on CF content. The regression obtained was as follows:

DMd (%) = 77.7 - 0.98 CF (% DM) (7)
r^2 = 0.31
rds = 3.50

The correlation and the precision of equation (7) were much lower than in equation (1). It was also much lower compared to the equation obtained by Ortiz and de Blas (1989) for predicting energy digestibility (n = 219; ED (%) = 84.02 - 1.42 CF (% DM); r^2 = 0.71)

The low correlation found in our work could be the consequence of the narrow variability range in the CF content of diets (table 1) respect to the results of Ortiz and de Blas (1989), where the range was from 2.34 to 59.05% of CF (% DM). So, more data seem to be necessary to confirm if prediction from "in vitro" data is better than prediction based on a single chemical component of the diet.

With regard to crude protein digestibility, in the third step we tested the same two treatments as for dry matter. The prediction equation obtained with only Viscozyme had better correlation and rsd (Table 2) than the obtained for the three enzyme treatment. Therefore, equation (3) was chosen for the prediction.

The correlation found for "in vivo" CP digestibility on the basis of "in vitro" CP digestibility was not as good as for DM digestibility.

The low correlation may be due to the fact that "in vitro" measurements could correspond to true more than to apparent digestibility, since endogenous losses of protein are not taken into account in the "in vitro" method. In this sense, the "in vitro" results were always higher (average 12.25 percentage points) than the CP "in vivo" digestibility. Similar results were obtained by Boisen and Fernandez (1991) in pigs. According to these authors endogenous losses are related to the fibre content of the diet. For this reason, we tried to improve the prediction including chemical parameters that could affect endogenous losses such as CF and CP. When a stepwise procedure was used, only CF was included in the second step to improve the correlation. The resulting equation was:

CPd (%) = 17.49 + 0.74 CPv (%) - 0.45 CF (% DM) (8)
r^2 = 0.64
rsd = 1.82

Crude fibre improved ($P < 0.03$) 10 points the determination coefficient (r^2) and reduced the residual standar deviation (rsd) with respect to equation (3). However these two variables only explain 64% of the total variation; this shows that more variables should be considered to simulate better "in vivo" CP digestibility. For instance, caecotrophy or different protein sources in the diet. De Blas et al (1984) obtained a prediction equation for CP digestibility based on the proportion in which concentrates contribute to the total protein of the diet (Pc). Other variables (ADF and CP) were not included in the stepwise procedure. The equation obtained was as follows:

CPd (%) = 56.48 + 18 Pc (%) (9)
n = 35
r^2 = 0.42

Besides, the results obtained by Boisen and Fernandez (1991) in pigs also give a worse prediction for CP than for energy digestibility (r^2 = 0.72 vs 0.84).

The "in vitro" analysis to determine CF digestibility were done using the three-enzyme procedure in the third step. Up to now, no data with the Viscozyme as the only enzyme cocktail are available. As for CP, "in vitro" CF digestibility is always higher than "in vivo" (average 13 percentage points). Therefore, a single enzyme treatment (Viscozyme) could be enough to simulate caecum digestion. However, the correlation obtained was relatively high (r^2 = 0.62) although the precision was not as good (equation 5). To improve the prediction, chemical analysis of the diet (CF and CP) were taken into account in a stepwise procedure. Only CF was included in the second step as follows:

CFd (%) = 4.38 + 1.17 CFv (%) - 1.33 CF (% DM) (10)
r^2 = 0.73
rsd = 3.98

Comparing equation (10) with equation (5) the inclusion of CF explained 11% more of total variation and improved the precision.

Conclusions

In general, the described multienzyme "in vitro" method predicts DM digestibility with good accuracy. However, the correlations found for CP and CF were always lower than for DM. In all cases the CF content of the diet improves the prediction. From the results obtained for DM and CP the use of Viscozyme on its own may be a simplified alternative to simulate caecum digestion.

References

Battaglini, M. and Grandi, A. (1984). Stima del valore nutritivo dei mangini composti per conigli. Proc. III World Rabbit Congress, Rome. Vol. 1, 252-264.

Boisen, S. (1991). A model for feed evaluation based on in vitro digestible dry matter and protein. In: Digestion in vitro (M.F. Fuller, Ed.). Commonwealth Agricultural Bureaux International, Slough (in press).

Boisen, S. and Fernandez, J.A. (1991). In vitro digestibility of energy and amino acids in pig feeds. In: Digestive Physiology in Pigs (M.W.A. Verstegen, J. Huisman and L.A. den Hartog, eds.), p. 231-236.

De Blas, J.C., Rodriguez, J.M., Santomá, G. and Fraga, M.J. (1984). The nutritive value of feeds for growing fattening rabbits. J. Appl. Rabbit Res., 7: 72-74.

Maertens, L., Moermans, R. and De Grotte, G. (1988). Prediction of the apparent digestible energy content of commercial pelleted feeds for rabbits. J. Appl. Rabbit Res., 11: 60-67.

Ortiz, V. and De Blas, J.C. (1989). Predicción de la digestibilidad de la energía de los piensos para conejos a partir de su contenido en fibra. Invest. Agrar.: Prod. Sanid. anim. 4: 197-205.

Van der Meer, J.M. and Perez, J.M. (1990). In vitro evaluation of European diets for pigs. Prediction of the organic matter digestibility by an enzymatic method or by chemical evaluation. EAAP 41 st Ann. Meet. Toulouse, 9-12 July 1990, 16 pp.

Table nº 2 Regression of "in vivo" on "in vitro" dry matter (DM), crude protein (CP) and crude fibre (CF) digestibility

	n	Equation	r^2	rsd	
(I)	21	DMd = - 2.5 + 0.95 DMv (±5.52) (±0.08)	0.88	1.47	(1)
(II)	21	DMd = - 14.22 + 1.06 DMv (±7.9) (±0.11)	0.83	1.74	(2)
(I)	21	CPd = - 9.5 + 0.97 CPv (±17.55) (±0.20)	0.54	2.01	(3)
(II)	19	CPd = - 7.43 + 0.9 CPv (±20.65) (±0.24)	0.48	2.16	(4)
(II)	13	CFd = - 12.27 + 0.98 CFv (±7.37) (±0.23)	0.62	4.54	(5)

(I) Viscozyme was used in the third step.
(II) Viscozyme, Celluclast and Novozyme were used in the third step.
DMd, CPd and CFd correspond to "in vivo" digestibility of DM, CP and CF, respectively.
DMv, CPv and CFv correspond to "in vitro" digestibility of DM, CP and CF, respectively.

Proceedings 5th World Rabbit Congress, 25-30 July 1992, Corvallis – USA, 938-946.

Fig. 1. Regression of "in vitro" (DMv) on "in vivo"(DMd) dry matter digestibility of 21 rabbit diets.