WORLD RABBIT SCIENCE 1996, 4(4), 205-209.

ENERGY, PROTEIN AND FIBRE DIGESTIBILITY OF SUNFLOWER HULLS, OLIVE LEAVES AND NaOH-TREATED BARLEY STRAW FOR RABBITS

GARCIA J., VILLAMIDE M.J., DE BLAS J.C.

Dpto. Producción Animal. E.T.S.I. Agrónomos. Universidad Politécnica de Madrid, 28040 MADRID, Spain.

ABSTRACT : One hundred and sixty nine New Zealand White x Californian rabbits were used to determine the nutritive value of sunflower hulls, olive leaves and NaOH-treated barley straw. These feedstuffs were substituted at 6, 12, 18 and 24% in a basal diet formulated for a high energy and protein content. Digestible energy (DE) values calculated by difference for the highest substitution levels (18 and 24%) were 4.77(±0.32), 6.16(±0.37) and 4.10(±0.32) MJ kg^{-1} DM for sunflower hulls, olive leaves and NaOH-treated barley straw, respectively. Standard errors of these estimations decreased with level of substitution (by 83% on average from 6 to 24% of inclusion level). Crude protein digestibility values obtained were not consistent due to its high standard errors (±48 as average). The values estimated for neutral detergent fibre digestibility (%) were relatively low and had also high standard errors: 10.7(±5.70); 7.76(±7.89) and 5.76(±6.14) for sunflower hulls, olive leaves and NaOH-treated barley straw, respectively. An stepwise regression equation to predict DE value of fibrous feeds was developed from data of this work and from literature using as independent variables ash, CP, crude fibre (CF), ether extract (%DM) and gross energy. The equation obtained was: DE(MJ kg^{-1} DM)= 14.46 (±1.10) - 0.23(±0.032) CF (R^2=0.781; RSD=1.43; P<0.001).

RESUME : Digestibilité chez le lapin, de l'énergie, des protéines et des fibres contenues dans des coques de tournesol, des feuilles d'olivier et de la paille d'orge traitée à la Soude.
Cent soixante neuf lapins NZW x Californien ont été utilisés pour déterminer la valeur nutritives des coques de tournesol, des feuilles d'olivier et de la paille d'orge traitée à la soude. Ces aliments ont été substitués aux taux de 6, 12, 18 et 24% aux éléments d'un régime de base au contenu élévé d'énergie et de protéines. Les valeurs d'Energie Digestible calculées par différence pour les niveaux de substitution les plus élevés (18 et 24%) sont de 4,77 ± 0,32 - 6,16 ± 0,37 et 4,10 ± 0,32 Mj/kg^{-1} MS pour les coques de tournesol, les feuilles d'olivier et la paille d'orge traitée à la soude respectivement. L'erreur standard lieé à ces estimations décroît avec le niveau de substitution (de 83% en moyenne pour les niveaux d'inclusion de 6 à 24%). Les valeurs de digestibilité des protéines brutes obtenues ne sont pas fiables à cause de cette forte erreur standard (±48 en moyenne). Les valeurs estimées de la digestibilité des NDF (%) sont relativement basses et sont aussi assorties de fortes erreurs standards (10,7 ± 5,70 - 7,76 ± 7,89 et 5,76 ± 6,14) pour les coques de tournesol, les feuilles d'olivier et la paille d'orge traitée, respectivement. Une équation de prédiction de la teneur en Energie Digestible des aliments fibreux a été déterminée par régression multiple et progressive à partir des résultats de ce travail et des données de la littérature. Elle utilise comme variables indépendantes les cendres, les protéine brutes, les fibres brutes, l'extrait éthéré (% MS) et l'énergie brute. L'équation obtenue est la suivante : DE(MJ kg^{-1} DM)= 14,46 (±1,10) - 0,23(±0,032) CF (R^2=0,781; RSD=1,43; P<0,001),

