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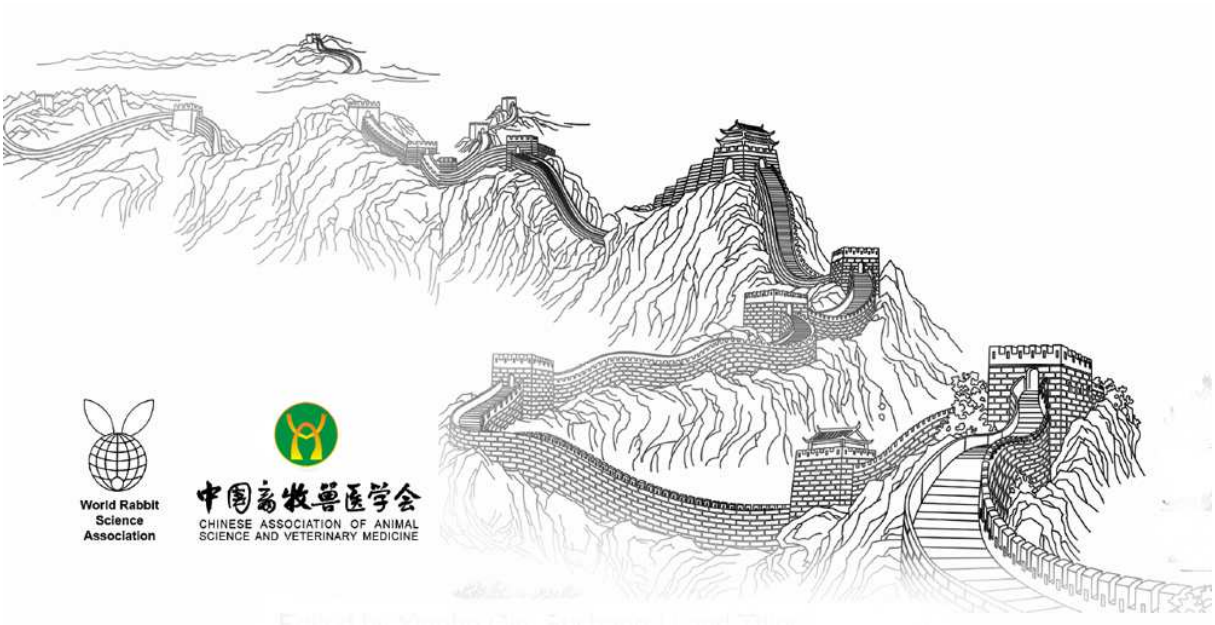

ESTIMATION OF DIGESTIBLE ENERGY CONTENT AND PROTEIN DIGESTIBILITY OF RAW MATERIALS BY THE RABBIT, WITH A SYSTEM OF EQUATIONS

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ABSTRACT

This study aimed to propose equations to estimate digestible energy or nitrogen digestibility of feedstuffs, when databases contain no value measured in-vivo, and particularly to formulate balanced diets with new feedstuffs. Three databases of raw materials composition (Feedipedia, Inra-Afz and Egran) with indication of digestible energy (DE-Rab) and nitrogen digestibility in the rabbit (N-Dig-Rab) were used to calculate equations that estimated DE-Rab and N-Dig-Rab for other raw materials. Estimation were based on chemical analyses and, when available, on the corresponding nutritive values for ruminants and growing pigs. The same calculations were made with a corpus of 82 published *in vivo* determination available in the literature. Selection of the most suitable equations was then obtained according to the correlation between the equation-estimated values and those issued of another corpus of 81 *in vivo* values. For DE-Rab as for N-Dig-Rab estimations, the inclusion of the nutritive values for other species, in addition to the chemical composition of raw materials, leads to the most precise equations. But frequently in the practice, only the chemical analyses of a raw material are available. For this reason 2 equations are proposed for each rabbit nutritive value, the first based on chemical analyses + other species nutritive values and the second on chemical analyses only. All data are expressed as % of DM and digestibility coefficients as values from 1 to 100..

