WORLD RABBIT SCIENCE 1995, 3(4), 171-178.

EUROPEAN RING-TEST ON *IN VIVO* DETERMINATION OF DIGESTIBILITY IN RABBITS : REPRODUCIBILITY OF A REFERENCE METHOD IN COMPARISON WITH DOMESTIC LABORATORY PROCEDURES

PEREZ J.M.[1], CERVERA C.[2], FALCAO E CUNHA L.[3], MAERTENS L.[4], VILLAMIDE M.J.[5], XICCATO G.[6]

[1] INRA, Station de Recherches Cunicoles, BP 27, 31326 CASTANET-TOLOSAN Cedex - France.
[2] Universidad Politécnica de Valencia, Departamento de Ciencia Animal, Camino de Vera 14, 46071 VALENCIA - Spain.
[3] Instituto Superior de Agronomia, Universidade Técnica de Lisboa, Tapada da Ajuda, 1399 LISBOA codex - Portugal.
[4] Rijkscentrum voor Landbouwkundig Onderzoek, Rijksstation voor Kleinveeteelt, Burg. van Gansberghelaan 92, B-9820 MERELBEKE - Belgium.
[5] Departamento de Produccion Animal, ETSI Agrónomos, Ciudad Universitaria, 28040 MADRID - Spain.
[6] Università di Padova, Dipartimento di Scienze Zootecniche, via Gradenigo 6, 35131 PADOVA - Italy.

ABSTRACT : A collaborative study involving six European laboratories from five countries (Belgium, France, Italy, Portugal and Spain) was undertaken to assess the reproducibility of an European standard method for *in vivo* determination of diet digestibility and to compare the results obtained with domestic procedures. The European method was carried out on 49-day-old rabbits caged individually and fed ad libitum ; it included an adaptation period of 7 days followed by a collection period of 4 days. The digestibility coefficients of dry matter (dDM), organic matter (dOM), energy (dE), crude protein (dCP), crude fibre (dCF), and the digestible energy (DE) content of two diets were measured in each laboratory by both standard and domestic methods. On the whole, 260 digestive balances were carried out on the basis of 8 to 10 rabbits minimum per diet per each method. In comparison with the laboratory procedures the standard method improved the reproducibility notably for the digestibility of dry matter (among-laboratory standard deviation : S_L = 0.70 *vs* 0.92), crude fibre (S_L = 2.49 *vs* 5.02) and DE content (S_L = 0.18 *vs* 0.26 MJ/kg DM).

RÉSUMÉ : Ring-test européen sur la détermination in vivo ***de la digestibilité des aliments chez le lapin.***
Une étude concertée impliquant 6 laboratoires européens (Gand, Lisbonne, Madrid, Padoue, Toulouse, Valence) a été mise en place pour préciser la reproductibilité d'une méthode européenne standardisée de mesure in vivo *de la digestibilité des aliments et pour comparer les résultats obtenus à l'aide des procédures propres à chaque laboratoire. Dans la méthode européenne, les bilans digestifs sont réalisés sur des lapins de 49 jours d'âge placés en cages individuelles et nourris à volonté ; après une période d'accoutumance de 7 jours à l'aliment expérimental, les fèces sont récoltées quotidiennement pendant une période courte de 4 jours. Dans tous les laboratoires, et pour chaque méthode, on a mesuré la digestibilité de la matière sèche (dMS), de la matière organique (dMO), de l'énergie (dE), des protéines (dMAT), de la cellulose brute (dCB) et la teneur en énergie digestible (ED) de deux aliments fabriqués en un seul site à partir des mêmes matières premières. Au total, 260 bilans digestifs ont été réalisés à raison de 8 à 10 lapins minimum par aliment en fin d'essai. L'application de la méthode européenne standardisée permet d'améliorer sensiblement la reproductibilité des mesures par rapport aux méthodes de laboratoires particulièrement pour la digestibilité de la matière sèche (écart-type inter-laboratoires S_L = 0.70 vs 0.92) , de la cellulose brute (S_L = 2.49 vs 5.02) et la valeur énergétique (S_L = 0.18 vs 0.26 MJ d'ED /kg MS).*

