EUROPEAN RING-TEST ON THE CHEMICAL ANALYSES OF FEED AND FAECES : INFLUENCE ON THE CALCULATION OF NUTRIENT DIGESTIBILITY IN RABBITS

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Abstract - A ring-test on chemical analyses of rabbit diets and faeces was carried out by six European laboratories, members of EGRAN (European Group on Rabbit Nutrition), from five Countries (Belgium, France, Italy, Portugal and Spain). Four samples of different complete feeds (A, B, C, D) and 8 samples of faeces, collected during the digestibility trial (EGRAN method) on 2 feeds (A, B) were analysed. Methodologies for dry matter (DM) and ash (ASH) determination were previously harmonised among the laboratories; crude protein (CP), crude fibre (CF), crude fat (FAT), fibre fractions (NDF, ADF, ADL) and gross energy (GE) were analysed using domestic methodologies.

The feeds were significantly different in all chemical constituents with a good repeatability (coefficient of variation within laboratory from 0.5% for DM and GE to 7.5% for ADL). A significant laboratory effect was also observed for all chemical constituents. Anyway, the reproducibility s.d. (S_L) was good for DM (coefficient of variation among laboratories, CV_L = 0.5%) and GE (0.7%), medium for CF (5.4%) and fibre fractions and poor for FAT (17.8%). Similar results were observed for faeces analyses.

The digestibility coefficients of diets A and B showed a significant laboratory effect in most cases, even though the diet effect was always much higher. The CV_L was low for dOM (1.0%), dCP (1.5%) and dGE (1.0%) and high for dCF (15.0%), dFAT (10.5%) and dADF (14.5%). Finally, the variability among laboratories of dADL was exceptionally high (83.3%), ranging dADL from 7.7% to 22.7% in the different laboratories. The estimation of DE content of the two feeds was in good accordance among laboratories (CV_L = 1.4%), with a range between 11.70 and 11.99 MJ/kg DM ($P<0.01$).

Further efforts are needed in the harmonisation of analytical methodologies among laboratories, especially in fat and fibre determination.

INTRODUCTION

The harmonisation of research methodologies is important in the exchange and comparison of information among laboratories and research teams. It appears to be fundamental for a rapid progress in the knowledge also on rabbit science and technique. Till now, some efforts on the harmonisation on scientific methods of rabbit research have been done on carcass and meat quality by the WRSA Commission (BLASCO *et al.*, 1992) and on "in vitro" digestibility evaluation of nutritive value of rabbit feeds (XICCATO *et al.*, 1994). In 1995 the EGRAN (European Group on Rabbit Nutrition) members proposed the European reference method for *in vivo* determination of diet digestibility in rabbits (PEREZ *et al.*, 1995b). On that occasion, a collaborative study was also carried out, comparing the results obtained with the common or domestic methodologies (PEREZ *et al.*, 1995a). EGRAN members noticed that DM digestibility coefficients were in a good accordance among laboratories, but nutrient digestibility coefficients were often very divergent, because of the high analytical variability both in feeds and faeces. Unfortunately, in the above mentioned research the variability due to the animals in digestibility trial was confounded with that due to the different analytical methodologies adopted by the six laboratories. Therefore, a second collaborative study was carried out to better separate the different sources of variation. It was devoted to describe the variability associated with the different analytical procedures.

MATERIAL AND METHODS

Laboratories and chemical analyses

Six laboratories, members of EGRAN, from 5 Countries (Belgium, France, Italy, Portugal and Spain) participated in this collaborative study. The French team prepared the samples of 4 complete feeds (A, B, C and D), different in crude protein (15.8 to 18.3% as fed), crude fibre (12.9 to 17.7%) and estimated digestible energy (10.3 to 11.7 MJ/kg). They performed also the digestibility trial on two feeds (A and B), involving 8 animals per feed, according to the European reference method (PEREZ *et al.*, 1995b). The faecal material excreted in a 4-day period was collected individually and the overall amount was immediately pre-dried (24 hours at 80°C). Because of the low quantity of dry matter excreted, the pre-dried faeces were joined 2 by 2, obtaining 4 samples per feed. Homogeneous subsamples (30 g) were obtained from these samples and mailed to the different laboratories together with the samples of all feeds (100 g) to be analysed.

