True Protein Digestibility and Digestibility-Corrected Amino Acid Score of Red Kidney Beans (*Phaseolus vulgaris L.*)

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True digestibility of protein (TDP) and digestibility-corrected amino acid score (AAS_{TDP}) for thermally processed red kidney beans were evaluated using rat balance assay. High fecal protein (based on Kjeldahl N) relative to low protein digestibility was observed. The fecal protein content was 43.8% from rats fed the raw bean diet and 25.6–36.0% from rats fed the processed bean diets. Heat processing appeared to have a greater ($P < 0.05$) impact on protein intake than on fecal protein output, which indicated that protein digestibility for beans may depend on protein intake rather than fecal protein output. AAS_{TDP} values of beans (ranging from 13.9 to 74.6) were similar to digestibility-corrected essential amino acid index ($EAAI_{TDP}$) and digestibility-corrected available lysine (AL_{TDP}) values. This suggests that $EAAI_{TDP}$ and AL_{TDP} might be considered another approach for assessing protein quality of beans. True digestibility of total fat, carbohydrate, and energy for beans was also measured.

Keywords: *True protein digestibility; digestibility-corrected amino acid score; red kidney beans*

INTRODUCTION

Net protein ratio (NPR) and relative NPR (RNPR) methods were considered to be improvements over the protein efficiency ratio (PER) method for measuring the protein quality of foods (Sarwar and McDonough, 1990). The protein digestibility-corrected amino acid score (AAS_{TDP}) method, based on the actual amino acid profile of a protein in comparison with the amino acid requirement of humans, was recommended to be the most suitable routine method for evaluating the protein quality of vegetable protein products (FAO/WHO, 1990). AAS_{TDP} is a required method for determining protein quality in foods intended for humans over 1 year old (FDA, 1991). The amino acid score (AAS) is calculated by dividing the amino acid content of a test food by amino acid requirements for preschool age children (FAO/WHO/UNU, 1985). TDP is determined by measuring nitrogen in food and feces using the rat balance assay (McDonough *et al.*, 1990). AAS_{TDP} is then calculated by multiplying the lowest AAS value by TDP.

Poor biological utilization of *Phaseolus vulgaris* protein is well documented and is known to be related to various factors (Durigan *et al.*, 1987), such as the low content of sulfur amino acids (Sarwar and Peace, 1986); the compact structure of native bean proteins, which may resist proteolysis (Deshpande and Nielsen, 1987a); the antinutritional compounds, which may modify digestibility and alter release of amino acids (Liener, 1979; Bressani *et al.*, 1982; Aw and Swanson, 1985); and increased endogenous nitrogen excretion (Deshpande and Nielsen, 1987b; Mongeau *et al.*, 1989; Marletta *et al.*, 1992). Low sulfur amino acids and low digestibility are the major factors contributing to poor protein quality of beans.

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Many studies on amino acid composition and TDP of dry beans have been reported (Elias *et al.*, 1976; Dryden *et al.*, 1977; Yadav and Liener, 1977; Evans and Bauer, 1978; Marletta *et al.*, 1992; Joseph and Swanson, 1993). Thermal effects on *in vitro* protein quality (Wu *et al.*, 1994) and NPR of beans have been investigated in our laboratory previously. NPR values were obtained in a separate sudy following the method of Bender and Doell (1957). Studies evaluating the protein quality of beans via the AAS_{TDP} method are limited.

The objectives of the present study were to evaluate the effect of thermal processing on TDP, AAS_{TDP}, $EAAI_{TDP}$, and AL_{TDP} of red kidney beans and to compare these values with NPR and *in vitro* protein digestibility (IVPD).

