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PREDICTION OF NUTRITIVE VALUE OF DIETS FOR RABBITS USING AN *IN VITRO* GAS PRODUCTION TECHNIQUE

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ABSTRACT: Thirty-one mixed diets for rabbits (DE concentration from 8.44 to 12.29 MJ/kg) were used to predict the digestibility of dry matter (dDM), organic matter (dOM), gross energy (dGE) and digestible energy concentration (DE) from some *in vitro* gas production parameters, using frozen caecal content from rabbits. Step-wise multiple regression analysis showed that the most significant contribution to the variation expressed by dDM, dOM, dGE and DE arises from crude fibre content (CF). Multiple regression analysis considered more than one independent variable, but it gave only marginally improvements in terms of the accuracy of digestibility prediction. The best equations in terms of R^2 and residual standard deviation (RSD) values were: DE (MJ/kg DM) = 0.75 - 0.291 CF - 0.208 ADL + 0.856 GE (R^2 = 0.895, RSD 0.279) and dOM (%) = 91.8 - 1.756 CF - 1.283 ADL (R^2 = 0.849, RSD 1.655) where CF = crude fibre (%DM), ADL = acid detergent lignin (%DM), GE = gross energy (MJ/kg DM). Dry matter loss (DMI, %) was the *in vitro* gas production parameter which correlated most closely with dDM, dOM, dGE and DE. The best prediction equations were: DE (MJ/kg DM) = -3.14 + 0.217 DMI + 0.114 B (R^2 = 0.734, RSD 0.437) and dOM (%) = - 6.80 + 1.078 DMI + 0.456 B (R^2 = 0.691, RSD 2.368), where B is the incubation time (h) at half potential gas. When data of the chemical composition and from fermentation parameters were included concurrently in the model, the most significant contribution to the variation explained of dDM, dOM, dGE and DE still arose from CF. These results suggest that *in vitro* gas production could be an interesting method of predicting the nutritive value of rabbit diets, but further investigations are required to increase caecal *inoculum* standardisation and its prediction ability.

Key words: rabbits, compound diets, gas production, digestibility prediction.

INTRODUCTION

The knowledge of nutrient digestibility, and especially of the digestible energy (DE) content, of a simple feedstuff or a mixed diet, represents the basis for sound nutrition for rabbits and is of great interest for the food industry. DE is usually

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determined by *in vivo* trials with the ingesta/excreta balance. This trials are expensive and time-consuming. Hence some regression equations have been proposed to predict the apparent digestibility coefficient and the DE by the chemical composition of feed or mixed diets (DE BLAS *et al.*, 1984; BATTAGLINI AND GRANDI, 1986; CORINO, 1987; MAERTENS *et al.*, 1988; DE BLAS *et al.*, 1992; FERNÁNDEZ-CARMONA *et al.*, 1996; VILLAMIDE AND FRAGA, 1998).

It is commonly reported that the acid detergent fibre (ADF), the neutral detergent fibre (NDF), and also the crude fibre (CF) are the chemical components mainly correlated with DE and digestibility of gross energy (GE). In considering simultaneously 2 or 3 chemical components the accuracy of DE prediction usually improves.

In recent years, other methods have been developed to predict the nutrient digestibility, i.e. *in vitro* methods (ADERIBIGBE *et al.*, 1992; FERNÁNDEZ-CARMONA *et al.*, 1993; RAMOS AND CARABAÑO, 1996) and also near-infrared reflectance spectroscopy (XICCATO *et al.*, 1999, 2003). The *in vitro* cumulative gas production technique (GPT) has been used to evaluate the nutritive value of ruminant feedstuffs (MENKE AND STEINGASS, 1988) and other animal species, such as horses (MACHEBOEUF *et al.*, 1997), chickens (KWAKKEL *et al.*, 1997) and pigs (WILLIAMS *et al.*, 1995). In rabbits, CALABRÒ *et al.* (1999) proposed the GPT to predict the nutritive value of rabbit diets, using fresh rabbit caecal content as *inoculum*. Their preliminary results on 10 compound diets indicated the possibility of accurately predicting the digestibility of DM, organic matter (OM) and GE in rabbit by *in vitro* GPT. At present, the main obstacle to GPT diffusion seems to be the variability in the *inocula* pattern (Williams *et al.*, 1998). By using frozen caecal content, as opposed to fresh, some of the problems could be solved, as researchers would work on a larger number of substrates with a standardized *inoculum*.