INTRODUCTION

Energy is the base of feed formulation and represents the largest part of the cost of rabbit feeding. A high precision is required for *in vivo* determination of diet digestibility mostly to assess digestible energy (DE) of single feedstuffs because the error of estimation shoots up as the level of inclusion decreases (VILLAMIDE *et al.*, 1991). At present, published DE values of feed ingredients are strongly divergent among laboratories (BELTRAN *et al.*, 1984 ; FEKETE and GIPPERT, 1986 ; CHEEKE, 1987 ; INRA, 1989 ; MAERTENS *et al.*, 1990). For complete feeds, alternative techniques to *in vivo* determination were proposed to estimate digestibility (e.g., prediction from chemical analyses, *in vitro* procedures) but their applicability is questionned (PEREZ and LEBAS, 1992 ; XICCATO *et al.*, 1994). In any case the *in vivo* determination remains the control method.

For all these reasons, it is essential to measure the dietary energy content of feeds with a precise *in vivo* method. Up to now, no standardized procedure was available to determine digestibility in rabbits. The procedures used for digestibility assays are usually

Table 1 : Composition of the experimental diets

Diet	R 1	R 2
Ingredients (g/kg)		
Barley meal	450	350
Wheat bran	100	100
Dehydrated lucerne meal	250	250
Wheat straw	50	150
Soyabean meal (48% CP)	120	120
Dicalcium phosphate	10	10
Limestone	10	10
Sodium chloride	5	5
Trace elements and vitamin mix	5	5
Calculated values		
Crude protein (% DM)	17,54	16,61
Crude fibre (% DM)	14,39	17,76
DE (MJ/kg DM)	11,54	10,46

inconsistent and the variability of digestibility determination among laboratories is still unknown. Accordingly, some European laboratories have devised a common reference method for the *in vivo* determination of dry matter digestibility of complete diets in rabbits (PEREZ *et al.*, 1995). A collaborative study was undertaken to assess the reproducibility of this common method and to compare the results obtained with domestic procedures. This work involved six laboratories from five countries : Belgium, France, Italy, Portugal and Spain (2 labs).

MATERIAL AND METHODS

Diets

Two experimental diets differing by their fibre content were prepared in pellet form at the French laboratory. Representative samples controlled by complete analysis were sent to the other 5 laboratories. The composition of the diets is given in Table 1. The two experimental diets contained the same ingredients. They differred by the substitution of 10 points of barley by wheat straw in R2 diet.

Digestibility procedures

Two digestibility procedures were carried out in each laboratory : the standard method and the domestic method.

The **standard method** is fully described in the above mentioned paper (PEREZ *et al.*, 1995). It includes an adaptation period of 7 days followed by a 4 days collection period. Rabbits were 42-45 days old at the beginning of the adaptation period and were weaned 7 days minimum prior to the start of this period. They did not receive the experimental diets previously. Twelve rabbits per diet were alloted at the beginning of the experiment by taking into account the sex, the origin of litters and the weight of animals :

Table 2 : Main characteristics of the standard and the laboratory methods

Characteristics	Standard method	Laboratory methods					
		1	2	3	4	5	6
Animals							
Age (days) (1)	49(2)	56	not controlled	91	56	59	56
Control of litters	yes	yes	no	yes	yes	yes	yes
Collection period							
Length (days)	4	7	4	7	2 x 4	7	7
Feeds							
Feeding	*ad lib.*	*ad lib.*	*ad lib.*	*ad lib.*	*ad lib.*	*ad lib.*	*ad lib.*
Control of feed intake	daily	weekly	daily	weekly	weekly	daily	daily
DM determination	3 x 50 g 24h-103°C	2 x 10 g 6h-105°C	2 x 3 x 4 g 20h-105°C	3 x 100 g 24h-80°C	2 x 40 g 24h-103°C	2 x 10 g 4h-105°C	2 x 2 g 20h-104°C
Feces							
Total collection	daily	daily	daily	daily	daily	daily	daily
Fresh weight	no	weekly	daily	no	weekly	weekly	daily
DM determination	total 24h-103°C	300 g 16h-65°C +6h-105°C	3 x 40 g 48h-80°C	total 24h-80°C	2 x 80 g 24h-103°C	2 x 500 g 96h-60°C +4h-105°C	total 48h-70°C +2x2g 20h-104°C

