In Vivo and *in Vitro* Digestibility of Soybean, Lupine, and Rapeseed Meal Proteins after Various Technological Processes†

Pascale Rozan,*,‡ Radia Lamghari,‡ Michel Linder,§ Christian Villaume,‡ Jacques Fanni,§ Michel Parmentier,§ and Luc Méjean‡

Institut National de la Santé et de la Recherche Médicale (INSERM U308), Equipe de Recherches Aliment et Comportement, 38 rue Lionnois, 54000 Nancy, France, and Laboratoire de Physicochimie et Génie Alimentaires, E.N.S.A.I.A., 2 Avenue Forêt de Haye, 54500 Vandoeuvre-lès-Nancy, France

Anti-nutritional factors and technological processes may modify the nutritional quality of plant proteins. Heated rapeseed meal, soybean, and lupine proteins dried by various processes were used to compare *in vivo* methods of nutritional quality measurement such as protein efficiency ratio and true digestibility (TD) to *in vitro* methods such as pH-stat giving degree of hydrolysis (DH) and digestion cell giving nitrogen digestibility (ND). Combined methods taking into account amino acid score were as follows: PDCAAS (protein digestibility-corrected amino acid score) derived from TD, NDCAAS (nitrogen digestibility-corrected amino acid score), and DHCAAS (degree of hydrolysis-corrected amino acid score). Correlations ($p < 0.001$) were 0.81 (TD vs DH) and 0.88 (TD vs ND). PDCAAS was significantly ($p < 0.001$) correlated with DHCAAS or NDCAAS ($r = 0.92$ and 0.98, respectively). Time-consuming and expensive TD determination could be supplanted by both *in vitro* methods; DHCAAS and NDCAAS could replace PDCAAS.

Keywords: *Plant proteins; nutritional protein value determination; in vivo methods; in vitro methods; combined methods; correlation between several methods*

INTRODUCTION

The protein nutritional value not only depends on the quality of protein (amino acids composition and protein structure) (Kakade et al., 1974) but also on the different components of food. High moisture, high fat, high protein, or low nitrogen content of food can result in erroneous estimates of protein quality (Chang et al., 1992). Protein quality is a function of the essential amino acid content of the protein, the specific species' essential amino acid requirements, and the bioavailability of the essential amino acids.

According to Kakade et al. (1974), amino acids near the reactive site of enzymatic hydrolysis as well as the presence of anti-nutritional factors (ANF) such as phytic acid, phenols, or fibers influence protein hydrolysis. The presence of protease inhibitors, i.e., trypsin inhibitors, and the nature of dietary fiber affect nutritional value. An increase in crude fibers has a negative effect on digestibility, particularly on the digestibility of non-proteic nitrogen and proteins (Eggum, 1973a).

Many protein sources such as seeds and leafs require extensive processes before their nutritional use to inactivate protease inhibitors, ANF, or concentrate proteins (heating, coagulation, precipitation) according to Zarkadas et al. (1995). These processes may often alter protein structures and generate a deleterious effect on the nutritive value of protein (Chango et al., 1993). Some protein sources such as rapeseed meal or soybean are relatively well-balanced in essential amino acids (EAA) but require treatments to remove ANF. The presence of toxic or indigestible components such as glucosinolates and polyphenols, i.e., lignin, prevents the use of rapeseed presscake in human food. Polyphenols, phytic acid, or fibers can bind protein and slow down proteolysis. Lupine seeds are attracting attention throughout the world as potential providers of protein and fat for the future (Lopez-Bellido and Fuentes, 1986). A factor that could limit the nutritional utilization of lupine seed is the presence of toxic alkaloids.

The ANF effects and the different heat treatments on nutritional value have been evaluated by *in vivo* and *in vitro* methods. Several bioassay methods such as protein efficiency ratio (PER) (Satterlee et al., 1979; Campos and Arêas, 1993), net protein ratio (NPR) (Pedersen and Eggum, 1983), or TD (Pedersen and Eggum, 1983; Campos and Arêas, 1993) have been used to determine protein quality of foods (Boisen and Eggum, 1991; Swaisgood and Catignani, 1991; Friedman, 1996a). *In vivo* methods, which are costly and time-consuming, make allowance for amino acids availability but not for human amino acid requirements. In this fact, different *in vitro* multi-enzyme methods are now rapidly growing (Hsu et al., 1977; Pedersen and Eggum, 1983; Savoie and Gauthier, 1986; Mac Donough et al., 1990) and give good prediction of nutritional value. Chemical methods based on essential amino acids have also been used (Zarkadas et al., 1995).