The aim of the present research was to predict the *in vivo* digestibility of rabbit compound diets from chemical composition and *in vitro* fermentation parameters, using a mixture of frozen caecal content from several rabbits as *inoculum*.

MATERIAL AND METHODS

Samples

Thirty-one compound rabbit diets (commercial diets) from 6 different feed producers were used as test substrates, 11 of which contained alfalfa as the main constituent and 16 of which were made by the same feed producer. These diets were ground to pass a 1 mm screen and their chemical composition was determined (AOAC, 1984).

In vivo trial

Apparent digestibility of DM (dDM), OM (dOM) and GE (dGE) was determined for each diet on 8 different New Zealand White rabbits which were 2-months old and 2-2.5 kg live weight. During the trial period, they were housed in metabolism cages and fed *ad libitum*. The adaptation period was 7 days and the faeces collection period was 5 days.

In vitro fermentation

The kinetics of fermentation were measured with the GPT using a new automated system, the APES (Automated Pressure Evaluation System, DAVIES *et al.*, 2000), in order to reduce the high labour input required for the manual gas production system. The APES consists of 48 x 120 ml culture bottles connected to pressure sensitive switches and solenoid valves.

The *inoculum* was prepared by mixing the caecal content of 24 New Zealand White rabbits (75 days old). The animals were fed a fattening diet with the following composition (on DM basis): OM 92.2%, CP 15.5%, EE 3.4% and CF 15.5%. This diet was always administered *ad libitum* from 50 days old. The feed was removed at 8 p.m. on the day before slaughter, in order to obtain constant microflora. Subsequently, the caeca were isolated by tying up the two extremities with a nylon string to prevent losses of digesta. The caecal content mixture was frozen at -18°C for 1 month; then it was defrosted, diluted 1:1 (v/v) with a basal medium (THEODOROU, 1993), which is made up by 4 solutions: macroelement solution (A) = 9.45 g of

Na_2HPO_4 + 6.2 g of KH_2PO_4 + 0.6 g of $MgSO_4$ dissolved in 1 litre of distilled water; microelement solution (B) = 13.2 g of $CaCl_2$ + 10 g of $MnCl_2$ + 1 g of $CoCl_2$ + 8 g of $FeCl_2$ dissolved in 1 litre of distilled water; tampon solution (C) = 35 g of $NaHCO_3$ + 4 g of $(NH_4)HCO_3$ dissolved in 1 litre of distilled water; resazurine solution (D) = 100 mg of resazurine in 100 ml of distilled water. The basal medium was prepared using the solution A, B and C in this proportion: 550 ml of distilled water + 200 ml of A solution + 0.1 ml of B solution + 200 ml of C solution + 10 ml of D solution. The caecal content diluted with the basal medium was squeezed through six layers of gauze to obtain the *inoculum*. During these procedures, microbial suspension was kept at 39°C under a stream of CO_2.

About 820 mg of sample was incubated with 74 ml of basal medium, 3.5 ml of reducing solution, prepared immediately before incubation with 0.625 g of cistein HCl in 100 ml of distilled water (THEODOROU, 1993) and 5 ml of *inoculum* at 39°C for 96 hours. Four bottles were incubated without the substrates to represent the control (blanks), and were used to correct dry (DMI) and organic matter losses (OMI) and gas production. For each sample 4 replications (bottles) were made. At the end of the fermentation period OMI and pH were determined. The cumulative gas data, related to incubated OM, were fitted to the monophasic model of GROOT *et al.* (1996): $G_{(t)} = A/(1+(B/t)^C)$ using a non-linear curve-fitting program (NLREG, Sherrod, 1995), where $G_{(t)}$ (ml/g OM incubated) is the gas produced at t time; A (ml/g OM incubated) represents the potential gas production; B (h) represents the time at which A/2 is produced; C is a constant defining the curve shape. Moreover, using B and C parameters, it was possible to calculate the maximum degradation rate (R_M, h^{-1}) and the time at which RM occurs (t_{RM}, h) according to GROOT *et al.* (1996). The following parameters were also calculated at the end of fermentation: the cumulative volume of gas per dry matter incubated (CV, ml/g) and the cumulative volume of gas per DM (Y_{DM}, ml/g) and degraded OM (Y_{OM}, ml/g).