MATERIALS AND METHODS

Diets. Red kidney beans (*P. vulgaris* L.) were thermally processed as described previously (Wu *et al.*, 1994). Nine treatments, e.g., raw beans (RAW), commercially canned beans (CAN, Progresso, Kellogg's), home-cooked beans (HOME, boiling, 100 °C for 120 min), autoclaved beans [121 °C for 10, 20, 40, 60, and 90 min (121–10, 121–20, 121–40, 121–60, and 121–90, respectively) and 128 °C for 20 min (128–20)] were applied. Diets were formulated according to the procedure of McDonough *et al.* (1990) and were composed of 10% protein from beans, 10% fat, 2% vitamin mix, 3.5% mineral mix, 0.005% BHA, 5% cellulose, and cornstarch to make up 100%. A protein-free diet was used to estimate metabolic nitrogen, and a diet with 10% protein from ANRC casein was used as control.

Animals. Forty-four male weanling Sprague-Dawley descended rats weighing 60 ± 5 g (Charles Rivers Laboratories, Research Triangle Park, NC) were housed in individual stainless steel cages with filter paper placed below the cages to minimize contamination of feces with urine and to catch spilled food during the balance period. The cages were in a room with temperature 22 ± 1 °C, relative humidity 65 ± 5%, and a 12-h light, 12-h dark cycle. The rats were fed a standard pelleted diet for an acclimation period of 4 days and then completely randomly distributed into 11 blocks each on the basis of equal mean body weights. The rats were provided

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distilled water *ad libitum*, but dietary intake was limited to 15 g/day. The rats were fed the diets for a 4-day preliminary period and a 5-day balance period with daily feed intake and weight gain recorded. On each day of the balance period, feces and spilled food for each rat were collected and deposited in open containers. Feces were dried in a vacuum oven at 40 °C for 24 h, weighed, ground to pass a 20-mesh screen, and stored in a freezer (−20 °C).

Chemical Analysis. Crude nitrogen, petroleum ether extractable material (total fat), ash, dietary fiber, and moisture of diet and feces were determined according to procedures described by the AOAC (1990). True N conversion factors (F_c) for diet and feces were obtained by using amino acid composition and Kjeldahl nitrogen content (Morr, 1982). The protein content of diet and feces was calculated by multiplying the Kjeldahl nitrogen content by F_c. The carbohydrate content was determined by difference. Energy intake and output were calculated using the Atwater factors of 4, 9, and 4 kcal/g for protein, fat, and available carbohydrate, respectively.

Calculation and Statistical Analysis. Apparent protein digestibility (ADP), true protein digestibility (TDP), and true protein digestibility-corrected amino acid scores (AAS_{TDP}) were calculated as follows:

$$ADP = [(P_i - P_f)/P_i] \times 100$$

$$TDP = [P_i - (P_f - P_{mf})]/P_i \times 100$$

$$AAS_{TDP} = AAS \times TDP$$

P_i is the protein intake of animals fed the test diet, P_f is the protein in feces from animals fed the test diet, and P_{mf} is the protein in feces from animals fed the protein-free diet. Digestibility-corrected essential amino acid index ($EAAI_{TDP}$) and digestibility-corrected available lysine (AL_{TDP}) values were also calculated in the same manner as AAS_{TDP}. AAS, EAAI, and AL data were reported previously (Wu *et al.*, 1994).

The experiment was conducted in a completely randomized design. Data were analyzed using analysis of variance, and means were separated using the least significant difference (lsd) test procedures when significant ($P < 0.05$) F values were obtained. Pearson correlation coefficients (Ott, 1988) were used to evaluate the relationship between *in vitro* chemical values (IVPD) and *in vivo* biological values (TDP, NPR, AAS_{TDP}, $EAAI_{TDP}$, and AL_{TDP}).

