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ESTIMATION OF DIGESTIBILITY OF COMPOUND DIETS FOR RABBITS USING THE *IN VITRO* GAS PRODUCTION TECHNIQUE

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ABSTRACT : The *in vitro* gas production technique was used to predict the digestibility of ten diets in rabbits. Using a step-wise linear regression approach, relationships were established between the *in vivo* coefficients of digestibility of dry matter (*d*DM), organic matter (*d*OM) and energy (dGE) and *in vitro* organic matter losses (OM*l*) and potential gas production (A, ml.g^{-1}). The best prediction equation was: *d*DM (%) = - 40.2 + 1.026 OM*l*(%) + 0.150 A (ml.g^{-1}) (R^2 = 0.748 and RSD = 3.66). In conclusion the results suggest that the *in vitro* gas production technique could be a promising method to predict the nutritive value of rabbit diets. Nevertheless because of the limited number of diets used for this study, the accuracy and the usefulness of this method has to be confirmed.

RÉSUMÉ : Estimation de la digestibilité d'aliments composés pour lapins, à l'aide de la technique de production de gaz *in vitro*.
La technique de production de gaz *in vitro* a été utilisée pour prévoir la digestibilité de 10 aliments pour lapins. La comparaison entre les coefficients de digestibilité *in vivo* de la matière sèche (*d*DM), de la matière organique (*d*OM) de l'énergie (*d*GE) les pertes de matières organiques (Om*l*,%) et la production potentielle de gaz (A, ml.g^{-1}) lors de la fermentation, a été effectuée par régression linéaire multiple et progressive. La meilleure équation prévisionnelle est : *d*DM (%) = - 40.2 + 1.026 OM*l*(%) + 0.150 A (ml.g^{-1}) (R^2 = 0.748 and RSD = 3.66). Les résultats obtenus montrent que la technique de production de gaz *in vitro* peut être intéressante pour prévoir la valeur nutritive des aliments pour lapins. Néanmoins, étant donné le nombre limité d'aliments utilisés dans cette étude, l'utilité et la fidélité de cette méthode devront être confirmées.

INTRODUCTION

The nutritive energy value of rabbit feeds is usually expressed as digestible energy. Its determination, whether in simple or compound diets, is usually carried out by *in vivo* trials of ingesta/excreta balance. Indeed, due to the easy collection of total faeces, the use of digestibility markers is not justified in this species. However, in terms of industrial production, rabbit diets are not formulated according to the digestible energy but on the basis of the energy content of each ingredient or using linear equations based on chemical composition.

Estimation of the energy value of the diet on the basis of digestible energy of each mixture ingredient is somewhat unreliable, chiefly for the following reasons. First, the calculation fails to consider food associative effects (positive or negative interactions) which can influence whole diet digestibility. Besides, it must be considered that the current food tables are incomplete and the energetic values of many foods are often different in various tables (XICCATO, 1989). For some foods, the energy value was obtained by the substitution method which does not account for associative effects.

To avoid *in vivo* digestibility trials, several researchers (DE BLAS *et al.*, 1984; BATTAGLINI and GRANDI, 1986; CORINO, 1987; MAERTENS *et al.*, 1988; FERNANDEZ-CARMONA *et al.*, 1996) have studied linear equations which predict the digestible energy (DE) of rabbit diets by their chemical characteristics. The ADF, NDF, but also the classic Weende crude fibre are the chemical components mainly correlated with the DE. In considering simultaneously 2 or 3 chemical components the accuracy of prediction of DE improved, but it was always lower than *in vivo* results.

However, *in vivo* experiments of digestibility are expensive, time-consuming and require relatively large amounts of feed. Therefore, it is of great importance to develop new methods to estimate easily and in a less costly way the nutritive value as alternatives to *in vivo* trials. *In vitro* techniques used to estimate the digestible energy content of feeds have been widely used in other animals but little in rabbits. Although this method has been used to a limited extent with rabbits, RAMOS *et al.* (1992) obtained a good estimation of DM digestibility from *in vitro* digestibility parameters.

Moreover, *in vitro* techniques have assumed great importance since interest in animal welfare is increasing, as is the tendency to avoid "uncomfortable" breeding conditions.