INTRODUCTION

The high requirements of fibre to regulate the transit time of the digesta in rabbits make them unique among the non-ruminant animals. Thus, fibrous feedstuffs are included in rabbit diets at levels around 65%, being most of them forages, mainly alfalfa hay (around 30%) and cereal middlings (around 25%). Several fibrous by-products (sunflower, rice and soybean hulls, cereal straw, barley rootlets, grape seed meal, olive leaves, paprika meal, etc.) are included in Spain at lower levels (10% in total) because of their unknown nutritive value. Furthermore, fibre acts as a binder in the pelleting process but the effect on pellet quality might vary from one source to other. Fibre is the second nutrient from an economic point of view, after the digestible energy, in rabbit diet formulation. Accordingly, there is an interest in the feed industry for a better knowledge of the feeding value of this type of ingredients.

Due to the high imbalance of these fibrous ingredients, the substitution method is recommended for their feeding evaluation (VILLAMIDE, 1996). The most important points with regard to this method are the nutrient composition of the basal diet and the substitution levels. Thus, the basal diet must be designed to avoid a great imbalance in all the experimental diets (VILLAMIDE *et al.* 1991). High substitution levels are also recommended to decrease the error of the estimation. However, high levels of dietary fibre lead to a high rate of passage of food throughout the intestine and decrease the digestibility of the other nutrients (DE BLAS *et al.*, 1989; GIDENNE, 1994). As a consequence, moderate levels of inclusion are recommended when using the difference method for fibrous by-products.

The aim of this work was to determine the nutritive value (digestible energy and fibre and protein digestibility) of three fibrous by-products: sunflower hulls, olive leaves and NaOH-treated barley straw using the substitution method with different substitution levels (6, 12, 18 and 24%).

MATERIAL AND METHODS

Diets

Chemical composition of the evaluated feedstuffs and the basal diet are shown in Table 1. Ingredient composition of the

Table 1 : Chemical composition (% DM) of the fibrous by-products studied and of the basal diet.

	Sunflower hulls	Olive leaves	NaOH-treated barley straw	Basal diet
Dry Matter	93.20	94.30	93.70	92.00
Ash	4.18	7.28	9.84	8.40
Ether Extract	3.95	4.23	1.11	2.18
Crude Protein	7.13	9.85	3.78	21.11
Crude Fibre	49.88	25.54	39.78	11.77
NDF	72.12	49.71	72.54	23.54
ADF	60.45	38.69	44.47	13.09
ADL	22.01	19.32	6.14	3.02
Gross Energy, MJ kg^{-1} DM	20.14	19.73	17.56	18.11

Table 2 : Digestible energy (DE, MJ kg^{-1} DM), NDF and CP content (%DM) of experimental diets.

		Substitution level (%)								
		0	6	12	18	24	SEM[1]	L[2]	Q[3]	C[4]
	[5]n	10	11	9	12	13	---	---	---	---
	DE	12.01	11.00	10.92	10.67	10.22	0.095	0.001	0.014	0.001
Sunflower Hulls	DNDF	4.59	3.45	5.16	5.14	5.31	0.36	0.005	0.471	0.023
	DCP	14.29	13.11	13.03	13.20	12.41	0.16	0.001	0.179	0.001
	n	10	12	12	9	12	---	---	---	---
	DE	12.01	11.75	10.94	10.88	10.59	0.083	0.001	0.018	0.013
Olive Leaves	DNDF	4.59	4.90	3.37	4.38	4.46	0.33	0.413	0.047	0.225
	DCP	14.29	14.39	12.77	12.96	11.69	0.19	0.001	0.276	0.437
	n	10	12	10	11	11	---	---	---	---
	DE	12.01	11.29	10.66	10.56	10.02	0.088	0.001	0.010	0.056
NaOH-treated	DNDF	4.59	5.40	4.86	4.02	5.11	0.35	0.673	0.825	0.003
Barley straw	DCP	14.29	13.48	12.60	12.44	12.00	0.17	0.001	0.035	0.736

[1] SEM: Standard error of means. [2] L: Significance of linear effect of inclusion level. [3]Q: Significance of quadratic effect of inclusion level. [4] C: Significance of cubic effect of inclusion level. [5]n: Number of rabbits per diet.