(1) age of the rabbits at the start of the collection period.
(2) 52 days for laboratory 5.

sexes and litters were balanced homogeneously among the treatments and within-litters, the rabbits of extreme weight being excluded. Only 10 rabbits per diet were considered for the balance trial. The exclusion of two rabbits among twelve was done at the end of the adaptation period on the basis of several criteria in this priority order : health problems, scraping, extreme feed intake, low weight gain and abnormal excretion of soft feces. In comparison with the published reference method, the standard procedure was applied in this ring-test with the following slight modifications : feeds and feces were both analyzed after drying at 103°C for 24 h, whereas in the reference method feeds were analyzed as fed-basis and feces after drying at 80°C for 24 h.

The **domestic methods** were briefly presented in Table 2. They were carried out in most laboratories 3 days after the end of the collection period of the common method, i.e. at 56-59 days of age. In the laboratory 3, which used 91-day-old rabbits, a new adaptation period of 7 days was performed before the balance trial. The same animals were used in the standard and domestic methods except in the case of the laboratory 2.

Chemical analyses

Dry matter (DM), ash, crude protein (CP) and Weende crude fibre (CF) contents were determined according to the methods of AOAC (1990). Gross energy (GE) content was measured with adiabatic or isoperibol bomb calorimeters. Cell wall fractions (NDF, ADF, ADL) were determined according to the general method proposed by VAN SOEST *et al.* (1991) with some variations between laboratories : direct determination *vs* sequential procedure with or without previous amylolytic treatment. In any case, no control or standardization of the analytical procedures was performed among the six laboratories.

Statistical methods

Statistical analysis of experimental data was performed by using the GLM procedure of SAS (SAS, 1988). Analyses of variance were performed in the first step separately for each method on individual and mean values. The model included the effects of diet, sex, laboratory, litter (within lab) and the interaction between diet and laboratory. Since no effect of sex and only a slight effect of litter ($P \approx 0.10$) on digestibility of main components were observed, these effects were excluded from the model. Components of variance were calculated using the rules for a mixed model assuming the diet x laboratory interaction and replicate effects to be random and the diet an effect to be fixed. Data were also analyzed by covariance with diet, laboratory and diet x laboratory as main effects and dry matter intake (DMI) as covariate. The among-laboratory standard deviations were estimated by following the guidelines of AOAC (1988). No outlying laboratories according to the test of DIXON (1953) were removed in the present exercise. Laboratory means were compared by using the Student-Newman-Keuls test (SAS, 1988).

RESULTS AND DISCUSSION

Analytical determinations

Analytical results were expressed on DM basis (Table 3). Since the diet x laboratory interaction was not significant for all parameters it was discarded from the statistical model. Significant differences were observed between laboratories for all chemical constituents ($P<0.01$; $P = 0.06$ for NDF). However none of the analytical results was so divergent to be removed as outliers. The coefficient of variation among laboratories (CV_L) varied from 1% for gross energy to 12.2% for ADL. The ranking was as expected. Crude

Table 3 : Among-laboratory variability of the analytical determinations of the experimental diets