The *in vitro* gas production technique has been used to predict the nutritive value (MENKE *et al.*, 1979, MENKE and STEINGASS, 1988) and study the fermentation kinetics (BEUVINK, 1993; BLÜMMEL and ØRSKOV, 1993; PELL and SCHOFIELD, 1993; THEODOROU *et al.*, 1994; CONE *et al.*, 1996) of ruminant feedstuffs. The technique uses a milled substrate, an anaerobic medium and an inoculum of a mixed microbial population from the rumen. Recently, it has has been applied successfully using other animal species, such as horses (MACHEBOEUF *et al.*, 1997) and chicken (KWAKKEL *et al.*,1997) as source of inoculum.

The aim of the present research was to predict the *in vivo* digestibility of rabbit diets from *in vitro* fermentation parameters using rabbit caecal content as inoculum.

MATERIALS AND METHODS

Substrates

Ten compound diets for rabbits were used as substrates. These diets were ground to pass a 1 mm screen and their chemical composition was determined (AOAC, 1984).

***In vitro* fermentation**

Cumulative gas production was measured according to the *in vitro* fermentation method of THEODOROU *et al.* (1994). This method allows measurements of cumulative gas production by use of a pressure transducer under strictly anaerobic conditions. About 820 mg of sample was fermented at 39°C in a 120 ml serum bottle, containing 74 ml of semi-defined medium D (THEODOROU, 1993) and 3.5 ml of reducing solution. The bottles were sealed with butyl rubber stoppers and aluminium crimp seals and warmed at 39° C until inoculation. Each substrate was tested in 4 replications.

Freshly collected samples of caecal content were used to prepare the inoculum. Five young New Zealand White rabbits (75 days old) showing a normal weight gain during the fattening period were randomly chosen prior to slaughtering. The animals were fed a fattening diet with the following composition (on DM basis): 15.8% crude protein (CP), 15.3% crude fibre (CF), 31.2% neutral detergent fibre (NDF), 9.5% acid detergent fibre (ADF) and 4.4% acid detergent lignin (ADL). This diet was fed ad *libitum* from weaning (30 days of age). The feed was removed at 6 pm. on the day before sampling, but water was still available *ad libitum*. The animals were sacrificed between 9 and 10 a.m. by cervical dislocation. The caeca was isolated by tying off the two extremities with nylon string to prevent losses of digesta.

The caecal content was diluted (1:1 v/v) with the medium D, then was squeezed through six layers of gauze to constitute the inoculum. During this procedure, to guarantee microbial activity, the microbial suspension was kept at 39°C anaerobically under a stream of CO_2 gas. Five ml of inoculum was added to each bottle, which was incubated at 39°C.

The production of gas resulting from fermentation was recorded at regular time intervals until the end of incubation to measure fermentation kinetics. Gas production was measured manually with a pressure transducer connected to the inlet of a disposable Luer lock three-way syringe valve. The first outlet of the syringe valve was connected to a disposable syringe needle and the second outlet to a disposable different capacity plastic syringe.

Pressure and volume were recorded twenty times at 2-24 h intervals throughout fermentation (96 h). At 96 h, the pH was measured and two bottles were used to determine the DM and OM losses by rinsing the contents through sintered glass filter crucibles (Scott Duran, porosity 2) with hot water. Neutral detergent fibre (GOERING and VAN SOEST, 1970) was determined for the remaining substrates of the other two bottles to calculate the NDF losses (NDF*l*).

Four bottles were incubated in the absence of substrates to represent the control, used to obtain the total correct gas production (CV, *ml*) and the correct amount of dry matter (DM*l*, *%*) and organic matter (OM*l*, *%*) loss. Gas yields (Y_{DM} as ml.g degraded DM and Y_{NDF} as ml.g^{-1} degraded NDF) were also calculated at the end of the fermentation.

Digestibility trial

The diets had been tested in previous trials (NIZZA *et al.*, 1993; NIZZA and DI LELLA, 1995) to determine the coefficients of apparent digestibility of dry matter (*d*DM), organic matter (*d*OM) and energy (*d*GE) by the ingesta/excreta balance. Apparent digestibilities of DM, OM and gross energy were determined for each diet on 10 New Zealand White rabbits which were 2 months old and 2-2.5 kg live weight, housed in metabolic cages and fed *ad libitum*. The adaptation period was 7 days and the faeces collection period was 5 days. In order to avoid intestinal disorders due to the low structural carbohydrate content of diet 3, the digestibility trial of this diet was carried out administering the diet 3 in a 1:1 ratio with diet 2. The DM, OM and energy digestibility coefficients of the diet 3 were obtained by the differences method (XICCATO, 1989).