basal diet was: barley (44.9%), soya-bean meal (18.0%) and lucerne hay (35.1%). This diet was formulated for a high digestible energy (DE) and protein (DCP) content to compensate the low digestible nutrient content of the sources of fibre studied in the experimental diets. Sunflower hulls, olive leaves and NaOH-treated barley straw were substituted in the basal diet at 6, 12, 18 and 24%. All the diets were supplemented with sodium chloride (0.5%), dicalcium phosphate (0.5%), calcium carbonate (0.5%) and a mineral-vitamin premix (0.5%) containing (g kg^{-1}): Mn, 13.4; Zn, 40; I, 0.7; Fe, 24; Cu, 4; Co, 0.35; riboflavin, 2.1; calcium pantothenate, 7.3; nicotinic acid 18.7; vitamin K_3, 0.65; vitamin E, 17; thiamine, 0.67; pyridoxine, 0.46; biotine, 0.04; folic acid, 0.1; vitamin B_{12}, 7 mg kg^{-1}; vitamin A, 6,700.000 IU kg^{-1}; vitamin D_3, 940,000 IU kg^{-1}.

Digestibility trial

The digestibility trial was conducted according with the European Reference Method (PEREZ *et al*, 1995). One hundred and sixty nine New Zealand White x Californian rabbits between 56-61 days old and weighing 1.4-1.7 kg were used. Animals were allotted randomly to the diets (13 rabbits per diet). Following a 7-d period of adaptation to each diet, feed intake was recorded and total faecal output collected during 4 consecutive days. Twenty five rabbits were eliminated of the assay due to feed waste, low intake, high soft faeces excretion and diarrhoea problems. Faeces produced daily were stored at -20°C, then dried at 80°C for 48 h and ground for their analyses. Faeces were analyzed for DM, NDF, CP and energy to determine diet digestibility.

The nutritive value of evaluated ingredients was calculated i) by difference between the digestible nutrient contents of experimental diets for each substitution level, and ii) by extrapolation of the linear regression between the digestible nutrient content and the substitution level to a 100% of inclusion of the ingredients.

Animals were housed in metabolism wire cages that allowed separation of faeces and urine. The rabbits were kept in a closed building with partial environmental control, under a 12-12h light-dark schedule.

Analytical procedures

Analyses were conducted according to AOAC (1984) for DM, ash, CP, crude fibre (CF) and ether extract, VAN SOEST *et al.* (1991) for NDF and GOERING and VAN SOEST (1970) for ADF and ADL. Gross energy was determined by adiabatic calorimetry.

Statistical Analysis

Data were analyzed using the GLM procedures of SAS (1985). Linear, quadratic and cubic effects of inclusion level were tested. Regression analysis between the digestible nutrient content of diets and inclusion level were performed. The standard errors (SE) of the extrapolated values derived from the linear regression equations were calculated according to the following formula:

$$SE = \sqrt{V(reg)[1/n + (1-0.12)^2/(\Sigma P_i^2 - (\Sigma P_i)^2/n)]}$$

where V(reg) is the variance of the regression, n the total number of data and P_i are numerical values of the substitution levels.

The standard error of the nutritive value of feedstuffs estimated by difference were calculated according to the following formula:

$$SE = 1/P \sqrt{[V(TD)/n_{TD} + (1-P)^2 V(BD)/n_{BD}]}$$

where P is the substitution level, V(TD) and V(BD) are the variances of the diet tested with a P proportion of the ingredient studied and of the basal diet, respectively.

Stepwise regression analyses of the DE of the studied feedstuffs, using its chemical composition as independent variables, were performed in order to obtain prediction equations.

