SUMMARY

The determination of the protein digestibility of a food is an important factor in estimating its quality. One of the main limitations of beans is the low nutritional value of their proteins due to low digestibility and a reduced content and bioavailability of sulphur amino acids. This work evaluated the protein quality and digestibility of different bean cultivars using a biological assay. The digestibility, Protein Efficiency Ratio (PER), Net Protein Ratio (NPR) and Net Protein Utilization (NPU) were determined for the “A 774”, “Carioca”, “Diamante Negro”, “Ouro Branco”, “RAO 33” and “Vermelho Coimbra” cultivars. The results obtained showed that the values for digestibility, PER, NPR and NPU were higher than those quoted in the literature, and that the “Ouro Branco” cultivar showed the highest nutritional performance, compared to the other varieties analysed. The most coloured varieties, such as “Vermelho Coimbra” and “Diamante Negro” showed lower values, indicating that the pigments and/or the compounds related to them were responsible for reducing the protein quality of the beans.

RESUMO

A determinação da digestibilidade protéica de um alimento é um importante fator para estimar sua qualidade. Um dos maiores problemas do feijão é representado pelo baixo valor nutricional de suas proteínas, decorrente, por um lado, da sua baixa digestibilidade e, de outro, dos reduzidos teor e biodisponibilidade de aminoácidos sulfurados. Este trabalho visou avaliar, através de ensaio biológico, a qualidade protéica e digestibilidade de diferentes cultivares, melhorados geneticamente. Foram feitas análises de digestibilidade, PER (Coeficiente de Eficácia Protéica), NPR (Razão Protéica Líquida) e NPU (Utilização Líquida da Proteína) para os cultivares “A 774”, “Carioca”, “Diamante Negro”, “Ouro Branco”, “RAO 33” e “Vermelho Coimbra”. Os resultados obtidos mostraram que os valores encontrados para digestibilidade, PER, NPR e NPU foram mais altos que os valores encontrados na literatura e que o cultivar “Ouro Branco” apresentou a melhor qualidade nutricional dentre as cultivares analisadas, e as variedades de maior coloração como “Vermelho Coimbra” e “Diamante Negro” tiveram a sua qualidade protéica reduzida.
1. INTRODUCTION

The legume seeds are widely consumed around the world and the acceptability depends on the climatic conditions in the area. In Latin America, beans (Phaseolus vulgaris) are the most consumed legume, supplying considerable amounts of nutrients to the diet of undernourished people (COELHO, 1991). BRESSANI et al. (1988) observed that besides increasing the protein content of the meal, beans have contributed to improving the protein quality of the diet by a factor of about 50 to 70%, when constituted predominantly of legumes and cereals, because bean proteins are rich in lysine, complementing proteins from cereals such as rice or corn, well known for being deficient in this amino acid. This fact means beans are of exceptional nutritional importance, especially when the restricted ingestion of food of animal origin among the low income population is considered, to whom the legume is a daily food.

Rice and beans are the staple foods of the Brazilian diet and mixed in adequate proportion, they may improve the biological value of the proteins consumed (VIEIRA, 1992).

The genus Phaseolus includes all species known as beans. The species Phaseolus vulgaris L. is the most common and includes many varieties such as “Carioca”, “Roxo” [Purple], “Mulatinho” [Brown], “Preto” [Black] and others (KANAMORI et al., 1982).

Although representing an important source of proteins, starch, vitamins, minerals and fiber (COELHO, 1991; GEIL; ANDERSON, 1994; BARAMPANA; SIMARD, 1994; MARTINEZ et al., 1998) bean nutrients are not readily available for use by the organism (EVANS; BAUER, 1978; SARWAR; PEACE, 1986; RODRIGUES, 1995; CARBONARO et al., 1997).

Digestibility is the first factor affecting the efficiency of diet proteins. When certain peptide links are not hydrolysed in the digestive process, part of the protein is either excreted in the faeces, or transformed into a metabolic product by microorganisms present in the large intestine (SGARBIERI; WHITAKER, 1982). One of the major limitations of beans is the low nutritional value of their proteins, due to their poor digestibility and reduced content of sulphur amino acids (NELSON, 1991; BRESSANI, 1993; GENOVESE; LAJOLO, 1996, 1998). Besides reduced sulphur amino acid contents, they also show reduced bioavailability (SGARBIERI; WHITAKER, 1982).