The same analyses were performed both on feeds and faeces. Dry matter (DM) and ash (ASH) determinations were previously harmonised among laboratories: 5 g of sample were dried in ventilated oven (103°C for 24 hours) to determine DM (for chemical analyses purposes) and then put in muffle (550°C for 5 hours) to measure ASH. These analyses were performed in triplicate. The other analyses, i.e. crude protein (CP), crude fibre (CF), crude fat (FAT), fibre fractions (NDF, ADF, ADL) and gross energy (GE) were performed in duplicate, by using the domestic methods. FAT was not determined in the laboratory 5.

The digestibility coefficients of the nutrients and energy and the digestible energy (DE) content were calculated by using the DM ingestion and excretion data determined by the French team during the digestibility trial. In this way the DM digestibilities were equal for all laboratories, i.e. 66.8% and 67.1% on average for feeds A and B, respectively. On the other hand, the digestibility coefficients of nutrients and energy were calculated on the basis of the chemical analyses (DM basis) of feeds and faeces (average of 2 or 3 replications) determined in each laboratory.

Statistical analyses

Different models were used for complete feeds, faeces and digestibility coefficients: model 1 (feed composition) included the fixed effect of the diet (n= 4) and the random effects of the laboratory (n= 6) and the diet x laboratory interaction; model 2 (faeces composition) included also the animals (n= 4 couples) as a random effect; model 3 (digestibility coefficients and DE content) considered the fixed effect of the diet (n= 2) and the random effect of the laboratory (n= 6) but excluded the diet x laboratory interaction because it was negligible. The statistical analysis was performed by ANOVA using the GLM procedure of SAS (1988), where the laboratory means were compared by the Newman-Keuls test. The repeatability (i.e. within-laboratory s.d., S_R) was estimated directly from the ANOVA error mean square: $S_R = \sqrt{(s_e^2)}$. The reproducibility (i.e. among-laboratory s.d., S_L) was estimated by the VARCOMP procedure of SAS, where the expected variance components of the laboratory (s_l^2) and the diet x laboratory interaction (s_{dxl}^2) were calculated: $S_L = \sqrt{(s_e^2 + s_l^2 + s_{dxl}^2)}$.

RESULTS AND DISCUSSION

The results of the chemical analyses of the feeds (avg. A, B, C, D) are given in Table 1. As expected, most of the chemical constituents differed significantly among diets. Significant laboratory and/or diet x laboratory interaction effects were also observed, but as a rule these were less important than the diet effect. The within-laboratory variability (S_R) was very small for DM and GE (<0.5% when expressed as a % of the mean), good for ASH, CP, CF, NDF and ADF (1 to 2%), while FAT (3.3%) and ADL (7.5%) analyses were less repeatable. The among-laboratory variability (S_L) showed a similar trend, when expressed as a % of the mean (CV_L): it was low (0.5-2.5%) for DM, GE, ASH, CP and ADF, medium (4.5-7.5%) for CF, NDF and ADL, while FAT showed a poor reproducibility (CV_L = 17.8%). However, the S_L generally decreased in comparison with that observed in the previous EGRAN collaborative study (PEREZ *et al.*, 1995a), especially for the Van Soest fibre fractions.

The analyses of faeces (Table 2) showed significant effects of diet and laboratory, the latter being less important than the former. Only FAT was characterised by a greater variability among laboratories than among

diets. Diet x laboratory interaction was not significant, while animal (couple) effect was, which confirms the importance of using an adequate number of rabbits in digestibility trials. Both S_R and S_L were similar to that observed on feed analyses. Only the S_L of NDF was lower in faeces than in feed analyses, in relation with the feed starch content and the incomplete efficacy of amylase pre-treatment. This difference was also noticed for the CF reproducibility s.d.