RESULTS

Digestible energy, NDF and crude protein contents of experimental diets (DE, DNDF and DCP, respectively), and the linear, quadratic and cubic effects of the substitution level are

Table 3: Linear equations (y = a + bx) relating the digestible energy (DE: y, MJ kg^{-1}DM), digestible CP and digestible NDF contents (%) of diets to the level of fibrous feedstuffs (x, %), and their solution when x = 100%.

	a	b	R.S.D.	r^2	F	y when x = 100 DE (MJ kg^{-1} DM)	Ed (%)
Sunflower hulls	11.91±0.089	-0.064±0.006	0.37	0.705	126.8	5.54±0.52	27.5±2.6
Olive leaves	12.22±0.075	-0.062±0.005	0.32	0.742	152.5	6.05±0.45	30.7±2.3
NaOH-treated barley straw	12.08±0.077	-0.077±0.005	0.32	0.815	229.6	4.33±0.45	24.7±2.6
	a	b	R.S.D.	r^2	F	y when x = 100 DCP	CPd (%)
Sunflower hulls	14.22±0.15	-6.15±0.97	0.62	0.430	40.1	8.07±0.87	113.2±12.2
Olive leaves	14.87±0.15	-11.62±1.02	0.64	0.711	130.2	3.25±0.90	33.0±9.1
NaOH-treated barley straw	14.37±0.14	-9.50±0.96	0.60	0.652	97.4	4.87±0.84	128.6±22.2
	a	b	R.S.D	r^2	F	y when x = 100 DNDF	NDFd (%)
Sunflower hulls	4.14±0.30	5.49±1.93	1.24	0.132	8.08	9.63±1.7	13.3±2.4

shown in Table 2. Digestible energy content of the experimental diets decreased cubic, quadratic and linearly with level of inclusion of the three ingredients studied. To propose a single DE value it has been only considered the linear equation that shows in all cases a higher significant effect. The linear equations obtained and the results of the extrapolation to a 100% inclusion are shown in Table 3. The energy value of these feedstuffs was low, from 4.33 to 6.05 MJ kg^{-1} DM for NaOH-treated barley straw and olive leaves, respectively, and an average energy digestibility of 27.6%.

The inclusion level affected differently the DNDF content of the experimental diets. A linear effect of substitution level was observed only in the sunflower hulls diets. The extrapolation to a 100% inclusion is shown in Table 3. Digestible crude protein (DCP) content of the experimental diets decreased linearly ($P<0.001$) with the inclusion level of the three ingredients studied, although a quadratic and a cubic trend were also found for treated straw and sunflower hulls, respectively. The values of CP digestibility (CPd, %) obtained from the extrapolated values of dietary DCP with the inclusion level are shown also in Table 3.

Values of digestible energy, CPd and NDF digestibility (NDFd, %) of the three feedstuffs studied were also calculated by difference for each substitution level (Table 4). Standard errors of these estimations decreased with level of substitution (by 83% as average from 6 to 24% of inclusion level). Estimations of DE and NDFd obtained at the 18 and 24% level of substitution were not significantly different, being as average: 4.77, 6.16 and 4.10 MJ kg^{-1} DM and 10.70, 7.76 and 5.76% for sunflower hulls, olive leaves and NaOH-treated straw, respectively.

Digestibility of CP of all the ingredients studied, and DE of olive leaves and NaOH-treated barley straw estimated either by difference or by extrapolation were no significantly different. However, the DE estimated for sunflower hulls by extrapolation was slightly higher than that estimated by difference (5.54 *vs* 4.77 MJ DE kg^{-1} DM). Finally, the NDFd values of sunflower hulls obtained by extrapolation or substitution were not significantly different.