Laboratory		1	2	3	4	5	6	Mean	S_R (1)	L (2)	S_L (3)
Ash	(% DM)	8.84b	8.74b	9.24a	8.42d	8.47cd	8.66bc	8.73	0.13 (1.5)	< 0.01	0.31 (3.6)
CP	"	17.12b	16.96b	17.03b	17.95a	17.63a	17.72a	17.40	0.20 (1.1)	< 0.01	0.45 (2.6)
CF	"	15.06b	16.58a	15.55b	15.25b	16.39a	15.48b	15.72	0.39 (2.5)	< 0.01	0.71 (4.5)
NDF	"	36.37	35.67	36.15	37.22	39.80	35.34	36.90	1.89 (5.1)	0.06	2.51 (6.8)
ADF	"	17.09c	19.62a	18.22b	17.63bc	20.08a	20.10a	18.88	0.35 (1.9)	< 0.01	1.28 (6.8)
ADL	"	4.08ab	4.29a	3.55b	3.59b	3.73b	4.54a	3.92	0.24 (6.2)	< 0.01	0.48 (12.2)
GE	(MJ/kg DM)	18.02bc	17.85c	18.29a	18.10b	17.99bc	17.92bc	18.03	0.11 (0.6)	< 0.01	0.18 (1.0)

(1) Within-laboratory standard deviation (repeatability) ; CV_R(%) in brackets.
(2) Laboratory effect ; means on the same line lacking a common letter differ ($P < 0.05$).
(3) Among-laboratory standard deviation (reproducibility) ; CV_L(%) in brackets.

Table 4 : Among-laboratory variability of the digestibility results obtained with the standard method

Laboratory	1	2	3	4	5	6	Mean	S_R (1)	Level of significance (2)			S_L (3)
	n = 20	*n = 20*	*n = 19*	*n = 20*	*n = 20*	*n = 20*			D	L	D x L	
DM intake (g/d)	127.3ab	100.5c	108.3a	131.9a	119.0b	119.7b	117.9	13.0 (11.0)	< 0.01	< 0.01	0.72	-
d DM (%)	60.99b	62.11ab	61.87ab	61.63ab	62.62a	60.87b	61.68	1.53 (2.5)	< 0.01	< 0.01	0.96	0.70 (1.1)
d OM "	61.07b	62.25ab	62.24ab	62.06ab	62.81a	61.18b	61.93	1.54 (2.5)	< 0.01	< 0.01	0.94	0.71 (1.1)
d GE "	59.58bc	61.18a	60.58ab	60.92a	61.61a	59.33c	60.53	1.67 (2.8)	< 0.01	< 0.01	0.95	0.94 (1.6)
d CP "	70.37c	71.74bc	73.54ab	71.42bc	74.53a	71.11bc	72.11	3.06 (4.2)	0.67	< 0.01	0.22	1.97 (2.7)
d CF "	8.37b	12.31a	11.70a	11.78a	14.26a	9.04b	11.24	3.38 (30.1)	0.01	< 0.01	0.30	2.49 (22.1)
d NDF "	23.24c	27.08b	31.79a	28.67b	31.50a	17.93d	26.66	2.67 (10.0)	< 0.01	< 0.01	< 0.01	5.68 (21.3)
d ADF "	7.48d	18.93a	14.77b	11.49c	18.27a	11.84c	13.79	3.07 (22.3)	< 0.01	< 0.01	0.03	4.65 (33.8)
d ADL "	10.84b	-	-	-3.07c	20.70a	8.07b	9.14	5.88 (64.4)	0.26	< 0.01	< 0.01	10.53 (115)
d HEM "	36.90b	37.04b	46.15a	43.31a	44.35a	26.03c	38.90	3.23 (8.3)	< 0.01	< 0.01	< 0.01	8.34 (21.4)
d CELL "	6.39d	-	-	15.11b	17.68a	12.94c	13.03	2.92 (22.4)	< 0.01	< 0.01	0.05	5.07 (38.9)
DE (MJ/kg DM)	10.76bc	10.91ab	11.13a	11.05a	10.96ab	10.65c	10.91	0.30 (2.8)	< 0.01	< 0.01	0.96	0.18 (1.6)

(1) Within-laboratory standard deviation (repeatability) ; CV_R (%) in brackets.
(2) D : diet effect ; L : laboratory effect ; D x L : diet x laboratory interaction
(3) Among-laboratory standard deviation (reproducibility) ; CV_L (%) in brackets.