Digestible energy (MJ/kg DM)

Equation 1: DE-Rab= 6.030 + 0.562 DE-Pig + 0.021 CP - 0.147 CF + 0.096 EE + 0.057 (ADF-ADL) ± 0.619.

Equation 2: DE-Rab = 15.818 - 0.0597 CF - 0.1325 ADF + 0.1002 EE - 0.2049 Ash + 0.00121 CP^2 + 0.00277 EE^2 ± 1.043.

Nitrogen digestibility (%)

Equation 3: N-Dig-Rab = 14.908 - 0.0200 ADF^2 + 0.7927 CF + 0.4107 N-Dig-Rum + 0.3661 N-Dig-Pig ± 5.60.

Equation 4: N-Dig-Rab = 63.064 + 1.958 CP + 0.757 CF - 1.918 ADL - 4.611 Ash - 0.0236 CP^2 + 0.2296 Ash^2 - 0.0232 $(NDF-ADF)^2$ ± 8.10.

Key words: Rabbit, equations, digestible energy, nitrogen digestibility, feedstuffs.

INTRODUCTION

In the most recent publications on rabbit nutrition (De Blas *et al*., 2010a; Gidenne *et al*., 2015) recommendations for the formulation of balanced diets are expressed, among many others, in form of digestible energy and digestible proteins. Data on the nutritive value and raw material suitability for rabbit feeding were gathered together with the chemical composition in different tables, available in the international literature as the EGRAN tables (Maertens *et al*. 2002), the FEDNA tables (De Blas *et al*, 2010b), the INRA-AFZ tables (Sauvant *et al.* 2004) or in the online encyclopaedic data basis "Feedipedia" (www.feedipedia.org). Unfortunately the digestible energy content or the protein digestibility was established for only few raw material, about 40-50, mainly used in European countries. As a consequence, for a great number of raw materials known to be suitable for rabbits, the value of rabbit digestible energy (DE-Rab) and protein digestibility (N-Dig-Rab) are lacking . Thus to help people to formulate balanced diets with "new feedstuffs", few years ago we proposed equations to estimate DE-Rab and N-Dig-Rab of a feedstuff for the rabbit (Lebas, 2013), based on values published only in Feedipedia database. Respect to calculations, for each raw material we included in the initial matrix the digestible energy, crude protein digestibility and the proximate chemical composition for crude protein (CP), crude fibre (CF, Weende method), NDF, ADF, ADL according to the method initially proposed by Van Soest (1963), ether extract (EE) and total minerals (Ash). When available, ruminant and growing pig digestible energy and protein digestibility were also added in the matrix.

The objective of this paper is to make with the other above mentioned sources of information, the same type of computation and to propose the most accurate equations, in comparison with that obtain from equations based on published experimental *in vivo* determinations for 72 raw materials (163 *in vivo* determination, included in 58 publications). Obviously, let us recall that in-vivo measurement of a nutritive value remains the reference method. Nevertheless, such equations may help to give an estimate of the nutritive value when any in-vivo data is available.

MATERIALS and METHODS

Data sources

For each of the 4 above mentioned databases (Feedipedia, Fedna and Inra-Afz), as for the 163 *in vivo* determinations, a matrix was constructed with the proximate chemical analysis of each raw material, digestible energy for rabbits and nitrogen digestibility in the rabbits. When available digestible or metabolizable energy for ruminants and growing pigs, and nitrogen digestibility for the same species were included in the matrix. When necessary, all data were recalculated to be expressed as percentage of dry matter (chemical composition) or Mega joules / kg DM using the coefficient 4.184 to transform kcal in kjoules when necessary. For databasis, the number of suitable raw material vectors was 40 from Feedipedia source, 111 from Fedna source, 46 from Inra-Afz source and 46 from the Egran source. The matrix of the 163 *in vivo* determinations was divided in 2 ~equal parts (82 and 81 items). The first part "a" was used to calculate equations (as for data obtained from databasis) and the second part "b" was used to compare values obtained *in vivo* and those obtained according to the different equations estimating DE-Rab and N-Dig-Rab. A separate variance analysis was performed to compare the nutritive value and the chemical composition of the 2 parts "a" and "b". As expected no significant difference was observed between the 2 parts ($P>0.30$).