Statistical analysis

The relationship between the apparent digestibility coefficient and nutritive value (dDM, dOM, dGE and DE) and the chemical composition and the fermentation parameters (A, B, pH, t_{RM}, R_M, CV, DMI, OMI, Y_{OM} and Y_{DM}) was established, using

step-wise linear regression (SPSS, 1986).

RESULTS AND DISCUSSION

Table 1 reports the mean values with standard deviation and variability coefficient (CV) of the chemical composition and *in vitro* fermentation parameters of the diets. Considering that the 31 compound diets were formulated to be fed to the rabbits as unique feedstuffs, the chemical composition presented high variability, especially in fibrous fraction content (CV = 11.4, 12.1 and 25.5%, respectively for NDF, ADF and ADL). Also *in vitro* fermentation parameters showed high CV, especially t_{RM} (64.8 h), R_M (35.7/h) and B (32.8 h).

Table 2 shows the apparent digestibility coefficients of DM, OM and GE and DE concentration. Digestibility coefficients recorded a variability according to the chemical composition range. Table 3 reports the correlation matrix between the *in vivo* digestibility and nutritive value with chemical composition or *in vitro* fermentation parameters. CF was the chemical component best correlated with the apparent digestibility coefficient and DE ($r>-0.90$; $P<0.01$); also ADL, ADF and NDF showed a close correlation (r = approx. - 0.80; $P<0.01$). The *in vitro* parameters showed lower correlation coefficient values compared with the chemical constituents. The best correlated parameters were DMI and OMI. Also A and CV showed significant correlations ($P<0.01$). These results confirm the findings of CALABRÒ *et al.* (1999) who, using fresh caecal content as *inoculum*, found DMI, OMI and A well correlated with the nutrient and energy digestibility coefficients.

Table 4 reports the regression equations which predict the *in vivo* apparent digestibility coefficients of DM, OM, GE and DE from the chemical composition of diets. Step-wise regression analyses showed that the most significant contribution to the variation explained of dDM, dOM, dGE and DE arises from CF. ADL was the second component which allows a further increase in estimation precision. Other components sporadically inserted in the equations were the GE for the estimation of dDM and DE.

Table 1: Chemical composition and *in vitro* fermentation parameters.

	Mean	Min.	Max.	SD	CV × 100
Chemical composition (%DM)					
Organic matter (OM)	90.4	88.0	92.7	1.26	1.39
Crude protein (CP)	17.7	15.3	19.9	1.06	5.96
Crude fibre (CF)	17.0	11.3	19.7	1.53	9.01
Ether extract (EE)	3.4	2.5	5.1	0.58	17.01
NDF	36.4	26.2	44.8	4.15	11.41
ADF	22.3	11.9	25.8	2.70	12.09
Cellulose	18.5	10.3	20.2	1.96	10.59
ADL	3.8	1.6	5.6	0.97	25.45
Gross energy, MJ/kg DM	17.36	16.96	18.05	0.28	1.62
In vitro parameter					
A, ml g^{-1}	266	140	365	47.23	17.73
B, h	7.8	5.5	16.8	2.57	32.83
R_M, h^{-1}	0.14	0.08	0.37	0.05	35.71
t_{RM}, h	6.50	1.59	21.29	4.21	64.78
CV, ml/g	349	210	489	56.50	16.18
pH	6.38	6.20	6.59	0.11	1.72
DMl, %	55.9	50.0	66.1	4.00	7.15
OMl, %	57.1	50.7	67.0	4.11	7.21
Y_{DM}, ml/g	570	364	683	69.8	12.25
Y_{OM}, ml/g	620	399	730	73.3	11.82

A: asymptotic gas production, B: time after incubation at which A/2 was formed, RM: degradation maximum rate, tRM: time at which RM occurs, CV: cumulative gas volume per organic matter incubated, DMl: percentage of dry matter loss, OMl: percentage of organic matter loss, YDM gas produced per degraded dry matter, YOM gas produced per degraded organic matter.
SD: stardard deviation, CV: coefficient of variation.

