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COMPARISON AMONG METHODS OF NUTRITIONAL EVALUATION OF FIBROUS INGREDIENTS

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COMPARISON AMONG METHODS OF NUTRITIONAL EVALUATION OF FIBROUS INGREDIENTS*

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ABSTRACT

A collaborative study was carried out by the EGRAN group in which two different Laboratories performed two assays designed to compare three methods of evaluation (direct, substitution of a basal diet or of a reference feedstuff) of the nutritional value of a fibrous ingredient (grape pulp, GP). A basal diet high in energy and protein and including 30% of alfalfa meal (AM) was progressively substituted (10, 20, and 30%) by GP in Lab 1. The AM of the basal diet was also substituted by GP (ratio AM:GP about 30:0, 20:10, 10:20, 0:30) in Lab 2. Another two diets with GP or AM as a sole ingredient were also evaluated in Lab 1 and 2, respectively, using growing rabbits for all diets. Digestibility of diets decreased linearly ($P<0.001$) with GP inclusion. Nutritional value of GP estimated by difference, substituting the basal diet and AM, were not different ($P>0.05$) and they were not significantly affected by the substitution rate, although values estimated for the lowest substitution rates had very high variation (CV>100%). Likewise, the nutritional value obtained by regression of basal diet and AM was similar ($P>0.05$). Its digestible energy (DE) was 5.553 ± 0.53 and 5.27 ± 0.53 MJ/kg DM, respectively, whereas CPd was not different from zero. The DE value of GP determined directly (7.41 MJ/kg DM) was significantly ($P<0.05$) higher than those obtained by the substitution method. As conclusions i) no additive nutritional values are obtained with the direct method for such imbalance ingredients, ii) substitution of AM instead of basal diet seems to have no advantage, iii) the substitution method could be applicable only with one and high substitution rate if this falls within its normal range of incorporation, but iv) using more substitution rates to estimate the nutritional value by regression is a much more reliable method.

INTRODUCTION

The nutritional value of feed ingredients for rabbit diets have been determined using methods proposed for other non-ruminant species, being the method accepted or not in based of the congruence of the results obtained. However, methodological studies on feedstuff evaluation have been scarcely done. Thus, MAERTENS and DE GROOTE (1981) compared the substitution and direct method for evaluating alfalfa hay obtaining similar results. Likewise, VILLAMIDE *et al.* (1991), and DE BLAS and VILLAMIDE (1990) studied the effect of different substitution rates and basal diets on the nutritional evaluation of feed ingredients. From these studies was deduced that most feed ingredients (cereals and their by-products and protein concentrates) could be evaluated by the substitution method, and alfalfa hay by the direct method (VILLAMIDE, 1996). Nevertheless, other fibrous feedstuffs with very high lignin content as straw, sunflower hulls, grape by-products are difficulty evaluated by the substitution method due to the important interactions that produce in the total mean retention time (GARCÍA *et al.*, 1999). This lead to different nutritive utilisation according to the substitution rate and the basal diet used. The direct method could be used in order to avoid these important interactions, or trying to decrease them, the substitution of a reference feedstuff of chemical composition more similar to test ingredient than the basal diet could be

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useful. Due to its characteristic only alfalfa hay could be used as reference feedstuff, because it could be evaluated directly. Therefore, a collaborative study was developed by the EGRAN, by which two different Laboratories carried out two assays which objective was to compare three methods of evaluation (direct, substitution of a basal diet and substitution of a reference feedstuff) for a fibrous feedstuff (grape pulp). Based on the results of the complete collaborative study the additivity of the values obtained was tested.

MATERIAL AND METHODS

Experimental design. A grape pulp and a medium quality alfalfa meal were chosen as test and reference feedstuffs, respectively. Their chemical composition is shown in Table 1. A basal diet (BD) including alfalfa meal (AM) as main source of fibre and with relatively high energy and protein content was designed to compensate the low nutritional value of grape pulp (GP). It was composed on dry matter basis (%DM) by 28.7 wheat, 10.3 sunflower meal, 30.6 alfalfa meal, 20.0 wheat bran and 10.4 full-fat soya bean.