Table 5 : Among-laboratory variability of the digestibility results obtained with the domestic methods

Laboratory	1	2	3	4	5	6	Mean	S_R (1)	Level of significance(2)			S_L (3)
	n = 12	*n = 18*	*n = 18*	*n = 20*	*n = 20*	n = 19			D	L	D x L	
DM intake (g/d)	147.6a	111.9c	90.4d	147.2a	120.5c	133.9b	124.4	14.6 (11.7)	< 0.01	< 0.01	0.64	-
d DM (%)	62.74a	61.71a	62.77a	60.85b	62.78a	62.71a	62.22	1.25 (2.0)	< 0.01	< 0.01	0.18	0.92 (1.5)
d OM "	62.96a	62.11ab	63.27a	61.56b	63.05a	62.96a	62.62	1.27 (2.0)	< 0.01	< 0.01	0.23	0.77 (1.2)
d GE "	60.98c	60.65c	62.58a	60.11c	62.12ab	61.28bc	61.30	1.32 (2.2)	< 0.01	< 0.01	0.11	1.03 (1.7)
d CP "	72.63a	70.56b	73.99a	69.58b	74.12a	73.26a	72.33	2.49 (3.4)	0.08	< 0.01	0.26	2.11 (2.9)
d CF "	9.46c	13.49b	12.45b	13.07b	20.73a	13.28b	14.10	3.44 (24.4)	< 0.01	< 0.01	< 0.01	5.02 (36.5)
DE (MJ/kg DM)	10.97b	10.84b	11.39a	10.87b	11.30a	10.96b	11.06	0.24 (2.2)	< 0.01	< 0.01	0.02	0.26 (2.3)

(1) Within-laboratory standard deviation (repeatability) ; CV_R (%) in brackets.
(2) D : diet effect ; L : laboratory effect ; D x L : diet x laboratory interaction.
(3) Among-laboratory standard deviation (reproducibility) ; CV_L (%) in brackets.

fibre determinations were more consistent (CV_L = 4.5%) than those of the detergent fibre fractions NDF and ADF (CV_L = 6.8%). Several collaborative studies involving numerous laboratories have shown that there are general relationships between the concentration of an analyte and the among-laboratory standard deviation (SAUVANT and BRETTE, 1983 ; PEREZ, 1991). The present results were very close to the predicted reproducibility values for most of the analytes. By comparison the repeatability (within-laboratory standard deviation expressed as a % of the mean) (CV_R) varied from 0.6% for GE to 5.1% for NDF. It has been suggested that between- and within-laboratory errors are generally in a ratio of about 2:1 (HORWITZ *et al.*, 1980). In this study the ratio varies from 1.3:1 for NDF to 3.7:1 for ADF. The higher ratio reflected excessive variation among laboratories for ADF determination.

Standard method

The analysis of variance of digestibility coefficients showed highly significant effects (P<0.01) due to laboratory for all the parameters (Table 4). Effect of diet was also significant except in the case of the digestibilities of crude protein (dCP) and lignin (dADL). The interaction diet x laboratory was significant only for the digestibility of fibre fractions. Liveweight of the rabbits had no influence upon DE content and digestibility of the main components of the diets : DM, organic matter (OM), GE and CP. Dry matter intake during collection period used as covariate improved the precision of the statistical model but only slightly (e.g. for DM digestibility : pooled SE = 0.337 *vs* 0.344).