The low biological usage of bean proteins is attributed to many factors such as: compact structure of the native proteins which may resist proteolysis; anti-nutritional compounds which can modify the digestibility, altering the liberation of amino acids and high excretion of endogenous nitrogen (WU; KUNKEL, 1995).

In general, the digestibility of bean proteins can be improved by thermal treatment (WU et al., 1974). However, studies indicate that the digestibility is limited, even after thermal treatment, due to the primary conformation of the proteins and/or to the presence of some components of the seed, such as thermo-stable protease inhibitors and polyphenols which complex with digestive enzymes and/or with bean proteins, reducing their susceptibility to hydrolysis (RODRIGUES; LAJOLO, 1993).

An improvement of digestibility does not necessary imply an increase in protein quality, such that the amino acids can be better absorbed, but not take part in protein synthesis, due to the deficiency of some essential amino acids. The nutritional value of a food is indicated by its chemical composition, although this does not characterize the food from the nutritional point of view. Food nutrients are available to the organism in their totality, only after their ingestion (SGARBIERI, 1989). Hence, biological analyses such as PER, NPR and NPU, which evaluate the protein quality based on the organism’s response to the protein ingestion, are important tools for better food characterization.

Several varieties of bean with great agricultural potential have been obtained from genetic breeding studies. Among these varieties are some that stand out due to the presence of more favourable characteristics, and thus deserve more attention for nutritional studies. Thus, the purpose of the present study was to evaluate the protein quality of the cultivars, A774, Carioca, Diamante Negro, Ouro Branco, RAO 33 and Vermelho Coimbra. These cultivars were kindly supplied by the Centro Nacional de Pesquisa de Arroz e Feijão da Empresa Brasileira de Pesquisa Agropecuária (EMBRAPA – CNPFA).

2. MATERIALS AND METHODS

2.1 Genetic material

The bean samples (Phaseolus vulgaris L.) of the varieties “A774” [brown group], “Carioca” [Carioca group], “Diamante Negro” [black group], “Ouro Branco” [white group], “RAO” 33 [purple group] and “Vermelho Coimbra” [red group] were obtained from the Empresa Brasileira de Pesquisa Agropecuária (EMBRAPA/CNPFA), Goiânia, GO, Brazil.

2.2 Preparation of bean flour

For the preparation of whole cooked flour, the grains were cleaned and cooked in water in the proportion of 1:1.5 (w/v) in a pressure cooker for forty minutes. The grains were then dried in an air circulating oven (24 h / 60°C) and ground in a mill.

2.3 Preparation of the diets

The composition of the experimental diets is shown in Table 1. Diets 1 to 6 were prepared by using bean samples as protein sources. Diet 7 [casein] was the standard and diet 8 was the protein free diet.

The nitrogen content of the mixture was determined by the semi-micro Kjeldahl method using the factor 6.25 to obtain the protein content (AOAC, 1984).
2.4 Biological assay

The evaluation of the protein quality of the experimental diets was performed by a biological assay. 48 male recently weaned Wistar rats, weighing 50 to 60g, from the Department of Nutrition and Health, Universidade Federal de Viçosa, Brazil, were used. The animals were divided into eight groups with six animals each, so that the weight difference between the groups did not exceed 5g. The rats were placed in individual cages and received water and their experimental diets ad libitum for 14 days (AOAC, 1975). The animals were maintained at 22 ± 3°C and the consumption of food was monitored weekly.

The true digestibility (AOAC, 1984) was determined by measuring the amount of nitrogen ingested in the diet, the amount eliminated in the faeces, and the metabolic loss in the faeces, which corresponds to the faecal nitrogen in the protein free group.

For the digestibility determination, the diets were labelled with indigocarmin in the proportion of 100mg/100g, and given to the animals on the 7th and 13th days. The faeces were collected from the 8th to the 13th days in individual containers for each animal and maintained under refrigeration.

At the end of the experiment, the faeces were dried in an air circulating oven at 105°C for 24h, cooled and weighed. Fat was extracted in a Soxhlet apparatus for 4h using petroleum ether as solvent. The nitrogen content of the carcasses was determined by the semi-micro Kjeldahl method. The samples were analysed in triplicate (AOAC, 1984).