Table 1. Chemical composition of raw materials (%DM)

Raw materials	GRAPE PULP		ALFALFA MEAL
Laboratory	1	2	2
Dry Matter	88.5	88.7	89.2
Ash	5.40	6.39	15.8
Gross Energy (MJ/kg DM)	20.4	20.9	17.4
Crude Protein	10.6	10.9	16.8
Crude Fibre	25.7	28.9	27.6
Neutral Detergent Fibre	57.6	55.0	48.7
Acid Detergent Fibre	46.6	43.9	30.7
Acid Detergent Lignin	33.0	31.0	12.6
Ether Extract	6.52	6.18	2.09

In Lab 1 the basal diet was substituted at 0.1, 0.2 and 0.3 by grape pulp (actual substitution rates on DM basis 0.099, 0.198 and 0.298 for GP-10, GP-20 and GP-30 diets, respectively) to determine by substitution its nutritional value. Also grape pulp was offered directly to rabbits (GP diet) to determine its nutritional value by the direct method.

In Lab 2 alfalfa meal was evaluated directly (ALF diet). Simultaneously the AM of basal diet was progressively substituted by GP (the ratio AM : GP was 0.306:0, 0.203:0.103, 0.103:0.203 and 0:0.306, for AG-10, AG-20 and AG-30 diets, respectively) to determine the nutritional value of grape pulp with respect to alfalfa meal. A mineral vitamin premix composed by 1.1% calcium carbonate, 0.3% dicalcium phosphate, 0.6% sodium chloride and 0.2% of a mineral-vitamin mixture was added to all diets, both in Lab 1 and 2, to avoid nutritional deficiencies with increasing substitution rates.

Chemical composition (Table 2) and nutritional value of experimental diets are expressed on the total weight basis. However, for calculation of the nutritional value of ingredients by the different methods, the energy and protein content of diets was corrected by the premix content.

The digestibility trial was conducted according to the European Reference Method (PÉREZ *et al.*, 1995) using one hundred and thirteen New Zealand White x Californian growing rabbits. Animals were allotted randomly to the diets (10-13 rabbits per diet) and were housed in a closed building with partial environmental control under a 12-12 h light-dark schedule.

Analytical Procedures. Analyses were conducted according to AOAC (1991) for DM, ash, crude protein (CP), crude fibre (CF) and ether extract (EE) after acid hydrolysis. Neutral detergent fibre (NDF), acid detergent fibre (ADF) and lignin (ADL) were analysed sequentially (VAN SOEST *et al*., 1991) with a thermo-stable amylase pre-treatment. Gross energy (GE) was determined by adiabatic calorimetry.

Table 2. Chemical composition of experimental diets (%DM)

Diets	BD	GP-10	GP-20	GP-30	GP	BD	AG-10	AG-20	AG-30	ALF
Lab	1	1	1	1	1	2	2	2	2	2
DM	88.9	88.9	88.9	89.3	88.7	89.8	89.2	89.6	88.7	92.1
ASH	8.28	8.29	8.14	7.99	7.05	9.13	8.84	7.85	6.91	17.1
GE (MJ/kg)	18.4	18.5	18.70	18.8	19.9	18.8	18.9	19.3	19.6	17.2
CP	17.7	16.9	16.4	15.8	10.2	18.4	17.7	17.3	16.3	16.3
CF	13.5	14.7	15.8	17.1	24.8	15.1	15.5	14.1	15.8	27.7
NDF	36.0	37.1	39.0	40.9	56.6	32.7	32.0	33.0	34.4	50.1
ADF	16.0	20.0	22.1	24.2	46.5	16.8	17.9	19.6	21.6	32.3
ADL	4.51	8.41	11.9	14.9	31.1	4.40	7.21	9.85	13.1	13.7
EE	4.17	4.45	4.62	4.88	4.81	4.65	5.12	5.60	5.91	1.97