The purposes of our investigation were (1) to examine the effect of heat treatments on the plant protein digestibility and (2) to correlate *in vivo* and *in vitro* digestibility data. Lastly, new combined *in vitro* methods were carried out to supplant AAS × TD (PDCAAS), by pH-stat or cell dialysis taking into account EAA, new parameters called DHCAAS and NDCAAS, respectively.

MATERIALS AND METHODS

Plant Protein Sources. The OO rapeseed meal (*Brassica napus)* was a gift of Delta Céréales (Les Angles, France).

† Presented in part as a poster at IX^{e} Eurobiology Congress, Pont à Mousson, 29/9-3/10/96.

* Author to whom correspondence should be addressed [telephone (33) 83-36-41-45; e-mail rozan@u308.nancy.inserm.fr].

‡ Institut National de la Santé et de la Recherche Médicale.

§ Laboratoire de Physicochimie et Génie Alimentaires.

S0021-8561(96)00723-6 CCC: $14.00 © 1997 American Chemical Society

Table 1. Diet Composition

overall (%)	
protein	10
corn oil	sufficient for 9
cellulose	5
salt mixture[a]	5
vitamin mixture[a]	2
corn starch	sufficient for 100
minerals (g)	
$CaHPO_4$	430
KCl	100
NaCl	100
MgO	10.5
$MgSO_4$	50
Fe_2O_3	3
$FeSO_4 \cdot 7H_2O$	5
trace elements	10
corn starch	sufficient for 1000
vitamins (mg)	
vitamin A	2000 UI
vitamin D3	250 UI
vitamin B1	2.0
vitamin B2	1.5
vitamin B3	7.0
vitamin B6	1.0
vitamin B7	15.0
vitamin B12	0.005
vitamin C	80.0
vitamin E	17.0
vitamin K3	4.0
vitamin PP	10.0
choline	136.0
folic acid	0.5
acidPAB acid	5.0
biotin	0.03
cellulose	sufficient for 1000

[a] Purchased from UAR (Villemoisson/Orge, France).

Soybean variety Kingsoy was supplied by ONIDOL (Toulouse, France). Lupine seed (*Lupinus albus*) variety Ares was purchased from CANA (Ancenis, France).

Protein Samples. Sodium caseinate (Sigma, C-8654) and gelatin (Merck 4078) were used as protein references (Satterlee et al., 1979; Pedersen and Eggum, 1983; Marshall et al., 1979).

Rapeseed meal (RM) was obtained after industrial oil extraction by pressure and hexane. The RM was then toasted. Fifty grams of crushed meal, humidified and homogenized with 70 mL of distilled water and acidified to pH 5 with acetic acid, was put in Petri dishes (190 mm ∅). Samples (HRM) were sterilized at 110 °C for 30 min, cooled, and steamed for 30 min. The products were freeze-dried (Bioblock, Christ loc-2) and crushed (Vertec).

Soybean and lupine seed protein concentrates were prepared by coagulation process (King, 1983). Lupine seeds and soybean were washed and soaked for 16 h in water at room temperature. The seeds were washed again, drained, and then ground with distilled water. The mixture was centrifuged at 3000*g* for 15 min. The supernatant was added to an alginic acid solution and then coagulated in a bath of calcium chloride. Fibrous concentrates of soluble soybean and lupine proteins were separated from the aqueous medium and dried at 50 °C for 16 h (HSP and HLP for soybean and lupine proteins, respectively) or freeze-dried (SP and LP for soybean and lupine proteins, respectively). ANF were eliminated according to Chango et al. (1993).

Crude Composition of Samples. Nitrogen, lipids, moisture, and ash assays were carried out according to the AOAC methods (1984). Ethanol-soluble carbohydrates were extracted with 80% ethanol at 80 °C and assayed with colorimetric method (Roe, 1955). Fiber composition was determined by Southgate procedure (1969). Glucosinolates content of RM were checked by liquid chromatography (Rozan et al., 1996). Amino acids assays (Moore et al., 1958) were carried out in the Protein Biochemistry Laboratory (INSERM U16, Lille, France).