RESULTS AND DISCUSSION

Rat Performance. During the balance period, diet and protein intake, feces and fecal protein output, fecal protein percent, and dietary protein retained were obtained from rats fed bean diets and casein control diet (Table 1). The average protein intakes were 3.0 g for raw beans, 3.4−4.7 g for processed bean diets, and 6.9 g for casein diet. The average fecal protein outputs were 2.6 g for the raw bean diet, 0.9−1.2 g for processed bean diets, and 0.4 g for the casein diet. Processing had a greater effect ($P < 0.05$) on protein intake as compared to fecal protein output (Table 1). Protein intake minus protein output, including metabolic protein output, equals protein retained (PR). The average values of protein retained were 0.5 g for raw bean diet, 2.6−3.6 g for processed bean diets, and 6.6 g for casein diet (Table 1).

Fecal Protein Excretion. Protein contents of diets were about 10% from bean and casein diets and 0% for protein-free diets as designed. High fecal protein contents from rats fed the bean diets were observed, as were high ratios of protein output to protein intake (P_o/P_i). Fecal protein content from rats fed the protein-free and casein diets was low (7.1 and 7.9%, respectively) and from rats fed the raw bean diet was high (43.8%), whereas that from rats fed the thermally processed bean diets (from 25.6 to 36.0%) were intermediate (Table 1). Rats fed the raw bean diet excreted a greater ($P < 0.05$) percentage of protein than rats fed the processed bean diets. A negative correlation coefficient ($r = -0.89$, $P < 0.001$) between the ratio of fecal protein output to protein intake (P_o/P_i) and TDP was observed. Rats fed diets containing severely thermally processed beans (121−90 and 128−20) had higher P_o/P_i values than rats fed diets containing mildly thermally processed beans (HOME, CAN, and 121−10). The raw bean diet had the poorest digestibility of 15.7% and the highest P_o/P_i value of 88.5% (Table 1). The low nutritional value and high fecal excretion of nitrogen in legumes have been attributed to the presence of residual antinutritional factors, deficiency of sulfur amino acids, and reduced protein digestibility (Richardson, 1980−1981; Pu, 1983; Sathe *et al.*, 1984; Marquez and Lajolo, 1990; Marletta *et al.*, 1992). On the other hand, increased fecal nitrogen of rats fed beans may be due to the enhancement of mucosal cell turnover rather than low digestion of bean proteins (Bender and Mohammadiha, 1981; Fairweather-Tait *et al.*, 1983). The increased excretion of nitrogen in the feces of rats fed bean diets may also be attributed to the increased microbial activity in the intestines utilizing indigestible carbohydrates and proteins from beans as substrates (Fairweather-Tait *et al.*, 1983).

Table 1. Effect of Thermal Processing of Red Kidney Beans on Protein Intake, Output, and Retained[a]

diet[b]	D_i[c] (g)	P_i[c] (g)	F_o[c] (g)	P_o[c] (g)	P_f (%)	P_o/P_i[c]	PR[c] (g)
RAW	32.9[c]	3.0[c]	6.0[a]	2.6[a]	43.8[a]	88.5[a]	0.5[d]
HOME	43.1[a]	4.4[a]	3.6[b]	0.9[b]	25.6[f]	21.3[c]	3.6[a]
CAN	42.8[a]	4.0[ab]	3.8[b]	1.1[b]	28.4[ef]	22.6[c]	3.0[abc]
121−10	43.7[a]	4.2[ab]	3.6[b]	1.0[b]	26.6[f]	22.6[c]	3.4[ab]
121−20	45.4[a]	4.4[a]	3.5[bc]	1.1[b]	30.7[de]	25.0[b]	3.4[ab]
121−40	34.5[bc]	3.4[bc]	2.6[bc]	0.9[b]	34.3[bcd]	25.6[b]	2.7[c]
121−60	38.2[bc]	3.7[bc]	3.2[bc]	1.0[b]	31.7[cde]	27.6[b]	2.8[bc]
121−90	36.9[bc]	3.6[bc]	3.3[bc]	1.2[b]	34.9[bc]	32.0[b]	2.6[c]
128−20	39.4[b]	3.9[b]	3.4[bc]	1.2[b]	36.0[b]	31.0[b]	2.8[bc]
SEM	33.9	0.3	0.4	0.05	0.1	0.2	0.3
CASEIN	68.6	6.9	4.7	0.4	7.9	6.3	6.6