The precision obtained with the above multiple regression equations in estimating dDM, dOM, dGE and DE is high. The determination coefficient (R^2) and RSD value, similar to those reported in the literature (CORINO, 1987; DE BLAS *et al.*, 1992; FERNÁNDEZ-CARMONA *et al.*, 1996; VILLAMIDE and FRAGA, 1998), indicate a variability range that does not differ from that observed in *in vivo* digestibility carried out on few animals and confirm the possibility of using dietary chemical components to estimate with high accuracy the digestibility coefficients and DE concentration. The linear regression equations to predict DE content or the digestibility of DM, OM and GE from the chemical composition of the feed generally considered a fibrous component as the best single predictor.

Contrary to what was observed in our study, where the best single predictor of DE was CF, FERNÁNDEZ-CARMONA *et al.* (1996) and CORINO (1987) reported ADF as the best single predictor of DE. The presence in the equation of CF or ADF could be due both to the strict correlation normally occurring between the two components, also found in our samples ($r=0.881$; $P<0.01$), and to the different concentrations and digestibilities of fibrous fractions found in the feeds tested by the various authors.

Table 5 reports predictions of *in vivo* apparent digestibility of DM, OM and GE and DE concentration from *in vitro* parameters of gas production. Step-wise regression analysis shows that the most significant contribution to the variation explained of dDM, dOM, dGE and DE arises from the DM matter losses after 96 hours of incubation. Parameter B supplied the highest marginal contribution, which allows estimation precision to be improved (R^2 from 0.624 to 0.727; from 0.612 to

Table 2: Apparent digestibility coefficient of DM, OM, GE and DE concentration.

	Mean	Min.	Max.	SD	CV × 100
dDM (%)	55.9	48.6	67.7	4.61	8.24
dOM (%)	57.1	49.6	69.6	4.72	8.27
dGE (%)	56.8	49.1	69.4	4.73	8.33
dDE (MJ/kg DM)	9.87	8.44	12.29	0.91	9.22

SD: stardard deviation, CV: coefficient of variation.

Table 3: Correlation matrix.

	dDM	dOM	dGE	DE
Chemical composition				
OM	+ 0.462**	+ 0.391*	+ 0.382*	+ 0.504**
CP	+ 0.547**	+ 0.595**	+ 0.588**	+ 0.576**
CF	- 0.902**	- 0.906**	- 0.910**	- 0.901**
NDF	- 0.783**	- 0.800**	- 0.797**	- 0.764**
ADF	- 0.810**	- 0.819**	- 0.822**	- 0.795**
Cellulose	- 0.621**	- 0.637**	- 0.641**	- 0.627**
ADL	- 0.873**	- 0.848**	- 0.849**	- 0.828**
GE	+ 0.609**	+ 0.554**	+ 0.545**	+ 0.678**
In vitro parameter				
A	+ 0.600**	+ 0.623**	+ 0.630**	+ 0.585**
B	+ 0.187	+ 0.150	+ 0.149	+ 0.223
CV	+ 0.546**	+ 0.552**	+ 0.555**	+ 0.519**
DMI	+ 0.790**	+ 0.782**	+ 0.776**	+ 0.780**
OMI	+ 0.777**	+ 0.775**	+ 0.770**	+ 0.758**
YDM	+ 0.377*	+ 0.406*	+ 0.413*	+ 0.340
YOM	+ 0.347	+ 0.380*	+ 0.388*	+ 0.310

*$P<0.05$; **$P<0.01$

0.691 from 0.602 to 0.681 from 0.608 to 0.734, for dDM, dOM, dGE and DE, respectively). The inclusion of B in the model, a variable poorly correlated with digestibility coefficients, may in our opinion be ascribed to greater independence compared with other variables. When data of the chemical composition and from fermentation parameters were included concurrently in the model, the most significant contribution to the explained variation of dDM, dOM, dGE and DE arose from CF and fermentation parameters that were never inserted in the equations.

These results are very similar to those reported by CALABRÒ *et al.* (1999) who,

Table 4: Prediction of in vivo apparent digestibility and DE concentration from chemical composition by step-wise regression.