***In Vivo* Methods.** A 10% protein diet is required for protein efficiency ratio (PER) determination (Block and Mitchell, 1946). Diet composition is given in Table 1. Groups of 10 22-day-old weanling Wistar male rats (weight 45 ± 5 g) (IFFA CREDO, L'Arbresle, France) were housed in individual cages according to Eggum (1973b) and were placed in a room at 21 ± 2 °C, 55−60% relative humidity, and under a 12-h light/12-h dark cycle. After a 3-day adaptation diet, experimental diets and water were supplied *ad libitum* for 28 days. At the start of the experiment, the differences between the average weights of the groups were less than 5 g.

The fecal index method is the classic procedure for determining apparent digestibility (AD). Nitrogen excreted in the feces and collected for 8 days (days 18−26) of the same rats was substracted from the amount ingested, and the value was expressed as a percentage of intake. Amount of fecal nitrogen excreted by rats consuming a protein-free diet (starch substituting for protein) was necessary to determine TD. Feces from each group of rats were collected, and the moisture-free weights and nitrogen content were determined by Kjeldahl procedure (AOAC, 1984). PER and NPR were calculated over 28 days, whereas AD and TD values were measured within 18−26 days.

The definitions of PER, AD, TD, and NPR are expressed in the following equations (Eggum, 1973b; Gaudard-de Weck et al., 1994) where I is the nitrogen intake 18−26 days (g); F is the fecal nitrogen 18−26 days (g); and Fe is the endogen fecal nitrogen 18−26 days (g):

$$\mathrm{PER} = \frac{\text{wt gain of rats (g)}}{\text{protein intake (g)}} \quad (1)$$

$$\mathrm{NPR} = [\text{wt gain of rats (g)} + \text{wt loss of rats fed with protein/free diet (g)}]/\text{protein intake (g)} \quad (2)$$

$$\mathrm{AD} = \frac{I - F}{I} \times 100 \quad (3)$$

$$\mathrm{TD} = \frac{I - (F - \mathrm{Fe})}{I} \times 100 \quad (4)$$

***In Vitro* Methods.** Two *in vitro* methods were tested with a pH-stat (Linder et al., 1995) and with a dialysis cell digestion (Savoie and Gauthier, 1986).

The first used was a multi-enzyme system consisting of porcine pancreatic trypsin (Type IX, activity: 14 900 units/mg of protein, T-7418, Sigma), bovine pancreatic chymotrypsin (Type II, activity: 50 units/mg of powder, C-4129, Sigma), and porcine intestinal peptidase (activity: 102 units/g of powder, P 7500, Sigma) cocktail according to Mac Donough et al. (1990) to determine digestibility of proteins. Assays were performed in triplicate. A 5-mL of enzyme solution (23 100 units of trypsin, 186 units of chymotrypsin, and 0.052 unit of peptidase/mL) was prepared at pH 8 and 37 °C. A 50-mL distilled water protein suspension was prepared for each protein at the same pH and temperature, with 1 mg of nitrogen/mL (Hsu et al., 1977) to minimize experimental error. The enzyme mixture was added to the protein solution in a stirred and thermostated reactor, and the pH value was kept constant at 7.98 by the addition of 0.1 M NaOH during 10 min exactly. The NaOH volume added was used to calculate the degree of hydrolysis (DH) according to Alder-Nissen (1986) and Linder et al. (1997). Alkali consumed was directly proportional to the number of peptide bonds cleaved. Over pH 6.5, the dissociation of the amino groups became significant, and the equation relating DH to the alkali consumption during the course of hydrolysis was given by eq 5. DH was defined as the rate of the number of peptide bonds cleaved (h) over the total number of such bonds in the protein substrate (h_{tot}), which was calculated from the amino acid composition of the substrates; B was the alkali consumption in mL; M_B was the molarity of the alkali (0.1 M NaOH); MP was the grams of protein (N × 6.25) in the reactor. α was the degree of dissociation (eq 6). The pK value was 0.76 because the temperature was kept constant at 37 °C (eq 7).

$$\mathrm{DH}\ (\%) = \frac{1}{\alpha h_{\mathrm{tot}}} \frac{BM_{\mathrm{B}}}{\mathrm{MP}} \times 100 \quad (5)$$

$$\alpha = \frac{10^{\mathrm{pH}-\mathrm{pK}}}{1 + 10^{\mathrm{pH}-\mathrm{pK}}} \quad (6)$$

$$\mathrm{p}K = 7.8 + \frac{(298 - T)}{298T} \times 2400 \quad (7)$$

The second method using a multi-enzyme system consisted of a 30-min pepsin predigestion (Sigma, porcine stomach mucosa, 1:60000; activity: 3200 units/mg) of protein source (40 mg of total nitrogen) and was followed by a 6-h pancreatin proteolysis (Sigma, porcine pancreas, 18F-0809) into a dialysis cell (Model Ser-II, Quebec, Canada) using a dialysis bag (molecular mass cutoff 1000 Da).