For abbreviations see text

Calculation Procedures and Statistical Analysis. The nutritional value (equations for digestible energy, DE) of ingredients calculated by difference between the nutritional value of diets, for each substitution rate was done according to the following equations for a substitution of basal diet and reference feedstuff, respectively.

$$DE_I = \frac{(DE_{TD} - (1-P)xDE_{BD})}{P} \quad ; \quad DE_I = DE_{RF} + \frac{(DE_{TD} - DE_{BD})}{P}$$

where $DE_{I,\ TD,\ BD,\ RF}$, were the DE of ingredient, test diet, basal diet, and reference feedstuff, respectively, and P was the substitution rate.

Their standard errors (SE) were calculated according to the following expressions

$$SE_I = \frac{1}{P}\sqrt{\frac{VAR_{TD}}{n_{TD}} + (1-P)^2\frac{VAR_{BD}}{n_{BD}}} \quad ; \quad SE_I = \sqrt{\frac{VAR_{RF}}{n_{RF}} + \frac{1}{P^2}\left(\frac{VAR_{TD}}{n_{TD}} + \frac{VAR_{BD}}{n_{BD}}\right)}$$

where $VAR_{TD,\ BD,\ RF}$ were variance of test diet, basal diet and reference feedstuff, respectively, and $n_{TD,\ BD,\ RF,}$ were the number of rabbits used in test diet, basal diet, and reference feedstuff, respectively.

The nutritional value of ingredients was also calculated by regression between the digestible nutrient content of experimental diets and the substitution rates, and extrapolating to a total substitution. The standard error of the extrapolated values when GP substituted basal diet was calculated according to the following expression:

$$\text{SE (extrapolated value of GP substituting a basal diet)} = \sqrt{MSE_{reg}\left[\frac{1}{N} + \frac{(1-P_m)^2}{\Sigma P_i^2 - (\Sigma P_i)^2/N}\right]}$$

where MSE_{reg} was the mean square error of regression; P_m, and P_i were mean and individual rate of inclusions of test ingredient, and N was the number of total data. For the substitution of a reference feedstuff the regression slope represents the global difference between the nutritional value of reference and test ingredient. Therefore, the standard error of the extrapolated value, in this case, was calculated according to the following expression where $SE_{alf,\ slope}$, were the standard error of alfalfa meal and the slope of the regression, respectively.

SE (extrapolated value of GP substituting alfalfa meal) $= \sqrt{SE_{alf}^{\ 2} + SE_{slope}^{\ 2}}$

Data were analysed using the GLM procedures of SAS (1990). Regression procedures were performed to determine the evolution of digestibility coefficients and digestible nutrient content of diets with the substitution rate of grape pulp. Values obtained by the different methods were compared by using a T-test.

RESULTS AND DISCUSSION

Chemical composition of grape pulp and BD differed slightly between Labs, mainly in CF content (Tables 1 and 2), although they could be considered within the normal variation among laboratories observed in the last ring-test (XICCATO *et al*. 1996).

Digestibility coefficients (GE digestibility, GEd, CP digestibility, CPd etc.) of experimental diets are shown in Table 3 and 4. Nutrient digestibility of basal diet differed ($P<0.06$) between Labs in the same way than for chemical composition. The GEd and CPd were 2.2 and 3.4 points higher for Lab 1 than for Lab 2, but no difference was found for DE content ($P= 0.417$) because of the higher GE obtained in Lab 2. These differences in nutrient digestibility were not related with feed intake or growth, which were similar between laboratories. In general, digestibility values of all diets in Lab 2 were lower than expected in function of their ADF content. Diets in Lab 2 had to be stored for two months in a freezer (5ºC) before doing the digestibility trial, which could imply a decrease in the bioavailability of some essential nutrients as vitamins. Therefore, the nutritional value of grape pulp was calculated with the original data in each Lab and no corrections have been done because the other diets were evaluated only in one Lab.

