Abstract - One hundred and forty four 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 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 (±38 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.

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%). Furthermore, 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 and their effect on pellet quality. 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 several fibrous by-products: sunflower hulls, olive leaves and NaOH-treated barley straw using the substitution method with four 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 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

<table>
<thead>
<tr>
<th>Ingredient</th>
<th>Dry Matter</th>
<th>Ash</th>
<th>Ether Extract</th>
<th>Crude Protein</th>
<th>Crude Fibre</th>
<th>NDF</th>
<th>ADF</th>
<th>ADL</th>
<th>G.E, MJ kg⁻¹DM</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sunflower hulls</td>
<td>93.20</td>
<td>4.18</td>
<td>3.95</td>
<td>7.13</td>
<td>49.88</td>
<td>72.12</td>
<td>60.45</td>
<td>22.01</td>
<td>20.14</td>
</tr>
<tr>
<td>Olive leaves</td>
<td>94.30</td>
<td>7.28</td>
<td>4.23</td>
<td>9.85</td>
<td>25.54</td>
<td>49.71</td>
<td>38.69</td>
<td>19.32</td>
<td>19.73</td>
</tr>
<tr>
<td>NaOH treated barley straw</td>
<td>93.70</td>
<td>9.84</td>
<td>1.11</td>
<td>3.78</td>
<td>39.78</td>
<td>72.54</td>
<td>44.47</td>
<td>6.14</td>
<td>17.56</td>
</tr>
<tr>
<td>Basal diet</td>
<td>92.00</td>
<td>8.40</td>
<td>2.18</td>
<td>21.11</td>
<td>11.77</td>
<td>23.54</td>
<td>13.09</td>
<td>3.02</td>
<td>18.11</td>
</tr>
</tbody>
</table>

Table 1: Chemical composition (% DM) of the fibrous by-products studied and of the basal diet.
(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; biotin, 0.04; folic acid, 0.1; vitamin B\(_{12}\), 7 mg kg\(^{-1}\); vitamin A, 6,700.00 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 forty four 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 (9-13 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. Faeces produced daily were stored at -20°C. 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 effect of inclusion level was 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 these regression equations were calculated according to the following formula:

\[
SE = \sqrt{V(\text{reg})[\frac{1}{n} + (1-0.12)^2/(\sum P_i^2 - (\sum P_i)^2/n)]},
\]

where \(V(\text{reg})\) is the variance of the regression, \(n\) the 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:

\[
SE = \frac{1}{P} \sqrt{V(\text{TD})/n_{\text{TD}} + (1-P) V(\text{BD})/n_{\text{BD}}},
\]

where \(P\) is the substitution level, \(V(\text{TD})\) and \(V(\text{BD})\) 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.

### Table 2: Digestible energy (MJ Kg\(^{-1}\) DM), NDF and CP content (% DM) of experimental diets

<table>
<thead>
<tr>
<th>Substitution level (%)</th>
<th>0</th>
<th>6</th>
<th>12</th>
<th>18</th>
<th>24</th>
<th>SEM(^1)</th>
<th>L(^2)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sunflower</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>hulls</td>
<td>(\times n)</td>
<td>10</td>
<td>11</td>
<td>12</td>
<td>13</td>
<td></td>
<td></td>
</tr>
<tr>
<td>DE</td>
<td>12.01</td>
<td>11.00</td>
<td>10.92</td>
<td>10.67</td>
<td>10.22</td>
<td>0.095</td>
<td>0.001</td>
</tr>
<tr>
<td>DNDF</td>
<td>4.59</td>
<td>3.45</td>
<td>5.16</td>
<td>5.14</td>
<td>5.31</td>
<td>0.25</td>
<td>0.005</td>
</tr>
<tr>
<td>DCP</td>
<td>14.29</td>
<td>13.11</td>
<td>13.03</td>
<td>13.20</td>
<td>12.41</td>
<td>0.16</td>
<td>0.001</td>
</tr>
<tr>
<td>Olive</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>leaves</td>
<td>(\times n)</td>
<td>10</td>
<td>12</td>
<td>12</td>
<td>9</td>
<td>12</td>
<td></td>
</tr>
<tr>
<td>DE</td>
<td>12.01</td>
<td>11.75</td>
<td>10.94</td>
<td>10.88</td>
<td>10.59</td>
<td>0.083</td>
<td>0.001</td>
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<tr>
<td>DNDF</td>
<td>4.59</td>
<td>4.90</td>
<td>3.37</td>
<td>4.38</td>
<td>4.46</td>
<td>0.33</td>
<td>0.413</td>
</tr>
<tr>
<td>DCP</td>
<td>14.29</td>
<td>14.39</td>
<td>12.77</td>
<td>12.96</td>
<td>11.69</td>
<td>0.15</td>
<td>0.001</td>
</tr>
<tr>
<td>NaOH-treated</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>barley straw</td>
<td>(\times n)</td>
<td>10</td>
<td>12</td>
<td>10</td>
<td>11</td>
<td>11</td>
<td></td>
</tr>
<tr>
<td>DE</td>
<td>12.01</td>
<td>11.29</td>
<td>10.66</td>
<td>10.56</td>
<td>10.02</td>
<td>0.088</td>
<td>0.001</td>
</tr>
<tr>
<td>DNDF</td>
<td>4.59</td>
<td>5.40</td>
<td>4.86</td>
<td>4.02</td>
<td>5.11</td>
<td>0.35</td>
<td>0.673</td>
</tr>
<tr>
<td>DCP</td>
<td>14.29</td>
<td>13.48</td>
<td>12.60</td>
<td>12.44</td>
<td>12.00</td>
<td>0.17</td>
<td>0.001</td>
</tr>
</tbody>
</table>