The true digestibility was calculated according to the following formula (HEGSTED, 1977):

\[
\text{NPU} = \frac{\text{Ni} - \text{Na} \times 100}{\text{Ni}}
\]

where:
- \(\text{Ni}\) = body nitrogen of the test group
- \(\text{Na}\) = body nitrogen of the free protein group
- \(\text{Ni}\) = nitrogen intake of the test group

The results obtained were submitted to an analysis of variance and the mean values compared by Tukey’s test at 5% of probability.

### TABLE 1. Composition of the diets used in the experiments with rats (g/100g of the mixture).

<table>
<thead>
<tr>
<th>Ingredients</th>
<th>D1</th>
<th>D2</th>
<th>D3</th>
<th>D4</th>
<th>D5</th>
<th>D6</th>
<th>D7</th>
<th>D8</th>
</tr>
</thead>
<tbody>
<tr>
<td>Whole and cooked Ouro Branco flour(^1)</td>
<td>50.82</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Whole and cooked Canoja flour(^1)</td>
<td>-</td>
<td>55.49</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Whole and cooked RAO 33 flour(^1)</td>
<td>-</td>
<td>-</td>
<td>52.14</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Whole and cooked A 774 flour(^1)</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>46.00</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Whole and cooked Verm. Coimbra flour(^1)</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>43.49</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Whole and cooked Diamante Negro flour(^1)</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>42.29</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Casein(^2)</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>12.30</td>
</tr>
<tr>
<td>Mineral mix(^2)</td>
<td>3.5</td>
<td>3.5</td>
<td>3.5</td>
<td>3.5</td>
<td>3.5</td>
<td>3.5</td>
<td>3.5</td>
<td>3.5</td>
</tr>
<tr>
<td>Vitamin mix(^2)</td>
<td>1.0</td>
<td>1.0</td>
<td>1.0</td>
<td>1.0</td>
<td>1.0</td>
<td>1.0</td>
<td>1.0</td>
<td>1.0</td>
</tr>
<tr>
<td>Soybean oil(^3)</td>
<td>7.0</td>
<td>7.0</td>
<td>7.0</td>
<td>7.0</td>
<td>7.0</td>
<td>7.0</td>
<td>7.0</td>
<td>7.0</td>
</tr>
<tr>
<td>Choline bitartrate(^3)</td>
<td>0.25</td>
<td>0.25</td>
<td>0.25</td>
<td>0.25</td>
<td>0.25</td>
<td>0.25</td>
<td>0.25</td>
<td>0.25</td>
</tr>
<tr>
<td>Cornstarch (q.s.p.)(^3)</td>
<td>8.93</td>
<td>4.26</td>
<td>7.61</td>
<td>13.75</td>
<td>16.25</td>
<td>17.46</td>
<td>42.45</td>
<td>54.75</td>
</tr>
<tr>
<td>L-cystine(^2)</td>
<td>0.3</td>
<td>0.3</td>
<td>0.3</td>
<td>0.3</td>
<td>0.3</td>
<td>0.3</td>
<td>0.3</td>
<td>0.3</td>
</tr>
<tr>
<td>Fibre(^2)</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>5.0</td>
</tr>
<tr>
<td>Dextrinised cornstarch(^2)</td>
<td>13.2</td>
<td>13.2</td>
<td>13.2</td>
<td>13.2</td>
<td>13.2</td>
<td>13.2</td>
<td>13.2</td>
<td>13.2</td>
</tr>
<tr>
<td>Sucrose(^3)</td>
<td>15.0</td>
<td>15.0</td>
<td>15.0</td>
<td>15.0</td>
<td>15.0</td>
<td>15.0</td>
<td>15.0</td>
<td>15.0</td>
</tr>
</tbody>
</table>

\(^1\) Flour produced from the bean samples analysed
\(^2\) Obtained from RHOSER- Industry and commercial Ltda
\(^3\) According to REEVES et al. (1993)

The Protein Efficiency Ratio (PER) was determined from the ratio of weight gain of the animals and the consumption of protein (AOAC, 1975).

The Net Protein Ratio (NPR) was determined by dividing the weight gain of the test group plus the weight loss of the protein free group by the consumption of protein of the test group (BENDER; DOELL, 1957).