Digestibility of protein samples was calculated by means of eq 8 and was represented by nitrogen digestibility (ND):

$$\mathrm{ND}\ (\%) = \frac{\text{N in dialysate (mg)}}{\text{N in protein sample (40 mg)}} \times 100 \quad (8)$$

Amino Acid Combined Methods. PDCAAS calculation needs an amino acids reference, which is the FAO/WHO/WHO reference for preschool-aged (2−5 years) (Satterlee et al., 1979; FAO/WHO, 1991; Madl, 1993) and essential amino acid content of samples. For a given essential amino acid, amino acid score (AAS) was calculated by dividing the content of this amino acid in a sample by the content of this amino acid in the reference (eq 9). PDCAAS was the product of the lowest AAS (AAS_l) in a food by TD of the food according to the procedure of Madl (1993) (eq 10). Two other new calculations modes were done using *in vitro* methods and AAS_1 (eqs 11 and 12).

$$\mathrm{AAS} = \frac{\text{amino acid composition}}{\text{amino acid reference}} \quad (9)$$

$$\mathrm{PDCAAS} = \mathrm{AAS}_l \times \mathrm{TD} \quad (10)$$

$$\mathrm{DHCAAS} = \mathrm{AAS}_l \times \mathrm{DH} \quad (11)$$

$$\mathrm{NDCAAS} = \mathrm{AAS}_l \times \mathrm{ND} \quad (12)$$

Statistical Treatment. Results were given as mean ± SD of samples and were compared with one-way analysis of variance (ANOVA) with a 95% confidence interval (Statview II software program, 1988) and with regression analysis.

RESULTS

Crude Composition. Nitrogen and crude composition of the seven samples are given in Tables 2 and 3. RM and HRM, which contained a high amount of total fibers (Table 3), were not concentrated as soy or lupine samples (70−84% proteins). HRM contained less glucosinolates and moisture than RM.

Amino Acid Analysis. The amino acid composition of samples, laboratory rat requirements, and required preschool-aged EAA composition are reported in Table 4. Compared to human requirements, sodium caseinate, RM, and soy protein had balanced amino acid compositions, and the EAA ratios were higher than the reference. Lupine protein was deficient in methionine, cysteine, and lysine; gelatin was deficient in sulfur amino acids and lacking in tryptophan. Compared to laboratory rat requirements, all of these samples were deficient for at least three amino acids. Most deficient amino acids were tryptophan (0.0−1.68 g/16 g of N) and sulfur amino acids (0.25−5.18 g/16 g of N); rat requirements were 5 g/16 g of N for each. All EAA of gelatin were deficient. As for lupine proteins, heating did not significantly modify the EAA composition, and composition is reported only for HLP.

Table 2. Nitrogen Content of Protein Sources (%)

protein source		nitrogen content (%)[a]
sodium caseinate (Sigma)	C	14.50 ± 0.50
gelatin (Merck)	G	15.76 ± 0.30
soybean protein (Kingsoy)	SP	11.37 ± 0.50
heated soybean protein	HSP	11.33 ± 0.25
sweet lupine seed protein (*Ares*)	LP	13.12 ± 0.25
heated sweet lupine seed protein	HLP	12.13 ± 0.41
rapeseed meal (*Brassica napus*)	RM	6.68 ± 0.05
heated rapeseed meal	HRM	6.50 ± 0.98

[a] Means of four samples ± SD.

Nutritional Value. The results concerning the nutritional value of various protein sources as determined by *in vivo*, *in vitro*, and combined determination methods are reported in Tables 5 and 6.