Table 1 : Chemical composition (%DM) and GE content (MJ/kg DM) of the complete feeds (means of A, B, C, D) and interlaboratory precision

	DM	ASH	CP	CF	FAT	NDF	ADF	ADL	GE
No.repls.	72	72	52	52	40	52	52	52	52
LAB1	91.30^{a}	8.52^{c}	18.31^{c}	16.10^{b}	3.17^{bc}	32.75^{b}	18.96	4.40	17.83^{b}
LAB2	90.20^{c}	8.48^{c}	19.44^{a}	16.95^{ab}	2.87^{c}	35.65^{a}	19.54	3.85	18.06^{a}
LAB3	91.18^{ab}	8.66^{b}	18.85^{b}	18.79^{a}	4.24^{a}	32.77^{b}	19.32	4.61	18.06^{a}
LAB4	90.47^{c}	8.58^{bc}	18.85^{b}	15.74^{b}	2.78^{c}	32.09^{b}	19.37	4.06	18.00^{ab}
LAB5	90.86^{b}	8.85^{a}	18.77^{b}	17.34^{ab}	n.d.	32.18^{b}	19.30	4.05	17.95^{ab}
LAB6	91.16^{ab}	8.80^{a}	18.15^{c}	16.55^{ab}	3.47^{b}	31.60^{b}	19.80	4.54	18.14^{a}
Mean	90.86	8.65	18.73	16.91	3.31	32.84	19.38	4.25	18.01
F (Diet)	4.6*	421.4**	210.8**	21.1**	169.1**	348.0**	297.6**	<1	66.4**
F (Lab.)	22.3**	15.9**	16.8**	3.3*	30.7**	12.1**	1.6	<1	5.0**
F (D x L)	16.5**	1.5	2.6*	35.0**	8.1**	2.6*	3.2**	8.4**	3.2**
S_R[(1)]	0.08	0.10	0.20	0.29	0.11	0.73	0.35	0.32	0.08
S_L[(2)]	0.44	0.18	0.47	0.92	0.59	1.53	0.38	0.32	0.12
CV_L[(3)]	0.5	2.1	2.5	5.4	17.8	4.7	2.0	7.5	0.7
CV_L (1995)[(4)]		3.6	2.6	4.5		6.8	6.8	12.2	1.0

[(1)] Within laboratory s.d. (repeatability). [(2)] Among laboratory s.d. (reproducibility).
[(3)] CV_L = coefficient of variation among laboratory. [(4)] CV_L (1995) = CV_L observed by PEREZ et al. (1995a).

Table 2 : Chemical composition (%DM) and GE content (MJ/kg DM) of the faeces (means of F_A, F_B) and interlaboratory precision

	DM	ASH	CP	CF	FAT	NDF	ADF	ADL	GE
No. repls.	136	136	104	96	75	96	96	96	96
LAB1	94.54^{c}	8.25	11.98^{c}	42.31^{ab}	2.07^{c}	72.40	48.67^{c}	10.84	18.65^{a}
LAB2	94.67^{bc}	8.30	12.13^{bc}	40.28^{b}	1.78^{c}	74.86	50.57^{ab}	10.88	18.46^{b}
LAB3	94.77^{bc}	8.29	12.41^{b}	43.35^{a}	3.36^{a}	73.63	49.00^{bc}	11.18	18.75^{a}
LAB4	95.43^{a}	8.37	12.13^{bc}	41.57^{ab}	2.72^{b}	73.67	50.93^{a}	10.64	18.64^{a}
LAB5	94.98^{b}	8.36	13.07^{a}	42.60^{a}	n.d.	73.06	49.43^{abc}	11.24	18.70^{a}
LAB6	93.07^{d}	8.36	11.77^{c}	41.95^{ab}	2.83^{b}	72.08	49.07^{bc}	12.27	18.73^{a}
Mean	94.58	8.32	12.25	42.01	2.55	73.28	49.61	11.18	18.66
F (Diet)	12.5*	2082.9**	483.2**	29.1**	24.4**	113.6**	30.2**	12.4*	323.3**
F (Lab.)	107.3**	1.1	47.7**	6.5*	55.8**	3.4	7.7*	4.4	25.0**
F (D x L)	<1	<1	<1	1.1	2.0	2.6*	<1	1.2	<1
F (Anim.)	20.5**	18.2**	36.5**	13.7**	3.6**	6.7**	8.2**	2.7*	3.7**
S_R	0.10	0.10	0.14	0.43	0.12	0.52	0.54	0.61	0.10
S_L	0.82	0.11	0.51	1.05	0.63	0.99	1.02	0.80	0.14
CV_L	0.9	1.3	4.2	2.5	24.7	1.4	2.1	7.2	0.8