Calculations

Equations to estimate the DE-rab or N-dig-Rab were calculated by progressive multiple regression with SAS software (Confais and Le Guen, 2006). The model allowed the next iteration to remove a previously selected regressor. If necessary the square of each of the data of chemical analysis was included in the model to account for the possibility of non-linear linkage of various regressors (CP^2, ADF^2, ...) with the two dependent variables (DE-Rab and N-Dig-Rab). The probability threshold for the addition or removal of a variable when looking for the best regression was $P = 0.15$ as suggested by Confais and Le Guen (2006). For each matrix, estimation of DE-Rab and protein digestibility were calculated in 3 series using 1/ exclusively the corresponding nutritive values for ruminants and pigs as regressors, 2/ the same regressors + chemical analyses, and 3/ exclusively the chemical analysis as regressors. At the end, correlations were calculated between the *in vivo* observed value (part "b") and those issued of the different equations (Pearson correlation coefficients R).

RESULTS and DISCUSSION

Accuracy of databasis

When calculating the estimation of DE-Rab with corresponding values for ruminants and pigs in the Fedna database, a surprising result was obtained: DE-Rab was very highly correlated with that of pigs, $R^2 = 0.9916$ with a residual standard deviation of ±0.41 MJ/kg DM. This result makes us suspicious about the construction of the Fedna database. For example, a DE-Rab value were proposed for raw materials for which no *in vivo* results are available in the international literature, *e.g.* for lentil seeds (Heuzé *et al*, 2015). In the web site used to collect the data, no information was available about the methodology employed for the calculation of DE-Rab values.

According to these observations it seems clear that, in the Fedna databases, the DE-Rab values derive directly from the pig values, with some modulation most probably in relation with the international literature. This is a good solution to provide for feed manufacturers data for a maximum of raw material (twice more numerous as in other database) which may be used to compute quite balanced diets. But it makes also the data of this database non relevant to estimate the rabbit DE from composition of the raw materials. That is why, in this paper, the data of Fedna were discarded for all further calculations. Direct contacts with at least one of the main authors of each of the 3 others databasis (Sauvant, Tran and Perez, 2016, personal communications) make clear that data were obtained after a critical compilation of data obtained *in vivo* and published in the international literature. For the Inra-Afz and the Egran databasis, the final values of DE-Rab and N-Dig-Rab were included after discussion of an expert group trying to provide a nutritive values vector as coherent as possible for each raw material. On the contrary

in the Feedipedia basis, the values are the average of data collected from all publications with only elimination of publications considered as "non-reliable".

Estimation of rabbit digestible energy

With Feedipedia database, the 3 types of estimations of the DE-Rab are relatively similar (table 1). On the contrary with Inra-Afz database, the best estimation is clearly obtained with the combination of pig digestible energy associated with chemical analyses. Ruminant digestible energy initially included in the equation at 2nd iteration just after de DE-Pig, was eliminated at the 7th and last iteration. Estimation with data of the chemical values of the Egran table (the only potential regressors available) provide an estimation similar to that obtained with the chemical analyses of the 2 other databases. And finally the equation obtained from the *in vivo* determinations is the less precise.

Table 1: Precision of the estimation of the rabbit digestible energy using 3 different databases and *in vivo* determinations.

	Source of data			
Estimation of DE-Rab	**Feedipedia**	**Inra-Afz**	**Egran**	***In vivo***
With Ruminant and Pig values	*n = 31*	*n = 45*		-
R^2	0.860	0.936	-	-
rSTD MJ /kg DM	± 1.135	± 0.982	-	-
With chemical analyses	*n = 40*	*n = 46*	*n = 46*	*N=82*
R^2	0.912	0.938	0.926	0.828
rSTD MJ /kg DM	±1.250	± 1.043	± 1.277	± 1.861
With pig & chemical analyses	*n = 31*	*n = 45*		-
R^2	0.889	0.976	-	-
rSTD MJ /kg DM	±1.191	±0.619	-	-