Statistical analysis

The monophasic modified model of Michaelis-Menten (GROOT *et al.*, 1996) was adopted to describe the kinetics of the gas production profiles:

$$G(t) = \frac{A}{1+\left(\frac{B}{t}\right)^C}$$

where G (ml.g^{-1} OM) denotes the amount of gas produced per g of organic matter incubated, at time t after incubation. A (ml.g^{-1} OM) represents the asymptotic gas production. B (h) is the time after incubation at which half of A has been formed. C is a constant determining the curve sharpness. Gas production profiles were described with the model using a non-linear curve-fitting program (NLREG, SHERROD, 1995).

It was also possible to calculate the maximum degradation rate (h^{-1}, R_M) and the time at which R_M occurs (h, t_{RM}) using the following formula (GROOT *et al.*, 1996):

$$t_{RM} = B(C-1)^{1/C} \qquad RM = \frac{Ct_{RM}^{C-1}}{B^C + t_{RM}^C}$$

The relationship between apparent digestibility coefficients of DM, OM and energy and fermentation parameters (i.e. A, t_{RM}, RM, CV, DM*l*, OM*l*, NDF*l*) of the diets was established, using step-wise linear regression (SPSS, 1986).

RESULTS AND DISCUSSION

Table 1 reports the chemical composition and the apparent digestibility coefficients of DM, OM and gross energy of the diets (*d*DM, *d*OM and *d*GE, respectively). Due to the different chemical composition of the diets (CP: from 17.0 in diets 2 and 8 up to 23.4 in diet 3; NDF: from 21.8 in diet 3 up to 48.9 in diet 2), a wide range of variation for digestibility coefficients of DM, OM and energy was observed (*d*DM: from 51.7 in diet 2 up to 75.0 in diet 3; *d*OM from 52.8 in diet 2 up to 76.1 in diet 3; *d*GE from 52.2 in diet 2 up to 75.6 in diet 3).

The monophasic model (GROOT *et al.*, 1996) gave a good description curve (R^2: from 0.95 in diet 9 up to 0.999 in diet 7; RSD: from 2.90 in diet 7 up to 6.46 in diet 5) obtained by incubating the diets with buffered caecal content of rabbit. Figure 1 shows gas production over time for diet 1.

The parameters from the model and the *in vitro* fermentation characteristics after 96 h of incubation are reported in the table 2. The potential gas production showed a fairly narrow range (A. 322-255 ml.g^{-1}), probably because we tested ten diets of a single category (compound diets for rabbits). By contrast, higher variability was observed for the

Table 1 : Chemical composition and apparent digestibility coefficients of diets

Diets	1	2	3	4	5	6	7	8	9	10
					Chemical composition (%DM)					
CP	20.1	17.0	23.4	18.3	17.3	18.7	18.6	17.0	18.8	18.8
CF	16.2	22.8	9.5	17.0	15.6	14.9	14.9	14.7	15.0	14.9
NDF	35.3	48.9	21.8	33.9	33.9	31.1	33.6	33.9	30.0	30.1
ADF	20.2	28.3	12.1	21.2	21.2	19.2	19.3	20.4	19.3	19.1
ADL	4.5	6.3	2.8	4.8	3.8	4.1	4.6	4.0	4.1	4.0
Ash	9.7	11.2	8.3	9.5	7.3	8.4	8.6	7.4	8.5	8.0
					Digestibility coefficient (%)					
*d*DM	62.6	51.7	75.0	59.5	67.8	68.4	64.3	68.3	67.9	69.7
*d*OM	64.4	52.8	76.1	60.7	68.9	69.0	65.2	69.3	68.7	70.8
*d*GE	63.4	52.2	75.6	59.8	68.0	68.5	64.6	68.5	68.0	69.9

*d*DM, *d*OM and *d*GE = *in vivo* apparent digestibility coefficient of dry matter, organic matter and energy, respectively.