DISCUSSION

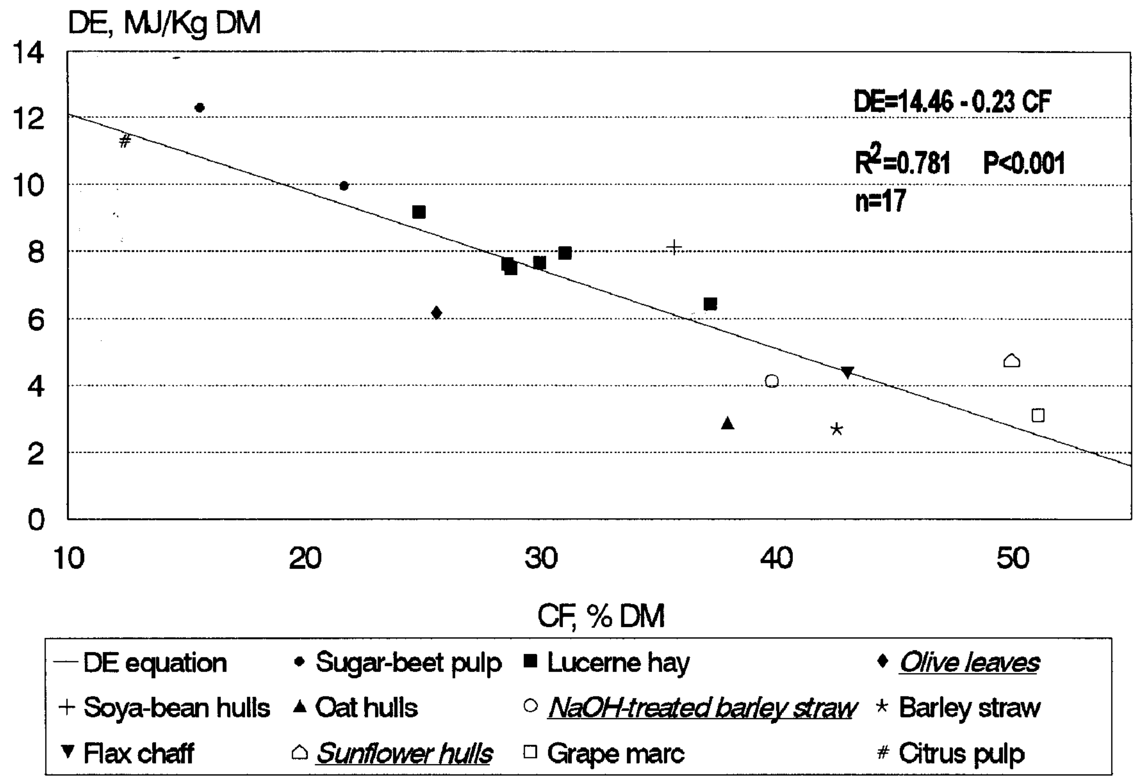
The quadratic and cubic tendencies observed for dietary DE with the substitution level of the ingredients studied imply

Table 4 : Digestible energy (DE, MJ kg^{-1} DM), NDF and CP digestibility (%) of the three fibrous by-products calculated by difference

		Substitution levels 6	12	18	24
Sunflower Hulls	DE	-4.68 ± 2.52[1]	3.14 ± 0.89	4.78 ± 0.50	4.76 ± 0.39
	NDFd	-19.75 ± 44.70	13.08 ± 15.93	10.70 ± 9.17	10.69 ± 6.94
	CPd	-69.11 ± 203.5	57.86 ± 84.10	119.98 ± 48.6	94.41 ± 43.09
Olive Leaves	DE	7.96 ± 1.86	3.32 ± 0.78	5.97 ± 0.68	6.35 ± 0.35
	NDFd	17.39 ± 47.07	-10.90 ± 20.00	7.16 ± 13.45	8.35 ± 8.87
	CPd	160.2 ± 143.7	19.20 ± 61.12	73.01 ± 37.07	38.07 ± 26.32
NaOH-treated Barley straw	DE	0.16 ± 2.08	1.00 ± 0.92	4.21 ± 0.55	4.00 ± 0.36
	NDFd	25.06 ± 35.94	9.58 ± 18.05	2.09 ± 10.38	9.43 ± 6.98
	CPd	30.22 ± 451.1	12.84 ± 145.4	113.2 ± 95.6	133.3 ± 72.1

[1] Standard error

Figure 1. Relationship between digestible energy (DE) and crude fibre (CF) of 17 fibrous feedstuffs.