[a] D_i, diet intake; P_i, protein intake; F_o, feces output; P_o; fecal protein output; P_f, fecal protein content; PR, protein retained. [b] RAW: diet from raw bean; HOME, diet from home-cooked beans (boiled, 100 °C for 120 min); CAN, diet from canned beans (Progresso, Kellogg Inc.); 121(128)−(10−90), diets from autoclaved beans [121(128) °C for 10−90 min, respectively]; SEM, mean square for error; CASEIN, diet from ANRC casein. [c] Means based on data from four rats, lsd test only for bean diets, not casein diet. Means followed by different letters, within a column, are significantly different ($P < 0.05$).

Apparent and True Protein Digestibility. TDP values (71.7−82.0%) were similar to IVPD values of heat-processed beans (78.9−82.2%) reported by Wu *et al.* (1994). The correlation coefficient between *in vivo* and *in vitro* protein digestibility was 0.99 ($P < 0.001$), indicating that both methods ranked the digestibility of bean protein and casein similarly. TDP of raw beans was different ($P < 0.05$) from IVPD (15.5 vs 43.2%), although both values were lower than for processed beans. This suggested that IVPD of raw beans could not be used to predict *in vivo* protein quality. The heat-processed beans were more ($P < 0.05$) digestible than the raw beans. This is consistent with suggestions that proteolytic enzyme inhibitors and toxic factors in beans are inactivated by heat processing (Uebersax *et al.*, 1991). Diets containing mildly thermally processed beans, HOME and 121−10, had higher TDP values (80.7 and 82.0%, respectively) and severely thermally processed diets 121−90 and 128−20 had lower TDP values

Table 2. Protein Digestibility and Digestibility-Corrected Amino Acid Score, Essential Amino Acid Index, and Available Lysine of Red Kidney Beans[a]

diet[b]	ADP[c]	TDP[c]	AAS_{TDP}[c]	$EAAI_{TDP}$[c]	AL_{TDP}[c]
RAW	11.0[c]	15.7[c]	13.9[d]	11.4[c]	13.6[f]
HOME	78.8[a]	82.1[a]	74.6[a]	63.2[a]	74.0[a]
CAN	73.0[ab]	76.6[ab]	62.7[bc]	58.1[ab]	65.7[c]
121−10	77.5[a]	81.0[a]	70.1[ab]	61.8[a]	71.0[b]
121−20	74.3[ab]	77.2[ab]	66.3[abc]	59.0[ab]	66.0[c]
121−40	73.3[ab]	77.1[ab]	68.4[abc]	59.0[ab]	65.7[c]
121−60	72.2[ab]	75.9[ab]	67.3[abc]	57.9[ab]	63.6[d]
121−90	67.9[b]	71.6[b]	65.5[bc]	55.4[b]	58.9[e]
128−20	68.8[b]	72.1[b]	60.5[c]	54.4[b]	58.4[e]
SEM	29.6	28.6	35.7	4.3	2.6
CASEIN	94.6	96.5	100.0	77.2	82.7

[a] ADP, apparent protein digestibility; TDP, true protein digestibility; AAS_{TDP}, true protein digestibility-corrected amino acid score; $EAAI_{TDP}$, true protein digestibility-corrected essential amino acid index; AL_{TDP}, true protein digestibility-corrected available lysine. [b] Diet same as in Table 1. [c] Means same as in Table 1.

(71.7 and 72.3%, respectively) than diets CAN, 121−20, 121−40, and 121−60. Thus, while mild thermal processing treatments improve digestibility by inactivation of certain inhibitors, overprocessing perhaps damages the protein, reducing digestibility and/or absorption.