Table 2 : Parameters estimated by the model and characteristics of *in vitro* fermentation at 96 hours

Diets	1	2	3	4	5	6	7	8	9	10
A, ml.g^{-1}	294	260	287	261	255	301	298	299	322	304
B, hours	16.6	15.9	15.7	14.9	15.9	14.6	13.3	15.5	14.5	12.8
C	1.33	1.25	1.31	1.24	1.32	1.42	1.42	1.50	1.24	1.45
RM, hours^{-1}	0.046	0.048	0.048	0.051	0.048	0.053	0.059	0.051	0.053	0.061
t_{RM}, hours	7.12	5.16	6.46	4.64	6.82	7.91	7.19	9.66	4.67	7.27
CV, ml.g^{-1}	122	119	131	121	118	154	154	160	157	166
PH	7.08	7.14	7.15	7.14	7.18	7.05	6.72	6.60	6.81	6.84
DM*l*, %	49.6	58.4	70.8	57.4	60.1	63.8	63.4	63.1	61.4	65.0
OM*l*, %	53.8	58.2	70.8	59.0	62.4	61.4	59.5	61.9	58.7	62.9
NDF*l*, %	21.8	35.2	30.0	32.9	24.0	26.1	38.0	41.6	21.6	33.3
Y_{DM}, ml.g^{-1}	371	291	295	289	259	337	335	341	351	357
Y_{OM}, ml.g^{-1}	382	328	318	310	281	439	459	429	466	454
Y_{NDF}, ml.g^{-1}	2767	896	2485	1399	1999	2844	1763	1587	3485	2281

A= asymptotic gas production.; B= time after incubation at which half of A was formed; C= constant determining the curve sharpness; RM= degradation maximum rate; t_{RM}= time at which R_M occurs; CV= cumulative gas production; DM*l*= dry matter loss (% of incubated); OM*l*= organic matter loss (% of incubated); NDF*l*= neutral detergent fibre loss (% of incubated); Y_{DM}= gas produced per degraded dry matter; Y_{OM}= gas produced per degraded organic matter; Y_{NDF}= gas produced per degraded NDF.

time at which the maximum degradation rate occurs (t_{RM}: 4.67-9.66 h) and for cumulative gas production (CV: 118-166 ml). It is difficult to account for the behaviour of these two parameters as the tested diets, except diets 2 and 3, showed similar chemical composition. Chemical composition alone cannot sufficiently explain such behaviour, and further information should be acquired regarding the quality of the structural carbohydrates in the substrates. Moreover, the commercial origin of the diets and the possible presence of some additives may have influenced the pattern of fermentation. However, this problem lies outside the scope of the present paper, which sets out to predict *in vivo* digestibility from *in vitro* fermentation parameters.

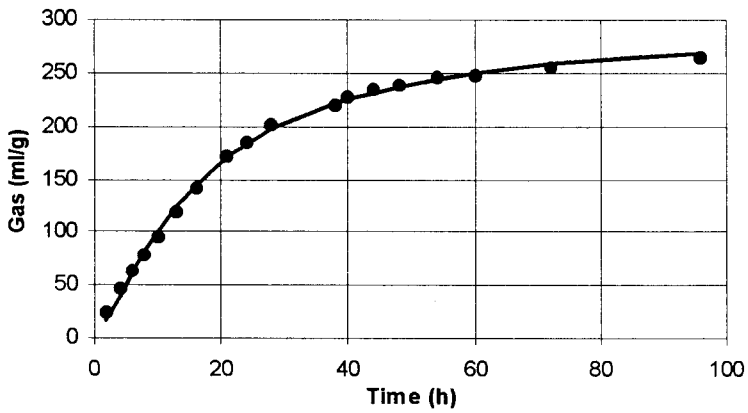

Figure 1 : An example of the raw data and resulting fitted curve for diet 1

At the end of incubation, the values of pH were optimal for cellulolytic fermentation and microbial growth (SATTER and SLYTER, 1974; SLATTER and ROFFLER 1977) for each diet.