The Net Protein Utilization (NPU) was determined by the nitrogen retention of the carcass of the animals. For the calculation of NPU, the animals were sacrificed by inhaling CO2 at the end of the experiment. The carcasses were dried in an air circulating oven at 105°C for 24h, cooled and weighed. Fat was extracted in a Soxhlet apparatus for 4h using petroleum ether as solvent. The nitrogen content of the carcasses was determined by the semi-micro Kjeldahl method. The samples were analysed in triplicate (AOAC, 1984).

The NPU was calculated according to the formula (HEGSTED, 1977):

\[
\text{NPU} = \frac{\text{Ni} - \text{Na} \times 100}{\text{Ni}}
\]
3. RESULTS AND DISCUSSION

3.1 Digestibility

The results for true digestibility are summarised in Table 2.

It was observed that the true digestibility obtained from the varieties analysed ranged from 85.66% for “RAO 33” to 93.97% for “Ouro Branco”.

Polyphenols, one of the pigments responsible for the different colours of legumes, have been studied with the purpose of evaluating their significance according to the nutritional as well as the technological points of view (ELIAS, 1982). Their ability to form complexes and to precipitate proteins makes them important from the nutritional point of view. JAFFÉ (1950), quoted by SGARBIERI, WHITAKER (1982), reported values of 76.8; 79.5 & 84.1% for in vivo digestibility of proteins from black, pink and white beans (Phaseolus vulgaris), respectively.

JAFFÉ; FLORES (1975) found in vivo and in vitro digestibility values of 68.9 and 70.1%, respectively, for cooked black beans.

Comparing the results obtained for the varieties analysed with those of casein (Table 2), it was observed that casein digestibility was significantly higher than that of the varieties studied, except for the “Ouro Branco” cultivar, which was not statistically different from casein. HUGHES et al. (1996) found 90.9% digestibility for white beans, which was much lower than the value obtained for the “Ouro Branco” variety.

### TABLE 2. True digestibility (TD) of the casein diet and of the whole cooked bean varieties in the experiment with rats.

<table>
<thead>
<tr>
<th>Diet</th>
<th>Group</th>
<th>DV (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Casein (D7)</td>
<td>-</td>
<td>99.00</td>
</tr>
<tr>
<td>Ouro Branco (D1)</td>
<td>White</td>
<td>93.97</td>
</tr>
<tr>
<td>Diamante Negro (D6)</td>
<td>Black</td>
<td>91.50</td>
</tr>
<tr>
<td>Vermelho Coimbra (D5)</td>
<td>Red</td>
<td>90.44</td>
</tr>
<tr>
<td>A 774 (D4)</td>
<td>Brown</td>
<td>88.67</td>
</tr>
<tr>
<td>Caricola (D2)</td>
<td>Caricola</td>
<td>88.05</td>
</tr>
<tr>
<td>RAO 33 (D3)</td>
<td>Purple</td>
<td>85.66</td>
</tr>
</tbody>
</table>

*Means within the same column followed by the same letter, are not statistically different, according to Tukey test at 5% of probability.

BRESSANI (1989), in a review, showed that cooked white beans were more digestible than the coloured cooked beans, similar to the results found in the present study.

In general, the values for true digestibility observed here were higher than those found in the literature for other varieties and these results can be attributed to many factors such as: low concentration of anti-nutritional compounds present in the grain [NIELSON, 1991], different varieties analysed, sowing conditions, storage time and heat treatments, which may have produced, physicochemical changes in the proteins of the legume seed, affecting their final nutritional properties [ALONSO et al., 2000]. These factors may have contributed to increase the digestibility of these cultivars.

The improvement of digestibility is not necessarily related to an increase in protein quality, particularly when the protein is deficient in essential amino acids. For this reason, biological assays such as PER, NPR and NPU were performed, in order to evaluate the protein quality, based on the organism’s response to the protein intake.

3.2 PER, NPR and NPU

The results for PER and RPER (relative PER); NPR and RNPR (relative NPR); NPU and RNPU (relative NPU) of the experiment are summarised in Table 3.

The values for PER and RPER ranged from 1.2 to 2.36 and from 30.92% to 60.82%, respectively, and were statistically different (P < 0.05) from the values obtained for casein.