In Vivo Results. The lowest performance in protein intake, weight gain, and PER was obtained with rats fed the gelatin diet. Freeze-dried coagulated soy and lupine proteins (SP and LP, respectively) had values significantly higher than their heated homologues; corrected PER was 0.9 ± 0.1 for SP versus 0.7 ± 0.1 for HSP and 0.8 ± 0.1 for LP versus 0.3 ± 0.1 for HLP. PER for isolated soy protein usually ranges from 1.65 to 1.8 when corrected to a casein PER of 2.5. The discrepancy could be due to the coagulation process of soy and lupine proteins by means of alginates. The evolution of NPR results was similar to PER for each sample and their respective treatments. Data of AD and TD are reported in Table 5. Nitrogen intake of RM and HRM was similar to that of sodium caseinate, but their fecal nitrogen content was significantly the highest of all groups. The fecal nitrogen content of other samples was included within 0.02 ± 0.00 g/d for gelatin to 0.04 ± 0.01 g/d for soy proteins samples. Concerning LP and HLP, the nitrogen intake was significantly different, whereas fecal nitrogen content was similar. Sodium caseinate exhibited the best digestibility (AD and TD), whereas gelatin and RM protein samples had the weakest. Heating of RM did not modify digestibility. Significant differences between results of SP/HSP and LP/HLP were observed for AD and TD, except for TD of lupine samples.

In Vitro Results. Comparisons have not been done (Table 6) because of the small number of assays (three or four assays for each sample). DH, which represented the percentage of peptidic bonds cleaved by enzymatic cocktail at pH 8 and 37 °C for 10 min was high for sodium caseinate (14.3 ± 0.5%). Heating of coagulated protein samples decreased DH of soy proteins but not DH of lupine proteins. Heating of RM involved an increase of DH. Gelatin result was the lowest. The most important nitrogen digestibility (ND) was observed with dialysis cell digestion method and was for casein and soy samples. Heating of lupine and rapeseed samples led to a decrease of ND.

Combined Methods (Table 6). Remarkable results with combined methods were noted: an important NDCAAS (nitrogen digestibility-corrected amino acid score) decrease was observed with heating soy and lupine coagulated proteins and with HRM. The results obtained for sodium caseinate with combined methods were better when compared with other samples. On the contrary, those of gelatin were nil because of the absence of tryptophan. Correlations between methods are presented in Figures 1 and 2, and the correspondent coefficient of correlation is in Table 7. High significant

Table 3. Crude Composition of Samples (%w/w, Dry Matter Basis)

	C	G	SP	HSP	LP	HLP	RM	HRM
protein	90.6 ± 3.1	98.5 ± 1.9	73.7 ± 0.3	70.0 ± 0.8	84.0 ± 2.3	76.4 ± 1.3	41.6 ± 0.9	41.8 ± 1.0
lipid	nd[a]	−[b]	8.9 ± 0.5	8.8 ± 0.8	3.6 ± 0.2	3.8 ± 0.9	2.1 ± 0.1	4.2 ± 0.1
ethanol-soluble carbohydrate	−	−	nd	nd	2.0	2.0	10.1 ± 1.4	11.8 ± 0.2
total fibers	−	−	nd	nd	nd	nd	30.6	30.0
moisture	nd	−	0	8.0 ± 0.5	0	8.1 ± 0.8	9.1 ± 0.5	3.3 ± 0.0
ash	nd	−	nd	nd	6.0 ± 1.0	6.0 ± 1	6.6 ± 0.4	7.8 ± 0.0
glucosinolates (μmol/g)	−	−	0	0	0	0	12.2	8.0

[a] nd, not determined. [b] −, not contained in sample.

Table 4. Amino Acid Composition (mg/g of Protein), Kjeldahl Factor (g/16 g of Nitrogen), and h_{tot} Evaluation (mequiv/g) of Protein Sources

	casein	gelatin[a]	SP & HSP	LP	HLP	RM & HRM	laboratory rat[b]	preschool aged[b]
ALA	30.7	113.0	43.0	40.5	47.2	38.5		
ARG	37.5	89.0	83.0	77.8	68.6	64.5	50.0	
ASX	71.3	66.0	119.0	125.4	125.6	71.0		
GLX	220.4	114.0	177.0	246.0	234.2	165.4		
GLY	19.7	276.0	42.0	65.0	68.2	54.7		
HIS	29.2	7.6	27.0	13.7	14.1	36.7	25.0	19.0
ILE	54.1	17.0	45.0	45.9	47.2	32.8	42.0	28.0
LEU	95.1	33.6	77.0	75.9	78.3	64.2	62.0	66.0
LYS	81.2	57.4	**61.0**	31.5	34.1	68.3	58.0	58.0
MET + CYS	**31.5**	8.2	28.0	**2.5**	**3.6**	51.8	50.0	25.0
PHE + TYR	110.6	25.6	87.0	84.0	78.4	42.9	66.0	63.0
PRO	115.7	165.0	48.0	50.3	50.0	95.0		
SER	60.3	41.5	52.0	67.1	68.8	41.7		
THR	46.6	22.7	39.0	35.4	38.1	45.0	42.0	34.0
VAL	67.4	26.3	45.0	39.1	43.6	45.6	125.0	35.0
TRP	16.8	**0.0**	13.0	nd[c]	nd	**8.9**	50.0	11.0
Kjeldahl factor	6.38	5.55	6.25	6.25	6.25	6.25		
evaluation of h_{tot}	8.20	11.10	7.35	7.40	7.49	8.00		