The digestibility coefficients and the DE content of the feeds (avg. A and B) are listed in Table 3. The laboratory effect was always less significant than the diet effect, while the reproducibility s.d. was very different depending on the chemical constituent, being very good (CV_L = 1 to 2%) for dOM, dASH, dCP and dGE, and poor (CV_L = 10 to 15%) for dCF, dFAT and dADF. Finally, the reproducibility s.d. of dADL was exceptionally high (CV_L = 83.3%). In some cases, the S_L of digestible coefficients was better than that observed

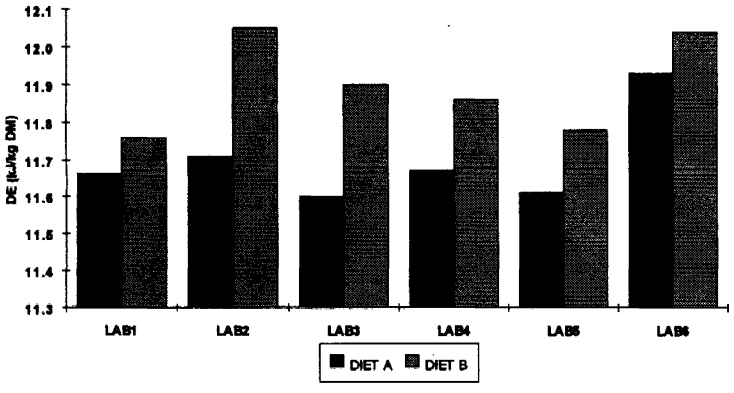
for feed and faeces analyses, as a consequence of the application within laboratory of the same analytical methodologies both on feeds and faeces. For example, FAT digestibility was more reproducible (CV_L = 10.5%) than FAT content on feeds (17.8%) and faeces (24.7%). In fact the analytical methods for FAT differed strongly among laboratories: three laboratories determined FAT by extraction with ethylic ether, two used petrol ether. Another source of variability was the acid hydrolysis pre-treatment, used for feeds and faeces in all laboratories, except one, which pre-treated only the faecal matter. Other differences in FAT determination methodology were observed (e.g. sample weight, extraction time, etc.), but in general the variations in FAT values showed the same trend for feeds and faeces.

Table 3 : Digestibility coefficients (%) and DE content (MJ/kg DM) of 2 complete feeds (means of A, B) and interlaboratory precision

	dOM	dCP	dCF	dFAT	dNDF	dADF	dADL	dGE	DE
No. repls.	48	48	48	40	48	48	48	48	48
LAB1	66.95	77.01^{a}	20.75^{ab}	72.71^{a}	35.04^{ab}	22.57^{ab}	22.73^{a}	65.51	11.71^{c}
LAB2	67.01	78.23^{a}	24.50^{a}	73.30^{a}	37.01^{a}	22.62^{ab}	8.10^{b}	66.07	11.88^{ab}
LAB3	66.92	77.01^{a}	23.11^{ab}	70.74^{ab}	33.92^{b}	22.43^{ab}	10.04^{ab}	65.47	11.75^{bc}
LAB4	66.98	77.25^{a}	19.76^{b}	57.16^{c}	32.13^{b}	20.46^{b}	18.15^{ab}	65.65	11.77^{bc}
LAB5	66.87	75.70^{b}	24.13^{a}	n.d.	34.03^{b}	22.67^{ab}	7.66^{b}	65.43	11.70^{c}
LAB6	66.92	77.31^{a}	23.85^{a}	67.85^{b}	34.05^{b}	25.62^{a}	13.61^{ab}	65.96	11.99^{a}
Mean	66.94	77.09	22.68	68.35	34.36	22.73	13.38	65.68	11.80
F (Diet)	<1	71.7**	220.7**	62.0**	618.0**	198.1**	26.6**	1.3	32.1**
F (Lab.)	1.4	7.0**	3.5*	38.9**	4.6**	2.4*	2.9*	1.5	6.8**
S_R	0.66	0.87	2.97	3.00	2.12	3.04	10.01	0.63	0.12
S_L	0.68	1.15	3.40	7.18	2.55	3.29	11.14	0.65	0.16
CV_L	1.0	1.5	15.0	10.5	7.4	14.5	83.3	1.0	1.4
CV_L (1995)	1.1	2.7	22.1		21.3	33.8	115.2	1.6	1.6