Equation	Step no.	Intercept	CF (%DM)	ADL (%DM)	GE (MJ/kg DM)	R^2	RSD
Y = dDM (%)	1	+ 95.7	- 2.343			0.814	1.746
Y = dDM (%)	2	+ 87.3	- 1.482	- 1.622		0.862	1.530
Y = dDM (%)	3	+ 33.7	- 1.225	- 1.607	+ 0.0495	0.892	1.379
Y = dOM (%)	1	+ 98.5	- 2.437			0.821	1.771
Y = dOM (%)	2	+ 91.8	- 1.756	- 1.283		0.849	1.655
Y = dGE (%)	1	+ 98.6	- 2.457			0.823	1.738
Y = dGE (%)	2	+ 92.1	- 1.792	- 1.252		0.845	1.625
Y = DE (MJ/kg DM)	1	+ 18.1	- 0.481			0.811	0.362
Y = DE (MJ/kg DM)	2	+ 1.74	- 0.401		+ 0.861	0.876	0.298
Y = DE (MJ/kg DM)	3	+ 0.75	- 0.291	- 0.208	+ 0.856	0.895	0.279

R^2: multiple coefficient of determination, RSD: residual standard deviation.

using fresh caecal content, obtained regression equations where OMI was the most correlated variable with dDM, dOM and dGE. This parameter is strictly correlated to the DMI of our equations. On comparing the regression equations obtained with the chemical components of the diets (Table 4) and those obtained from the gas production technique (Table 5), greater estimation precision was evidenced (higher R^2 and lower RSD values) with the chemical components of the diets.

Comparison among the other *in vitro* techniques reported in literature is not easy, whether due to the different methods used or the kind of *inoculum* used in this research. PASCUAL *et al.* (2000) obtained regression equations of dOM with high R^2 (0.949 and 0.888, respectively) and high RSD (3.98 and 4.86%) using the multi-enzyme method described by RAMOS AND CARABAÑO (1996) and the caecal method described by FERNÁNDEZ-CARMONA *et al.* (1993), respectively. By contrast, the faecal technique supplies equations with low R^2 (0.678) and high RSD (10.47%). Also previous research carried out by RAMOS *et al.* (1992) and by RAMOS AND CARABAÑO (1996) with the multi-enzyme method always supplied estimation of DM digestibility

Table 5: Prediction of in vivo apparent digestibility and DE concentration from in vitro parameters by step-wise regression.

Equation	Step no.	Intercept	DMI	B	R^2	RSD
Y = dDM (%)	1	+ 0.40	+ 0.992		0.624	2.480
Y = dDM (%)	2	- 7.25	+ 1.059	+ 0.501	0.727	2.153
Y = dOM (%)	1	+ 0.16	+ 1.017		0.612	2.608
Y = dOM (%)	2	- 6.80	+ 1.078	+ 0.456	0.691	2.368
Y = dGE (%)	1	+ 0.19	+ 1.013		0.602	2.650
Y = dGE (%)	2	- 6.75	+ 1.073	+ 0.455	0.681	2.417
Y = DE (MJ/kg DM)	1	- 1.40	+ 0.201		0.608	0.521
Y = DE (MJ/kg DM)	2	- 3.14	+ 0.217	+ 0.114	0.734	0.437

R^2: multiple coefficient of determination, RSD: residual standard deviation.

with high R^2 (0.90 and 0.87) and low RSD (1.36 and 1.52, respectively). PASCUAL *et al.* (2000) report that the low precision of the caecal technique compared with the multi-enzyme technique indicates the need to improve the standardisation of caecal content.

CONCLUSIONS

The results of this study indicate that the *in vitro* gas production technique, using caecal content as a source of *inocula*, can estimate the DM, OM, GE and DE digestibility of compound diets with moderate precision (60 to 70% of explained variability). The best estimation obtained from the chemical composition of diets confirm the findings of previous research and may be due to better standardisation of the analytical method and to the fact that a large part of digestible nutrients are not involved in caecal fermentation. However, given that the gas production technique may also provide information on the kinetics and fermentation characteristics of diets through determination of VFA, useful indicators of caecal microbial activity, the method clearly has considerable future potential. In the meantime, better standardisation of the caecal content should be included among research objectives.

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