[a] According to Eastoe and Leach (1977). [b] EAA recommendation, FAO/WHO (1991). [c] nd, not determined. h_{tot}, total number of peptide bonds of protein sources. Amino acid values in boldface have served for calculation of $AAS_1$and PDCAAS.

Table 5. *In Vivo* Determination of Nutritional Value of Samples

	during 28 days				between 18 and 26 days			
	protein intake (g/d)	weight gain (g/d)	corrected PER (c = 2.5)	NPR[a] related	nitrogen intake (g/d)	fecal nitrogen (g/d)	AD (%)	TD[b] (%)
C	1.6 ± 0.1^{e}	5.6 ± 0.5 ^{g}	2.5 ± 0.1 ^{f}	100.0 ± 6.3^{e}	0.15 ± 0.0^{e}	0.03 ± 0.01^{b}	79.5 ± 3.5^{e}	81.8 ± 3.4^{d}
SP	1.4 ± 0.1^{d}	1.8 ± 0.2^{c}	0.9 ± 0.1^{d}	30.2 ± 3.1^{c}	0.14 ± 0.0^{d,e}	0.04 ± 0.01^{d}	72.3 ± 5.3^{d}	74.5 ± 5.3^{c}
HSP	1.3 ± 0.1^{c}	1.4 ± 0.2^{b}	0.7 ± 0.1^{c}	21.7 ± 3.9^{b}	0.13 ± 0.0^{d}	0.04 ± 0.01^{d}	69.4 ± 5.6^{c}	71.7 ± 5.5^{b}
LP	1.4 ± 0.1^{c,d}	1.6 ± 0.2^{c,b}	0.8 ± 0.1^{c,d}	26.1 ± 3.3^{b,c}	0.11 ± 0.0^{c}	0.03 ± 0.01^{c}	69.9 ± 6.0^{c}	72.6 ± 6.0^{b}
HLP	1.0 ± 0.2^{b}	0.5 ± 0.1^{d}	0.3 ± 0.1^{b}	1.2 ± 3.4^{a}	0.06 ± 0.0^{b}	0.02 ± 0.01^{a,b,c}	60.8 ± 11.0^{b}	66.2 ± 10.8^{b,c}
RM	1.7 ± 0.1^{f}	4.5 ± 0.6^{f}	1.9 ± 0.2^{e}	73.7 ± 8.1^{d}	0.16 ± 0.0^{e}	0.07 ± 0.01^{e}	54.6 ± 7.0^{a,b}	56.8 ± 6.6^{a}
HRM	1.6 ± 0.1^{e}	4.1 ± 0.5^{e}	1.8 ± 0.1^{e}	70.3 ± 5.4^{d}	0.15 ± 0.0^{e}	0.07 ± 0.02^{e}	55.0 ± 8.6^{b}	57.1 ± 8.7^{a}
G	0.3 ± 0.0^{a}	−0.7 ± 0.1^{a}	−1.5 ± 0.3^{a}	5.3 ± 1.1^{a}	0.04 ± 0.0^{a}	0.02 ± 0.00^{a}	48.2 ± 7.5^{a}	56.3 ± 6.8^{a}

[a] Weight loss of 25 rats fed with free/protein diet during 28 days = 0.46 ± 0.13 g/d. [b] Fecal nitrogen of free/protein diet group between 18 and 26 days = 0.003 g/d. On the same column, two values are significantly different (p < 0.05) when no superscripts are identical.