Among-laboratory standard deviation was extremely high for fibre fractions digestibility (CV_L = 20 to 40%). Notably, ADF digestibility (dADF) was very variable, the highest laboratory means (Lab 2) being 2.5 times the lowest (Lab 1). This is probably due to the high variability observed among laboratories for theses analyses that were not standardized both for feeds and feces. By contrast, the reproducibility was excellent (CV_L = 1.1%) for digestibility of DM and OM. The reproducibility was also very good (1.6%) for digestibility of energy and DE content (Fig. 1) and quite good for dCP (2.7%). In comparison with the analytical data (Cf. Table 3) this has to be judged very satisfactory. Actually, despite some variations among laboratories, our results showed that for the major components (DM, OM, GE) the standardized method for the determination of *in vivo* digestibility allowed a precision at least comparable to that obtained with the chemical analyses.

Finally, this collaborative study demonstrated that a simplified and quick method with only a 4 days collection period can replace usual procedures including a longer period of feces collection (e.g. 2 x 4 days, COLIN and LEBAS, 1976) provided there are enough (at least 8) rabbits per diet (VILLAMIDE and RAMOS, 1994). It should be noted also that the reproducibility standard deviation calculated in the present European ring-test for DE (0.18 MJ/kg DM) is lower that the corresponding value obtained for metabolizable energy in poultry (0.38 MJ/kg DM ; CV = 2.9%) (BOURDILLON *et al.*, 1990).

Domestic methods

The analysis of variance showed significant diet and laboratory effects for all the criteria (Table 5). Both main effects and interaction were significant for dCF (P<0.01) and DE (P<0.05). As for the standard method, liveweight had no effect upon digestibility and the use of DM intake as covariate improved the precision of the statistical model only slightly. As

Figure 1 : Digestible energy content : standard method

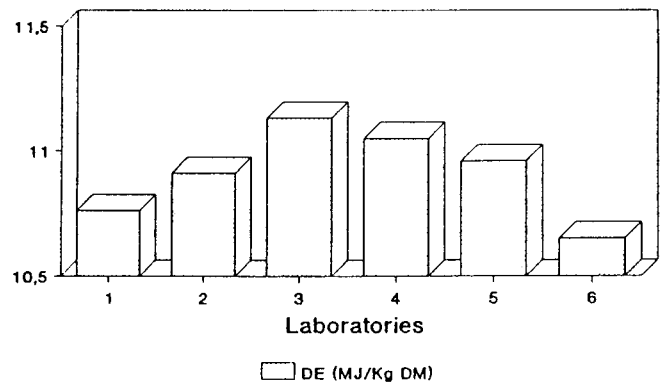

Figure 2 : Digestible energy content : domestic methods

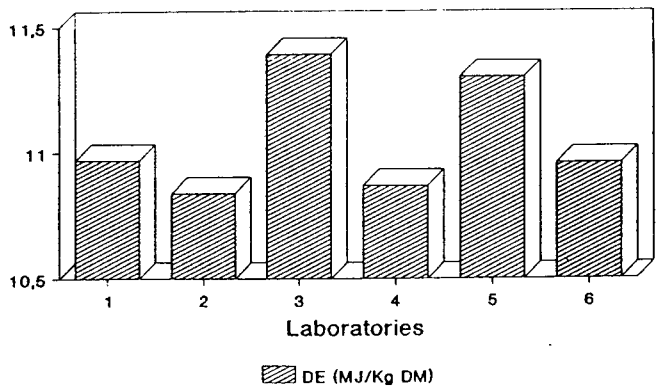

Table 6 : Comparison of the results obtained with the standard and the domestic methods (Domestic method *minus* standard method)

Laboratory	1	2	3	4	5	6
d DM (%)	+ 1.75**	- 0.40	+ 0.90	- 0.78	+ 0.16	+ 1.84**
d OM "	+ 1.89**	- 0.14	+ 1.03	- 0.50	+ 0.24	+ 1.78*
d GE "	+ 1.40**	- 0.53	+ 2.00**	- 0.81	+ 0.51	+ 1.95**
d CP "	+ 2.26**	- 1.18	+ 0.45	- 1.84*	- 0.41	+ 2.15
d CF "	+ 1.09	+ 1.18	+ 0.75	+ 1.29	+ 6.47**	+ 4.24**
DE (MJ/kg DM)	+ 0.21**	- 0.07	+ 0.26*	- 0.18*	+ 0.70**	- 0.05*