Among the bean samples analysed, the “Ouro Branco” variety showed the highest nutritional value based on its PER and RPER values, which were significantly higher than the other varieties. The “Diamante Negro” showed the lowest PER and RPER values. These results are similar to those obtained by BRESSANI; ELIAS (1984), where white beans showed better nutritional performance than purple, black or brown beans.

In the present work, significant differences in RPER were observed among beans and between the bean varieties and casein diets.

### TABLE 3. PER, RPER (relative PER), NPR, RNPR (relative NPR), NPU, RNPU (relative NPU) of casein and whole cooked bean diets.

<table>
<thead>
<tr>
<th>Diet</th>
<th>Group</th>
<th>PER (%)</th>
<th>RPER (%)</th>
<th>NPR</th>
<th>RNPR</th>
<th>NPU</th>
<th>RNPU</th>
</tr>
</thead>
<tbody>
<tr>
<td>Casein (D7)</td>
<td>-</td>
<td>3.89</td>
<td>100</td>
<td>4.56</td>
<td>100</td>
<td>66.73</td>
<td>100</td>
</tr>
<tr>
<td>Ouro Branco (D1)</td>
<td>White</td>
<td>2.36</td>
<td>60.82</td>
<td>3.49</td>
<td>76.53</td>
<td>52.11</td>
<td>78.09</td>
</tr>
<tr>
<td>Diamante Negro (D6)</td>
<td>Black</td>
<td>2.18</td>
<td>56.18</td>
<td>3.49</td>
<td>76.53</td>
<td>54.22</td>
<td>81.32</td>
</tr>
<tr>
<td>Vermelho Coimbra (D5)</td>
<td>Red</td>
<td>1.55</td>
<td>40.20</td>
<td>2.89</td>
<td>63.37</td>
<td>47.60</td>
<td>71.42</td>
</tr>
<tr>
<td>A 774 (D4)</td>
<td>Brown</td>
<td>1.44</td>
<td>37.11</td>
<td>2.76</td>
<td>60.52</td>
<td>46.38</td>
<td>69.50</td>
</tr>
<tr>
<td>Caricola (D2)</td>
<td>Caricola</td>
<td>1.32</td>
<td>34.00</td>
<td>2.57</td>
<td>56.35</td>
<td>42.61</td>
<td>63.85</td>
</tr>
<tr>
<td>RAO 33 (D3)</td>
<td>Purple</td>
<td>1.21</td>
<td>30.92</td>
<td>2.53</td>
<td>55.48</td>
<td>49.61</td>
<td>74.34</td>
</tr>
</tbody>
</table>

Means within the same column followed by the same letter, are not statistically different, according to Tukey’s test at 5% of probability.

PER and RPER were determined on the 14th day of the experiment.

The NPR and RNPR of the bean varieties were significantly lower than for casein and ranged from 2.53 to 3.49 and 55.48% to 76.53%, respectively (Table 3), indicating that statistically significant differences were detected only for casein. This is explained by the fact that PER does not consider the use of the protein for maintenance, only for growth promotion, tending to underestimate the quality of inferior proteins and, for this reason, enhances existing differences.
among proteins of higher and smaller nutritional values, contrary to the NPR value (SGARBieri, 1989).

The “Ouro Branco”, “Carioca” and “A 774” varieties showed the highest values for NPR. Although the “Diamante Negro” variety showed the lowest value for NPR in the present study, this value was higher than those observed by other authors working with black beans (Bressani et al., 1981; Durigan et al., 1987; Chiaradia 1997).

The higher values for NPR and RNPR compared to those found in the literature may be due to a decrease in anti-nutritional factors during the heat treatment process or to differences amongst varieties. Several studies confirm that heat treatment can be efficient in reducing trypsin inhibitor activity (Kakade; Evans, 1966; Antunes; Sgarbierei, 1980; Mendez et al., 1993), although it may not be responsible for the reduced protein digestibility in cooked beans.

The results for NPU and RNPU ranged from 49.61 to 52.11 and from 74.34% to 78.09%, respectively. The “Carioca” and “Ouro Branco” varieties were statistically equal to casein. The most coloured varieties, such as “Vermelho Coimbra” and “Diamante Negro” showed lower values, indicating that the pigments and/or the compounds related to them, were responsible for reducing the protein quality of the beans.

ACKNOWLEDGMENT

The authors thank FAPEMIG for the financial support.

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