Table 3. Digestibility of diets (%), (Laboratory 1)

Diets	BD	GP-10	GP-20	GP-30	RSD	L[1]	GP
n	11	12	11	12			12
DMd	67.9	64.3	61.1	56.5	2.1	0.0001	40.8 ± 2.4[2]
OMd	68.3	64.4	61.0	56.4	2.1	0.0001	40.0 ± 2.4
GEd	66.5	62.6	58.8	54.2	2.4	0.0001	36.4 ± 2.8
CPd	74.8	69.7	64.8	58.9	2.9	0.0001	11.6 ± 3.3

For abbreviations see text, [1]L: Significance of linear effect of dietary level of inclusion of GP, [2]standard deviation

Table 4. Digestibility of diets (%) (Laboratory 2)

Diets	BD	AG-10	AG-20	AG-30	RSD	L[1]	ALF
n	10	13	11	10			11
DMd	65.4	63.0	62.9	59.5	1.9	0.0001	47.4 ± 1.4[2]
OMd	65.6	63.1	62.8	59.5	1.8	0.0001	43.6 ± 1.5
GEd	64.3	61.4	61.1	57.9	1.8	0.0001	41.3 ± 1.8
CPd	71.4	66.2	66.0	58.1	3.7	0.0001	55.9 ±1.7

For abbreviations see text, [1]L: Significance of linear effect of dietary level of inclusion of GP, [2]standard deviation

All digestibility coefficients decreased linearly ($P<0.0001$) with the substitution of both basal diet and alfalfa hay by grape pulp. Dry matter, organic matter (OM) and GE digestibility decreased 0.41 points on average for each 1% increment of dietary GP that substituted the basal diet. When GP substituted AM the digestibility coefficients were less affected (0.19 decreased). MOTTA FERREIRA *et al*., (1996) also observed a linear decrease in GE and CP digestibilities when substituted alfalfa hay by grape pomace, but in that case there were no differences in dietary DE content. Crude protein digestibility decreased sharply, 0.54

and 0.40 points for each 1% of substitution of GP by basal diet and AM, respectively, resulting in a negative CPd of grape pulp.

The nutritional values of GP calculated by the different methods are shown in Table 5. Digestible energy values of grape pulp estimated by difference respect to the basal diet (5.94 MJ/kg DM, on average) did not differ (P>0.05) from those estimated by difference respect to AM (4.33 MJ/kg DM, on average). Despite the low value obtained for the lowest substitution rate of AM (2.517), it was also associated to a very high standard deviation (4.54), which prevented a statistical difference. In fact, the latter value is also not different from zero (P<0.05). The standard error of the estimates decreased proportionally to the substitution rate (from 1.63 to 0.61 MJ/kg DM on average). Thus, the coefficient of variation of the values estimated at 10% of substitution rate were higher than 100%, which indicates the high uncertainty of these values. Other values observed in the literature for similar products using the substitution method were: 3.09 MJ DE/kg DM, that was estimated by substituting a basal diet by 40% of grape marc (MAERTENS and DE GROOTE, 1984), and 7.61 MJ/kg DM that was estimated by substituting dietary AM included at 30% by grape pomace (MOTTA FERREIRA *et al*., 1996).