* Difference significant at $P < 0.05$
** Difference significant at $P < 0.01$.

a rule the reproducibility was less favourable than in the case of the common method for all parameters. Among-laboratory standard deviations were respectively 0.92 (1.5%) *vs* 0.70 (1.1%) for dDM and 0.26 MJ/kg DM (2.3%) *vs* 0.18 (1.6%) for DE (Fig. 2). These data were consistent with the findings of the French ring-test comparing the results from 4 diets measured in 4 laboratories (PEREZ *et al.*, 1994).

Standard method *vs* domestic procedures

In comparison with the standard method, domestic procedures used in laboratories 1 and 6 tended to overestimate the digestibility for almost all the criteria (Table 6). That was also the case for dGE ($P<0.01$) and DE ($P<0.05$) in laboratory 3 and dCF and DE in laboratory 5 ($P<0.01$). Conversely dCP and DE were underestimated ($P<0.05$) with the procedure used in laboratory 4. The reasons for these discrepancies are not clear. In particular the difference in feed intake cannot explains the variations observed.

Within-laboratory variability observed with the domestic methods is quite similar to that obtained with the standard method (CV_R = 2.0 *vs* 2.5% for dDM and 2.2 *vs* 2.8% for DE) but it is difficult to compare the repeatabilities because one laboratory (Lab 1) eliminated the extreme values in its own method by retaining only 6 rabbits per diet for final calculation.

CONCLUSION

From this collaborative study involving six laboratories and including 260 digestive balances several conclusions can be drawn : (*i*) Among-laboratory variability of the digestibility results obtained with the domestic methods showed the need to standardize the digestibility procedures ; (*ii*) A simplified and quick method with only a 4 days collection period can replace usual procedures including a longer period of feces collection if there are enough rabbits per diet (at least 8) ; (*iii*) Sex has no effect and litter a slight effect on digestibility of main dietary components ; (*iv*) As the standard method adopted by several European laboratories gave reproducible digestibility values for main components it can be regarded as a baseline of calibrating domestic procedures in order to be able to compare digestibility data from different laboratories ; (*v*) Variability of the analytical data brought out the need to standardize the chemical procedures among the European laboratories more especially for fibre fractions.

Acknowledgements : We are indebted to J. De Dapper (INRA, Toulouse) for preparing the experimental diets and to Béatrice Lamboley (INRA, Toulouse) for her technical assistance in calculations. The authors like further to express their gratitude to the technical staff in the different laboratories involved.

Received : September 1995
Accepted : November 1995

REFERENCES

AOAC, 1988. Guidelines for collaborative study procedure to validate characteristics of a method of analysis. *J. Assoc. Off. Anal. Chem., 71, 161-171.*

AOAC, 1990. Official methods of analysis (15th Ed.). *Association of Official Analytical Chemists , Arlington, VA.*

BELTRAN M.T., MARTINEZ J., FERNANDEZ J., CERVERA C., 1984. Estimacion del valor nutritivo de diversas materias primas para conejos. *Proc. 3rd World Rabbit Congress, vol.1, 265-272.*

BOURDILLON A., CARRÉ B., CONAN L., DUPERRAY J., HUYGHEBAERT G., LECLERCQ B., LESSIRE M., MCNAB J., WISEMAN J., 1990. European reference method for the *in vivo* determination of metabolisable energy with adult cockerels. *Br. Poult. Sci., 31, 557-565.*

CHEEKE P.R., 1987. Rabbit feeding and nutrition. *Academic Press Inc., Orlando, USA, 376p.*

COLIN M., LEBAS F., 1976. Méthodes d'études de la digestibilité des aliments chez le lapin. I. Périodicité des collectes. *Sci. Tech. Anim. Lab., 1, 129-133.*