To complete tables for raw material presently with blanks for DE-Rab values, the proposed equation is that issued of the Inra-Afz tables including pig DE and chemical composition (equation 1):

DE-Rab = 6.030 + 0.562 DE-Pig + 0.021 CP - 0.147 CF + 0.096 EE +0.057 (ADF-ADL) ± 0.619 MJ/kg DM

Correlations between the *in vivo* measures of DE-Rab and the values estimated with the different equations based on raw material chemical composition (n=81) were as follows : Feedipedia R=0.812, Inra-Afz R=0.845, Egran R=0.838 and *in vivo* (part "a") R = 0.832. All were statistically significant (P<0.0001). If only chemical analyses are available, for practical use everywhere, the equation based on Inra-Afz tables would be preferred because of a slightly better precision of this equation: ± 1.04 MJ/kg DM and a better correlation with the *in vivo* direct determinations (equation 2)

DE-Rab = 15.696 + 0.05751 CP - 0.03929 NDF - 0.12995 ADF + 0.2003 EE - 0.2416 Ash ± 1.043 MJ/kg DM

This equation 2 is more precise (±1.043 vs ±1.32 MJ) than that proposed some year ago by Wiseman *et al.* (1992) on the basis of 31 individual raw materials. It must be also underlined that the equation obtained from the *in vivo* determinations (subgroup "a") is clearly the less precise and provides values not better correlated with other values also determined *in vivo* (subgroup "b") than the other equations.

Estimations of nitrogen digestibility by rabbits

The most accurate equation for the estimation of N-Dig-Rab (table 2) was obtained with data of the Feedipedia and inclusion of ruminant and pig digestibility + chemical analyses in the model: $R^2 = 0.751$ and precision of ±5.60 points of digestibility. But this equation is based on only 27 raw materials (Equation 3): N-Dig-Rab = 14.907 - 0.0200 ADF^2 + 0.7927 CF + 0.4107 N-Dig-Rum + 0.3661 N-Dig-Pig ± 5.60 points.

If only chemical analyses are available, the most precise equation was that calculated with the Inra-Afz database (table 2). Correlations between the *in vivo* measures of N-Dig-Rab and the values estimated with the different equations based on raw material chemical composition (n=71) were as follows: Feedipedia R=0.481, Inra-Afz R=0.410, Egran R=0.222 and *in vivo* (part "a") R = 0.388. Three of the 4 equations were statistically significant (P<0.001), but with the Egran values, the probability of the correlation to be different of zero was only P=0.062. It is clear that theses correlations are lower than that observed for the digestible energy evaluation. Despite a little bit lower correlation with *in vivo* data, we consider that the equation obtain with the Inra-Afz values is the most suitable for practical use because of its better precision (Equation 4):

N-Dig-Rab = 63.064 + 1.958 CP + 0.757 CF - 1.918 ADL - 4.611 Ash - 0.0236 CP^2 + 0.2296 Ash^2 - 0.0232 $(NDF-ADF)^2$ ± 8.10 pts

Table 2: Precision of the estimation of nitrogen digestibility by rabbits, using 3 different databases and *in vivo* determinations, with indication of the number of raw materials (n) included in each analysis.

	Source of data			
Estimation of N-Dig-Rab	**Feedipedia**	**Inra-Afz**	**Egran**	***In vivo***
With Ruminant and Pig values	*n=27*	*n = 45*	-	-
R^2	0.679	0.697	-	-
rSTD (points of digestibility)	± 6.08	± 10.79	-	-
With Chemical analyses	*n=37*	*n = 46*	*n = 46*	*N=75*
R^2	0.830	0.877	0.787	0.582
rSTD (points of digestibility)	± 9.36	± 8.10	± 12.67	± 12.72
With Ruminant, pig & chemical analyses	*n = 27*	*n = 45*	-	-
R^2	0.751	0.836	-	-
rSTD (points of digestibility)	± 5.60	± 8.13	-	-

This equation 4 is also a little bit less precise than that proposed by Villamide and Fraga (1998): ± 8.10 *vs* 6.51 points. But because it includes the analysis of crude protein, crude fibre, NDF, ADF ADL and ash instead of only crude protein and ash in the Villamide and Fraga equation, the equation 4 is usable securely for a largely wider set of raw materials and then should be preferred.