TDP of foods and feedstuffs was recently determined according to the rat balance method. The mean value for TDP of cooked kidney beans was 80% (Tobin and Carpenter, 1978). Whole beans exhibited the poorest digestibility (62.8%) with rat fecal excretion of more than 30% of the ingested nitrogen (Marquez and Lajolo, 1990). These observations were confirmed by this study.

Since true digestibility of protein measurements take into account metabolic fecal nitrogen (0.13 g/5 days), the TDP of a food is always higher than apparent digestibility (ADP) (Sarwar, 1987). ADP was about 5% lower than TDP in the raw diet and 3−4% lower in processed-bean diets (Table 2).

Protein quality estimation by TDP was compared with estimation by NPR and by IVPD using a multienzyme assay (Wu *et al.*, 1994). There was a positive correlation between TDP and NPR ($r = 0.935$, $P < 0.001$), between TDP and IVPD ($r = 0.995$, $P < 0.001$), and between NPR and IVPD ($r = 0.905$, $P < 0.001$). The coefficient of variation was 7.66% for the TDP assay among rats within diets, whereas it was 16.57% for the NPR assay. This indicated that a higher degree of assay precision existed in the TDP assay, which is based on protein intake and fecal protein output in a 5 day balance period, than in the NPR assay, which is based on rat weight gain (and loss) and protein intake in a 10 day period.

Digestibility-Corrected Amino Acid Score. AAS_{TDP} was calculated by multiplying AAS by TDP. TDP was also used to correct values for essential amino acid index (EAAI) and available lysine (AL) as reported by Wu *et al.* (1994). The results are termed the digestibility-corrected essential amino acid index ($EAAI_{TDP}$) and the digestibility-corrected available lysine (AL_{TDP}). Thermal processing affected AAS_{TDP}, $EAAI_{TDP}$, and AL_{TDP} values (Table 2). AAS_{TDP} values for HOME and 121−10 were higher ($P < 0.05$) than that for 128−20. $EAAI_{TDP}$ and AL_{TDP} values for HOME, CAN, and 121−10 were higher than values for 121−90 and 128−20.

The similar ranking order and value for AAS_{TDP}, $EAAI_{TDP}$, and AL_{TDP} for beans suggest that while AAS_{TDP} was recommended to be the most suitable routine method for evaluating protein quality of vegetable protein products (FAO/WHO, 1990), $EAAI_{TDP}$ and AL_{TDP} might be considered alternative methods for assessing protein quality of beans.

Table 3. True Digestibility of Total Fat, Carbohydrate, Ash, and Energy of Red Kidney Beans

diet[a]	total fat[b]	carbohydrate[b]	energy[b]
RAW	93.7[b]	83.5[c]	86.4[c]
HOME	98.6[a]	94.6[ab]	95.9[ab]
CAN	99.3[a]	93.4[b]	95.1[b]
121−10	99.9[a]	94.6[ab]	96.1[ab]
121−20	98.7[a]	95.3[a]	96.3[ab]
121−40	98.4[a]	95.0[ab]	96.1[ab]
121−60	99.2[a]	95.4[a]	96.6[a]
121−90	99.6[a]	94.2[ab]	95.8[ab]
128−20	99.2[a]	94.1[ab]	95.6[ab]
SEM	1.8	1.2	0.8
CASEIN	98.4	98.9	99.0

[a] Diet same as in Table 1. [b] Means same as in Table 1.

True Digestibility of Other Nutrients. True digestibility values of total fat, carbohydrate, and energy were calculated similarly to TDP. True digestibility values for these nutrients and energy were higher than the true digestibility value for protein, especially for raw beans (Table 3). True digestibility values for total fat, carbohydrate, and energy of the raw beans were greater than 70% as compared to the true protein digestibility of 15.51%. This suggests that improvement in digestibility of protein was more important than improvement in digestibility of other nutrients for beans in human and animal nutrition.

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