Table 5. Nutritional value of grape pulp as affected by evaluation method (mean ± standard error)

	Substitution rate			Regression	Direct
	0.10	0.20	0.30		
Substitution of a basal diet					
DE (MJ/kg DM)	6.01 ± 1.9	6.18 ±.0.9	5.64 ± 0.6	5.55 ± 0.5	7.41±.0.2
GEd (%)	29.5 ± 9.1	30.7 ± 4.5	27.7 ± 2.8	27.3 ± 4.9	36.4 ±.0.8
DCP (%)	-0.48 ± 2.4	0.32 ± 1.0	0.42 ± 0.7	1.02 ± 5.9	1.2 1 ±.0.1
CPd (%)	-4.5± 21.6	3.01 ± 9.6	3.93 ± 6.0	9.59 ± 55.6	11.4 ±.0.9
Substitution of a reference feedstuff					
DE (MJ/kg DM)	2.52 ±1.4	5.63 ±.0.8	4.85 ± 0.6	5.27 ± 0.5	
GEd (%)	12.1 ± 4.8	27.0 ± 2.7	23.3 ± 2.2	25.3 ± 2.5	
DCP (%)	-5.16 ± 2.2	0.21 ± 1.2	-3.3 ± 1.1	-2.29 ± 5.0	
CPd (%)	-47.9 ± 20.3	1.9 ± 10.9	-30.5 ± 9.9	-21.1 ± 45.7	

For abbreviations see text

Likewise, there were no significant differences (P>0.05) in the DE estimated by regression of basal diet or alfalfa hay (5.55 and 5.27 MJ/kg DM, respectively). Despite the differences in both chemical composition and digestibility values observed for the diet used in both Labs (basal diet), the evolution of the digestible nutrient content with grape pulp inclusion was independent of the Lab, resulting the same nutritional value.

The DE value obtained for GP by the direct method (7.411 MJ/kg DM) was significantly higher (P<0.05) than those estimated by the substitution method, possibly due to the low intake observed for this diet (90 g DM/d for GP diet vs 103-144 g DM/d for the other diets). Nutritional value of ingredients determined directly seems to be inversely related to the feed intake. Thus, FERNÁNDEZ-CARMONA *et al* (1996) reported a lower DE value for a GP (5.8 MJ/kg DM) with a higher fat content (8.3%), using the direct method in adult animals, which showed a higher intake (49.2 g DM/kg $^{-0.75}$). Previously, MARTÍNEZ and FERNÁNDEZ, (1980) had obtained with the same methodology a DE content of 1.7 MJ/kg DM for a GP of similar composition of that of the current work, but, in that case the intake was much greater (63.6 g DM/kg $^{-0.75}$).

The complete collaborative study let us to estimate the additivity of the nutritional value of GP, these values (5.89 ±0.33 MJ DE/kg DM and -7.78±5.7% CPd) were significantly different than those obtained directly, but similar to those estimated by substitution

The digestible crude protein (DCP) content of grape pulp estimated by substitution is not different from zero, or even negative (for the lowest substitution rate of alfalfa). This might indicate a negative effect of tannins from GP on the protein digestibility of other dietary ingredients (MOTTA FERREIRA *et al*., 1996). In general, the DCP followed the same tendency of the DE. The lowest values and highest standard errors were obtained for the lowest substitution rates, there were no differences between the values obtained by difference and regression, and the highest value was obtained by the direct method, although it was not different (P>0.05) of values obtained by substitution. Moreover, the coefficient of variation was higher for DCP than for DE as a consequence of the higher standard deviation observed for the CPd of diets and the low value of DCP.

The main conclusions that can be derived for the current study were: i) despite the easiness and low variability of the direct method, it can not be used to evaluate so imbalance ingredients as grape pulp because the results obtained were not additive in complete diets; ii) the substitution method using alfalfa meal as a reference feedstuff seems to have no advantage with respect to the substitution of a basal diet; iii) the substitution method is preferable when a sufficiently high (>20%) substitution rate is used, if this falls within the normal range of incorporation of this feedstuffs in rabbit diets; iv) the multilevel assay, where the nutritional value is estimated by regression, is a much more reliable method because is based in the effect of the test ingredient in several diets and it does not depend of only one diet that could have a strange digestive behaviour.

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