DIXON W. J., 1953. Processing data for outliers. *Biometrics, 9, 74-89.*

FEKETE S., GIPPERT T., 1986. Digestibility and nutritive value of nineteen important feedstuffs for rabbits. *J. Appl. Rabbit Res., 9, 103-108.*

HORWITZ W, KAMPS L.R., BOYER K.W., 1980. Quality assurance in the analysis of foods for trace constituents. *J. Assoc. Off. Anal. Chem., 63, 1344-1354.*

INRA, 1989. L'alimentation des animaux monogastriques : porc, lapin, volailles. *INRA Editions, Paris, 282 p.*

MAERTENS L., JANSSEN W.M.M., STEENLAND E., WOLTERS D.F., BRANJE H.E.B., JAGER F., 1990. Tables de composition, de digestibilité et de valeur énergétique des matières premières pour lapins. *5èmes Journ. Rech. Cunicole Fr., Paris, 12-13 Décembre 1990. Communication n° 57, 57.1-57.9. ITAVI Ed., Paris.*

PEREZ J.M., 1991. Intérêt et limites des modèles de prévision de la valeur énergétique des aliments destinés au porc. *Thèse de Doctorat, Université de Montpellier II, 196 p.*

PEREZ J.M., LEBAS F., 1992. Peut-on estimer la valeur énergétique des aliments destinés aux lapins ? *Cuniculture, 19, 271-274.*

PEREZ J.M., BOURDILLON A., JARRIN D., LAMBOLEY B., LEBAS F., LE NAOUR J., WIDIEZ J.L., 1994. Application de la méthode européenne standardisée de mesure *in vivo* de la valeur énergétique des aliments destinés au lapin : comparaisons inter-laboratoires. *6èmes Journ. Rech. Cunicole Fr., La Rochelle, 6-7 Décembre 1994, pp. 365-374. ITAVI Ed., Paris.*

PEREZ J.M., LEBAS F., GIDENNE T., MAERTENS L., XICCATO G., PARIGI-BINI R., DALLE ZOTTE A., COSSU M.E., CARAZZOLO A., VILLAMIDE M.J., CARABAÑO R., FRAGA M.J., RAMOS M.A., CERVERA C., BLAS E., FERNANDEZ J., FALCAO e CUNHA L., BENGALA FREIRE J., 1995. European reference method for *in vivo* determination of diet digestibility in rabbits. *World Rabbit Sci., 3, 41-43.*

SAS, 1988. SAS/STAT User's Guide (Release 6.03). *SAS Institute Inc., Cary, NC, USA, 1028 p.*

SAUVANT D., BRETTE C., 1983. Les tolérances sur les caractéristiques portées sur les étiquettes. *In : L'évolution de la législation en alimentation animale, Cycle Approfondi d'Alimentation Animale, INA-PG, 24 mars 1983, H1-H16. ADEPRINA Ed., Paris.*

VAN SOEST P.J., ROBERTSON J.B., LEWIS B., 1991. Methods for dietary fiber, neutral detergent fiber, and non starch polysaccharides in relation to animal nutrition. *J. Dairy Sci., 74, 3583-3597.*

VILLAMIDE M.J., RAMOS M.A., 1994. Length of collection period and number of rabbits in digestibility assays. *World Rabbit Sci., 2, 29-35.*

VILLAMIDE M.J., FRAGA M.J., DE BLAS J.C., 1991. Effect of type of basal diet and rate of inclusion on the evaluation of protein concentrates with rabbits. *Anim. Prod., 52, 215-224.*

XICCATO G., COSSU M.E., CARAZZOLO A., CARABAÑO R., RAMOS M., 1994. Evaluation in vitro de la valeur nutritive des aliments pour lapins. Efficacité de différents enzymes digestifs. *6èmes Journ. Rech. Cunicole Fr., La Rochelle, 6-7 Décembre 1994, pp. 375-383. ITAVI Ed., Paris.*