CONCLUSION

From published feedstuff databases, eventually with knowledge of the digestibility of these ingredients by pigs and ruminants, and from *in vivo* published results, it is possible to propose equations to estimate the digestible energy and the digestible crude protein (CP content x N digestibility) contents to formulate balanced diets following current nutritional recommendations. These equations obviously did not replace any in-vivo measurements correctly conducted, since the best results were 2 to 4 times less precise than direct *in vivo* individual determinations. However, estimating nutritive values with such equations could be used when not any data is available in databases. This will help to formulate balanced diets for the rabbit, particularly to study *in-vivo* new or local materials.

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ESTIMATION OF DIGESTIBLE ENERGY CONTENT AND PROTEIN DIGESTIBILITY OF RAW MATERIALS WITH A SYSTEM OF EQUATIONS

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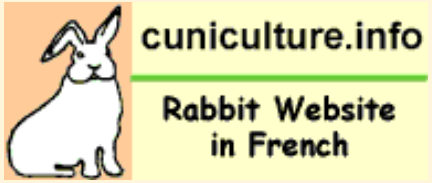

Background

Rabbit feeds formulation : *2 main criteria*

1. **Digestible ENERGY content (DE)**
2. **Digestible PROTEINS content (DP)**

Number of feedstuffs known to be suitable for rabbit feeding : **more than 1000** (Feedipedia, 2016)

Digestible Energy and Digestible Proteins are available for **about 50 feedstuffs only** (Egran, INRA-AFZ, Feedipedia,…)

Background

In the matrix of nutritive value of most feedstuffs,
data are available for

- Crude proteins
- Crude fibre
- NDF , ADF & ADL
- Ether extract
- total minerals (Ash)

In **addition** in some databases, **Digestibility** values are available for **Ruminants** and/or **Pigs**

Gross energy : rarely determined directly => easier to calculate directly **Dig. Energy**

Crude proteins content easy to determined => calculation of **Protein digestibility** to be multiplied by crude protein content => Digestible protein content for formulation

Aim of the study

To propose acceptable method (**equations**) to **fill blanks** in the feedstuffs matrix of composition and an idea of DE and DP content of **new feedstuffs**

Bases used for calculation :

Chemical composition
Values published for other species

Comparison with *in vivo* determinations

Methodology

3 published databases were used
+ 163 *in vivo* determination from literature

163 *in vivo* determinations => a/ 82 used to calculate equations
(2 sub-groups) b/ 81 used to test the calculated estimation

Equations to estimate the DE-rab or N-dig-Rab were calculated by **progressive multiple regression** (SAS software), with possibility of introduction or removal of regressor at each iteration.

With b/ subgroup MEASURED ⟷ CALCULATED
R = ?