Maertens and De Groote (1984), de Blas et al (1989), de Blas and Villamide (1990), García et al (1995)

interactions between these three sources of fibre and the basal diet and prevent to find a single digestible energy value from a quadratic or cubic equation. A quadratic tendency is often found in the literature (i.e. for fats, MAERTENS *et al.*, 1986) and can be explained by a positive or negative effect of the ingredient on basal diet digestibility, that is attenuated with higher inclusion levels. However, cubic trends have difficult biological explanation, because imply different effects of ingredients on basal diet digestibility for each substitution level, as can be observed in Table 4. The values calculated by difference at the two lowest levels of substitution were extreme (negative or higher than 100%) and showed very high standard errors. This can be due to the great influence of the interactions or of the analytical errors in dietary DE determinations on the values estimated by difference at so low levels of inclusion. Thus, 0.1 MJ in dietary DE determinations implied 1.7 and 0.83 MJ in ingredient DE estimations for 6 and 12% of substitution levels, respectively. Therefore, the values obtained from the extrapolation of the linear equations or those obtained by difference at the highest levels of inclusion are recommended.

Standard errors of CPd values calculated by difference (Table 4) were high for the three fibrous by-products evaluated. The confidence interval for CPd at 95% were: (33.8;180.4), (4.9;106.2) and (-10.7;257.3) for sunflower hulls, olive leaves and NaOH-treated barley straw, respectively. When CPd values were calculated by extrapolation of the linear equation, the standard errors decreased (by 23% as average) but remained still high. On the other hand, estimated CPd values either by difference or regression method had no a clear biological meaning. These results would suggest that the methods used in this study were not valuable to estimate CPd of ingredients with a very low protein content.

GARCIA *et al* (1996), using semipurified diets containing the same fibrous by-products as sole source of fibre, obtained lower standard errors of NDFd estimations than those observed in this study (1.33 *vs* 6.58 on average). No significant differences were observed between NDFd estimated by both methods for olive leaves and sunflower hulls. However, a significant ($P<0.01$) lower NDFd value for NaOH-treated barley straw was obtained by the substitution method (5.8 *vs* 16.6%).

An stepwise regression equation to predict the DE value of fibrous feeds was developed by the stepwise procedure using as independent variables its common available chemical composition (ash, CP, CF, ether extract and gross energy). The values of DE of the three feeds evaluated in this study, sunflower hulls, olive leaves and NaOH-treated barley straw, and the values obtained by other authors for soya-bean hulls, oat hulls, flax chaff, grape marc, lucerne hay, sugar-beet pulp (MAERTENS and DE GROOTE, 1984), citrus and sugar-beet pulps (DE BLAS and VILLAMIDE, 1990), barley straw (DE BLAS *et al*, 1989) and lucerne hay (GARCIA *et al*, 1995), were used. The regression equation obtained was (Figure 1):

$$DE(MJ\ kg^{-1}\ DM)=14.46(\pm1.10)-0.23(\pm0.032)CF\ (\%\ DM\ basis)$$
$$n=17 \quad R^2=0.781 \quad RSD=1.43 \quad P<0.001$$

The relatively lack of precision of this equation for the three sources of fibre studied in this work could be explained by other variables not measured by all the authors cited or by the different chemical significance of CF of the different sources of fibre.

Digestible energy of olive leaves (6.16±0.37 MJ kg^{-1} DM) was overestimated by the regression equation (by 38%). This could be due to the higher degree of lignification of its fibrous fraction (39% ADL/NDF). On the other hand, sunflower hulls showed a higher value of DE (4.77±0.32) than that predicted by the regression equation (2.79). This might be explained by its relatively higher ether extract content (3.95%). Digestible energy of NaOH-treated barley straw (4.10±0.32) had a closer value with that predicted, and higher than that observed for non treated barley straw (2.69±0.42, DE BLAS *et al*, 1989). This could be related to the effect of NaOH treatment on covalent linkages of lignin with carbohydrates.

Acknowledgements- This work has been supported by CICYT (AGF93-0870) and CDTI-Eureka Program (EU-619).

Received : June 3rd, 1996
Accepted : October 28th, 1996.

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