\(^1\) SEM: Standard error of means. \(^2\) L: Significance of linear effect of inclusion level. \(^3\) \(n\): Number of rabbits per diet.
RESULTS

Digestible energy, NDF and crude protein content of experimental diets, and the linear effect of the substitution level are shown in Table 2. Digestible energy content of the experimental diets decreased linearly ($P<0.001$) with level of inclusion of the three ingredients studied. The linear equations obtained were:

Sunflower hulls:
$$\text{DE (MJ kg}^{-1}\text{DM)} = 11.91 (±0.089) - 0.064 (±0.0057) \text{SL}$$
$$R^2 = 0.705 \quad P<0.001$$

Olive leaves:
$$\text{DE (MJ kg}^{-1}\text{DM)} = 12.22 (±0.075) - 0.062 (±0.0050) \text{SL}$$
$$R^2 = 0.742 \quad P<0.001$$

NaOH-treated barley straw:
$$\text{DE (MJ kg}^{-1}\text{DM)} = 12.08 (±0.077) - 0.077 (±0.0051) \text{SL}$$
$$R^2 = 0.815 \quad P<0.001$$

where $\text{SL}$ was the substitution level of the ingredients evaluated (%). The results of the extrapolation of these equations to a 100% inclusion were: 5.54 (±0.31); 6.05 (±0.27); 4.33 (±0.27) MJ kg$^{-1}$ DM for sunflower hulls, olive leaves and NaOH-treated barley straw, respectively.

Digestible NDF content of the experimental diets was not affected by the inclusion level, except for the sunflower hulls diets, where the low value obtained for the diet with 6% of inclusion might have produced the linear effect observed. Digestible protein (DCP) content of the experimental diets decreased linearly ($P<0.001$) with the inclusion level of the three ingredients studied. The values of CP digestibility (CPd, %) obtained from the extrapolated values of dietary DCP with the inclusion level were: 113 (±7.29); 33 (±5.38) and 129 (±13.18) for sunflower hulls, olive leaves and NaOH-treated barley straw, respectively.

Values of digestible energy, CPd and NDF digestibility (NDFd, %) of the three feedstuffs studied were also calculated by difference for each substitution level. Standard errors of these estimations decreased with level of substitution (by 83% as average from 6 to 24% of inclusion level). The values calculated at the two lowest levels of substitution were erratic and could not be explained on a biological basis. Estimations obtained at the 18 and 24% level of substitution were not significantly different, so that values are presented in Table 3 as an average.

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 not 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).

DISCUSSION

An equation regression to predict the DE value of fibrous feeds was developed by the stepwise procedure using as independent variables its 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):

$$\text{DE (MJ kg}^{-1}\text{DM)} = 14.46 (±1.10) - 0.23 (±0.032) \text{CF}$$
$$n=17 \quad R^2=0.781 \quad \text{RSD}=1.43$$
$$P<0.001$$

Table 3: Nutritive value of fibrous by-products calculated by difference at the highest levels of inclusion (mean of 18 and 24% level of inclusion).

<table>
<thead>
<tr>
<th></th>
<th>sunflower hulls</th>
<th>olive leaves</th>
<th>NaOH-treated barley straw</th>
</tr>
</thead>
<tbody>
<tr>
<td>ED (MJ Kg$^{-1}$ DM)</td>
<td>4.77±0.32$^1$</td>
<td>6.16±0.37</td>
<td>4.10±0.32</td>
</tr>
<tr>
<td>NDFd (%)</td>
<td>10.70±5.70</td>
<td>7.76±7.89</td>
<td>5.76±6.14</td>
</tr>
<tr>
<td>CPd (%)</td>
<td>107.12±32.42</td>
<td>55.54±22.41</td>
<td>123.3±59.31</td>
</tr>
</tbody>
</table>

$^1$Standard error of means
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% LADF/NDF). On the other hand, sunflower hulls showed a higher 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. 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.

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

![Graph showing the relationship between digestible energy (DE) and crude fibre (CF) of 17 fibrous feedstuffs.](image)


Standard errors of CPd values calculated by difference (Table 3) 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 biological meaning. These results would suggest that the methods used in this study were not valuable to predict 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%).
REFERENCES


Valor nutritivo de la cascarilla de girasol, hoja de olivo y paja tratada con Na-OH en conejos - Se utilizaron 144 conejos Neozelandés Blanco x Californiano para determinar el valor nutritivo de la cascarilla de girasol, hoja de olivo y paja de cebada tratada con sosa. Se formuló una ración basal con un contenido en energía y proteína elevado, sobre la que se substituyeron los alimentos estudiados al 6, 12, 18 y 24%. Los valores de energía digestible (ED) calculados por el método de diferencia fueron 4.77±(0.32), 6.16±(0.37) y 4.10±(0.32) MJ kg⁻¹ MS para la cascarilla de girasol, hoja de olivo y paja de cebada tratada con sosa, respectivamente. El error estándar de estas estimaciones se redujo con el nivel de sustitución (83% de media entre el 6 y 24%). La estimación de la proteína bruta digestible fue muy imprecisa debido al elevado error estándar (±38 de media). Los valores de fibra neutro detergente digestible (% MS) fueron bajos para todos los alimentos estudiados y también presentaron errores estándar elevados: 10.7(±7.0), 7.76(±7.89) y 5.76(±6.14) para la cascarilla de girasol, hoja de olivo y paja de cebada tratada con sosa, respectivamente.