Table 6. *In Vitro* and Combined Methods To Determine Nutritional Value of Samples

	in vitro			combined methods			
	pH-stat		digestion cell				
	NaOH vol (mL)	DH (%)	ND (%)	AAS_1	PDCAAS	DHCAAS	NDCAAS
C	2.85 ± 0.10	14.3 ± 0.5	48.4 ± 1.8	1.26	103.0	18.0	61.0
SP	1.60 ± 0.00	9.2 ± 0.0	44.6 ± 2.0	1.05	78.2	9.7	46.8
HSP	1.37 ± 0.03	7.8 ± 0.2	42.2 ± 1.7	1.05	75.2	8.2	44.3
LP	1.63 ± 0.17	9.3 ± 0.1	43.8 ± 2.0	0.10	7.3	0.9	4.4
HLP	1.73 ± 0.04	9.7 ± 0.2	28.0 ± 0.3	0.14	9.3	1.4	3.9
RM	1.22 ± 0.02	6.4 ± 0.1	27.8 ± 1.2	0.81	46.0	5.2	22.5
HRM	1.60 ± 0.00	8.4 ± 0.0	22.7 ± 2.1	0.81	46.3	6.8	18.4
G	1.35 ± 0.00	5.8 ± 0.0	33.9 ± 4.1	0.00	0.0	0.0	0.0

correlations (p < 0.001) were found with TD and the two *in vitro* methods (Figure 1; Table 7), and none were found with PER and *in vitro* methods (Table 7). Correlation between the different PDCAAS are presented in Figure 2 and Table 7. Highly significant correlations were found between PDCAAS and DHCAAS (degree of hydrolysis-corrected amino acid score) on one hand and between PDCAAS and NDCAAS on the other hand (p < 0.001).

DISCUSSION

Sodium caseinate was chosen as the balanced amino acid protein reference and gelatin as the unbalanced amino acids reference. Soybean is well known and usually used as the plant reference. The heating and coagulating treatment on the nutritional quality of this protein has been studied. Development of rapeseed oil methyl ester production from rapeseed for diesel engines

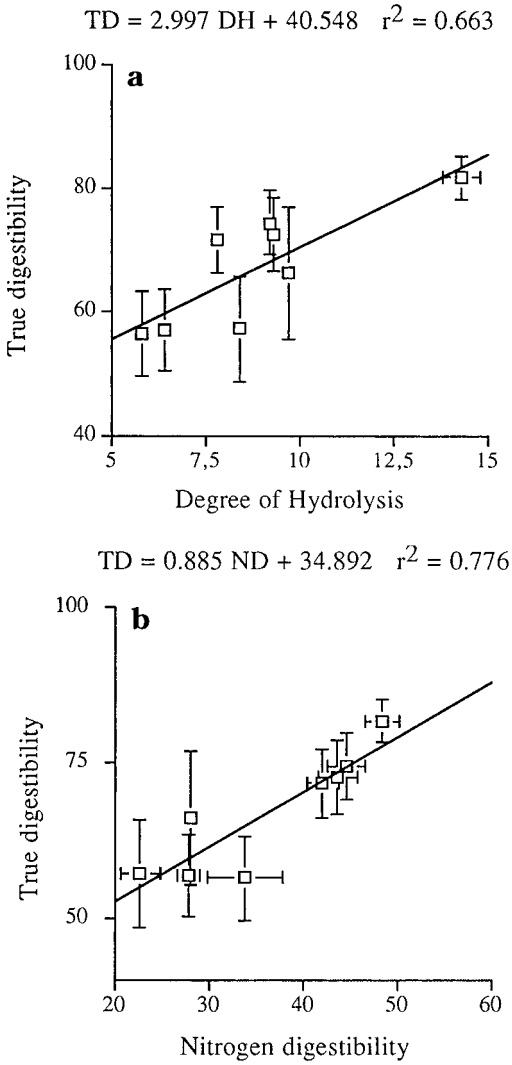

Figure 1. (a) Correlation between TD and DH. (b) Correlation between TD and ND.