On the contrary, CF and fibre fraction digestibilities were less reproducible than the chemical analyses on feeds and faeces. The laboratories used different methodologies: even though all the laboratories used the pre-treatment with amylase, they used different amounts and type of enzyme (thermoresistant or not). Also the washing technique (with or without acetone) and the desiccation time (4 to 12 hours) were not harmonised. The different methods affected more the analyses on the feeds than those on the faeces and caused a great variability in digestibility coefficients. ADL digestibility was mostly affected, this fraction being the last step of a sequential method with increasing analytical errors. It varied from 2.6% (lab. 5) to 32.0% (lab. 1) on the feed A (avg. 20.8%) and from -9.2% (lab. 3) to 13.4% (lab. 4) on the feed B (avg. 5.9%).

Figure 1 : Energy values of the diets in the different laboratories



The evaluation of DE content was in good accordance among laboratories, varying from 11.70 to 11.99 MJ/kg DM ($P<0.01$) on average for the two feeds (Table 3). Within the feeds, the energy value varied from 11.60 (lab. 3) to 11.93 (lab. 6) for the feed A (avg. 11.70 MJ/kg DM) and 11.76 (lab. 1) to 12.05 (lab. 2) for the feed B (avg. 11.90 MJ/kg DM) (Figure 1). All laboratories determined a nutritive value higher for the feed B than for A, but the difference between the two values was minimal in the lab. 1 (0.10 MJ/kg DM) and maximal in the lab. 2 (0.34 MJ/kg DM). In any case, the reproducibility s.d. of DE was very low (CV_L = 1.4%), according to the

low S_L of GE determination both in feed and in faeces. This suggests that different laboratories can obtain coherent DE evaluations when the methodology for *in vivo* DM digestibility is harmonised (PEREZ *et al.*, 1995a,b).

In conclusion, this interlaboratory study indicates that the nutritive values (DE content and OM and CP digestibility) of different feeds can be judged accurate in different laboratories when a common methodology like that proposed by EGRAN is utilised, which allows to reduce strongly the variability of DM digestibility. However, according with the conclusion of the first ring-test, further efforts are needed in the harmonisation of analytical methodologies among laboratories for other chemical constituents, especially crude fat, crude fibre and fibre fractions.

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REFERENCES

AOAC, 1990. Official methods of analyses (15th ed.). Association of Official Analytical Chemists, Arlington, VA (USA).

BLASCO A., OUHAYOUN J., MASOERO G., 1992. Study of rabbit meat and carcass criteria and terminology. *Proc. 5th World Rabbit Congress*, **Vol. B**, 775-786

PEREZ J.M., CERVERA C., FALCAO E CUNHA L., MAERTENS L., VILLAMIDE M.J., XICCATO G., 1995a. European ring-test on *in vivo* determination of digestibility in rabbits: reproducibility of a reference method in comparison with domestic laboratory procedures. *World Rabbit Sci.*, **3**, 171-178.

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., 1995b. 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*. SAS Institute Inc., Cary, NC, USA.

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. *Proc. 6èmes Journées de la Recherche Cunicole en France*, La Rochelle (France), 6-7/12/94, **Vol. 2**, 375-383.