83 feedstuffs studied in 163 *in vivo* determinations

Raw material	Nb	Raw material	NB	Raw material	Nb
Albizia falcata	1	Alfalfa	20	Alfafa+Fescue	3
Banana fresh	1	Barley grain	2	Barley straw	1
Beet pulp	4	Bermuda grass	1	*Bracharia brizantha*	1
Brewer's grain	2	*Bromus cartaticus*	1	*Brosmum alicastruni*	1
Carrot	1	Cassava peel	1	Cassava meal residue	1
Cassava meal	2	Cassava tops	2	*Centrosema pubescens*	1
Ceretatis capita	1	Chick peas	2	*Chloris gayana*	1
Cocoa hulls	1	Coconut meal	1	Corn gluten feed	2
Cucurbita pepo residues	1	*Desmodium heterophylum*	1	DDGS Maize	3
DDGS Barley	1	DDGS Wheat	1	Flax seeds	1
Grape pomace / marc	5	Grape seed meal	1	Grass hay	1
Hempseed meal	1	*Hibiscus rosa sinensis*	2	Lettuce leaves	1
Leucaena leucocephala	4	Maize whole plant	5	Maize grain	2
Maize by products (offals)	3	Maize ears	1	Maize stover	1
Milo/Sorghum	4	Mulberry leaves	1	*Neosotonia wightii*	1
Oat hulls	1	Oat grain	2	Oat green forage	1
Olive leaves	1	Olive oil cake	2	Orange tree leaves	1
Palm kernel meal	1	*Panicum maximum*	2	Peas	1
Pennisetum purpureum	1	*Potamogeton natans*	1	*Pueraria phaseolides*	1
Rapeseed meal	2	Rapeseed seeds	3	Rapeseed hulls	1
Rice bran	1	Rice hulls	1	*Robinia pseudoacacia*	1
Ray gras hay	1	Sainfoin hay	1	*Sesbania formosa*	1
Sesbania sesban	1	*Setaria splendida*	1	Soybean hulls	2
Soybean meal	1	Soybean seed whole	2	Stale bread	1
Stylosanthes guyanensis	1	*Sulla* forage	2	Sunflower meal	4
Sunflower hulls	2	Tobaco by-product	1	*Trifolium pratense* forage	1
Wetch-Oat hay	1	Wheat bran/Milurex	14	Wheat grain	1
Wheat shorts	1	Wheat straw	5		
Total	**47**		**79**		**37**

RESULTS

With values for other species

	Feedipedia	INRA-AFZ
D. Energy equation Pig DE + Chemical composition	N=31 **R^2 = 0.889** ±1,191 MJ/kg	N=45 **R^2 = 0.976** ±0.619 MJ/kg
N-Dig-Rab equation Rum. & Pig + Chemical composition	N=27 **R^2 = 0.751** ± 5.60	N=45 **R^2 = 0.836** ± 8.13

1 **DE-Rab** = 6.030 + **0.562 DE-Pig** + 0.021 CP - 0.147 CF + 0.096 EE + 0.057 (ADF-ADL) ± *0.619 MJ/kg DM*

3 **N-Dig-Rab** = 14.907 - 0.0200 ADF^2 + 0.7927 CF + **0.4107 N-Dig-Rum** + **0.3661 N-Dig-Pig** ± *5.60 points*

RESULTS

Equations based only on Chemical values

	Feedipedia	Inra-Afz	Egran	*in vivo*
DE Rabbit	N=40 **R^2 = 0.912** ± 1.250 MJ	N=46 **R^2 = 0.938** ± 1.043 MJ	N=46 **R^2 = 0.926** ± 1.277 MJ	N=82 **R^2 = 0.828** ± 1.861 MJ
N-Dig-Rabbit	N=37 **R^2 = 0.830** ± 9.36	N=46 **R^2 = 0.877** ± 8.10	N=46 **R^2 = 0.787** ± 12.67	N=75 **R^2 = 0.582** ± 12.72

RESULTS

Correlations between the **values measured** *in vivo* (sub-goup b) and **values calculated** with the different equations

	Feedipedia	Inra-Afz	Egran	*in vivo a/*
DE	0.812	**0.845**	0.838	0.832
N-Dig	0.481	**0.410**	0.222	0.388

2 **DE-Rab** = 15.696 + 0.05751 CP - 0.03929 NDF - 0.12995 ADF + 0.2003 EE - 0.2416 Ash ± *1.043 MJ/kg DM*

4 **N-Dig-Rab** = 63.064 + 1.958 CP + 0.757 CF - 1.918 ADL - 4.611 Ash - 0.0236 CP^2 + 0.2296 Ash^2 - 0.0232 $(NDF-ADF)^2$ ± *8.10 points*

Conclusion

- To fill blanks in the matrix of nutritional values of published databases, the best is to use equations 1 or 3 wich include nutritional values for pigs and/or ruminants

- **If only the chemical composition is known, equations 2 or 4 provide acceptable values for formulation of nearly balanced diets.**

Thanks

for your attention