The simple correlation matrices and equations obtained from step-wise linear regression analysis are presented in table 3. The organic matter losses at the end of incubation fitted well with *in vivo* digestibility of DM, OM and energy (R^2 = 0.691, 0.681 and 0.693, respectively ; $P<0.05$). No significant correlations were found between the

Table 3 : Prediction of *in vivo* apparent digestibility coefficients of dry matter (*d*DM), organic matter (*d*OM) and gross energy (*d*GE) from gas production parameters

Correlation matrix	*d*DM, %	*d*OM, %	*d*GE, %
A, ml.g^{-1}	+0.507	+0.498	+0.502
RM, h^{-1}	+0.435	+0.437	+0.435
t_{RM}, h	+0.223	+0.202	+0.204
CV, ml.g^{-1}	+0.486	+0.464	+0.468
DM*l*, %	+0.652	+0.627	+0.643
OM*l*, %	+0.691	+0.681	+0.693
NDF*l*, %	+0.166	+0.138	+0.149

Linear regression equations	Eq. No.	Intercept	a_1 (OM*l*,%)	a_2 (A, ml.g^{-1})	R^2	RSD
y = *d*DM (%)	1	+ 3.90	+ 1.013		0.478	4.92
	2	- 40.2	+ 1.026	+ 0.150	0.748	3.66
y = *d*OM (%)	3	+ 6.58	+ 0.986		0.464	4.92
	4	- 36.3	+ 1.000	+ 0.146	0.725	3.77
y = *d*GE (%)	5	+ 4.66	+ 1.006		0.480.	4.87
	6	- 38.7	+ 1.019	+ 0.148	0.744	3.65

A= asymptotic gas production.; RM= degradation maximum rate; t_{RM}= time at which R_M occurs; CV= cumulative gas production; DM*l*= dry matter disappearance (% of incubated); OM*l*= organic matter loss (% of incubated); NDF*l*= neutral detergent fibre loss (% of incubated); Y_{DM}= gas produced per degraded dry matter; Y_{OM}= gas produced per degraded organic matter; Y_{NDF}= gas produced per degraded NDF.

model parameters (A, B and RM) and *in vivo* digestibility. These apparently conflicting results are probably due to the fact that not all the *in vitro* degraded matter (organic matter loss) was fermented as gas and volatile fatty acids (VFA). Besides, the composition of VFA formed may have affected the amount of gas produced.

Because of the small number of diets sampled (10), it seemed appropriate to reduce the number of *in vitro* fermentation parameters in the regression analysis. We eliminated those that were correlated with each other, which gave scarce information regarding fermentation.

Step-wise multiple regression analyses showed that the most significant contribution to the variation explained of *d*DM, *d*OM and *d*GE arises from the organic matter losses after 96 hours of incubation. When A is added to the model, the closeness of the linear relationship improved and the prediction of DM, OM and energy digestibilities was more precise (RSD: 3.66; 3.77; 3.65 for *d*DM, *d*OM and *d*GE, respectively).

The equation best estimating *in vivo* DM digestibility from *in vitro* parameters is: *d*DM (%) = - 40.2 + 1.026 OM*l* (%) + 0.150 A (ml.g^{-1}); R^2 = 0.748 and RSD = 3.66. Our results appear less precise than that reported by RAMOS *et al.* (1992) who incubated 21 diets for rabbits using an *in vitro* enzymatic method:

$$DMd\ (\%) = 7.54 + 0.88\ DMv\ (\%) - 0.3\ CF\ (\%\ DM)$$
$$R^2 = 0.90 \text{ and } RSD = 1.36$$

where DMd and DMv are the dry matter *in vivo* and *in vitro* digestibility, respectively, and CF the crude fibre content.

The three DM, OM and energy equations were similar in estimation and precision, confirming the close correlation among the digestibilities of these parameters. However, where testing ten feeds of the same category (compound diets), the resulting equations are applicable only in the range of parameters used in our trials.

CONCLUSIONS

In general the results of this preliminary study indicate the possibility of accurately predicting the dry matter, organic matter and gross energy digestibilities in rabbits by the *in vitro* gas production technique using caecal content as a source of inoculum. The accuracy is satisfactory but it has to be confirmed with other categories of feeds and also the number of samples has to be increased. However, it was confirmed that until now the ingesta/excreta balance remains the most precise method to determine the apparent digestibility coefficients of rabbit diets.

Regarding the methodology, the present paper verified the validity of the system and the *in vitro* procedure adopted for the fermentation study using the caecal content of rabbit as inoculum. In general, we believe that the *in vitro* gas production technique could be useful for studying the nutritive characteristic of rabbit diets, supplying information regarding both the extent and the kinetics of the fermentation process, especially the dynamics of volatile fatty acid production.

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