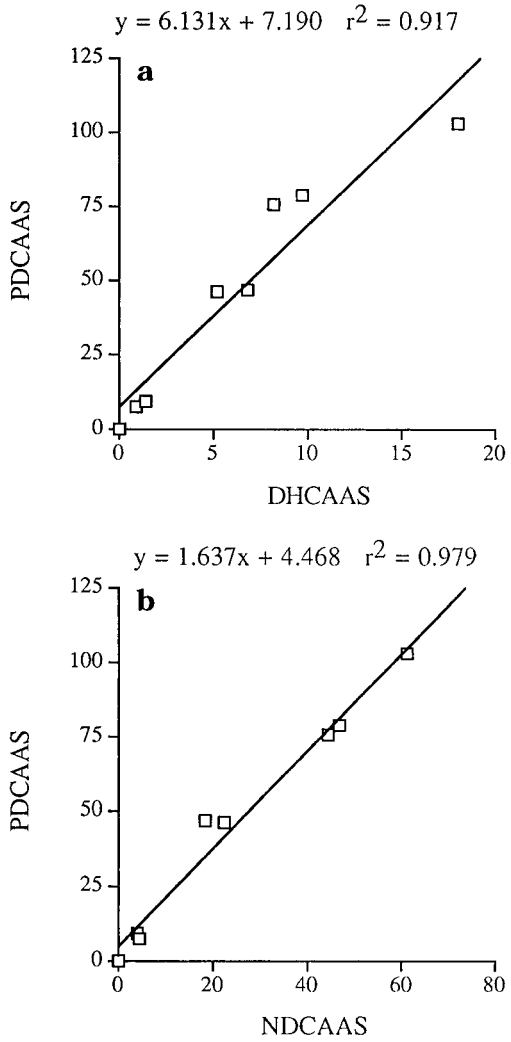

Figure 2. (a) Correlation between PDCAAS and DHCAAS. (b) Correlation between PDCAAS and NDCAAS.

Table 7. Coefficient of Correlation and Statistical Results with Degree of Liberty of Seven Samples[a]

		DH	DHCAAS	ND	NDCAAS
TD	*r*	0.814		0.881	
	b	*b*		*b*	
PER	*r*	0.579		0.095	
	p	ns		ns	
PDCAAS	*r*		0.958		0.989
	p		*b*		*b*

[a] *r*, correlation coefficient. *p*, signification of correlation. ns, not significant. [b] $p < 0.001$.

involves the production of an important rich protein presscake meal. These rapeseed proteins have an excellent balance of essential amino acids, but meal is generally used only in low quantities for monogastric and polygastric animals' diets. Glucosinolates are responsible for goiter formation, for liver and kidney dysfunctions, and for reduced growth performance (Kroll and Przybilski, 1991; Spiegel et al., 1993). The principal anti-nutritional fiber in rapeseed meal is lignin, which is not digested by monogastrics (Slominski and Campbell, 1990; Slominski et al., 1994). Genetic selection has produced sweet white lupines (*Lupinus albus*) that no longer requires treatment to reduce alkaloid toxicity (Ballaster et al., 1980). The most important anti-nutritional factors are found to be low and comprise lupanin, vicin, convicin, and saponin, but trypsin inhibitor activity is moderate (Schoeneberger et al., 1983).

Crude composition of samples were relatively similar, except for RM and HRM, which contained a high amount of fibers. The four plant sources contained at least 70% of protein whereas the presence of 30% of fiber involved only 40% protein for RM and HRM.

Casein and soybean having a balanced EAA composition are considered as animal and vegetable references for rat growth. Rapeseed proteins are balanced too. Lupine and gelatin proteins were chosen for their unbalanced EAA composition. Methionine and cysteine are very low in lupine protein as compared to FAO/WHO (1991) human reference. This reference is usually used, but this is misleading since the rat appears to have a much higher requirement for sulfur amino acids than does the human. In addition, it also requires high amounts of histidine, isoleucine, threonine, and valine (FAO/WHO, 1991). According to this FAO/WHO reference, EAA composition indicates low deficiencies for laboratory rat in all samples. Rat growth is influenced by the amino acid content of the casein, which provides only 63% of the sulfur amino acids required by the rat. The use of amino acid scores related to human requirements would provide a realistic basis for defining the value of food proteins based on human needs rather than on growing rat needs (FAO/WHO, 1991).

The more widespread procedure to determine protein quality is the protein efficiency ratio (PER) method. The official "prefered" method for evaluating protein quality

in the United States is PDCAAS for all foods for human, age 1 year and older. PER may be used and is required for foods for infants. But it is a costly and time-consuming method that does not take into consideration the amino acid availability. To better appreciate protein quality, biological parameters were calculated, i.e., net protein ratio (NPR), nitrogen retained, and apparent and true digestibility (AD and TD, respectively) (Campos and Arêas, 1993; Pedersen and Eggum, 1983). AD and TD are considered as the main characteristics of food or feed proteins (Block and Mitchell, 1946). AD is taken as the percentage of nitrogen intake that is absorbed, and TD takes into account endogenous nitrogen. In contrast to AD, TD is considered to be independent of the protein content of the diet, of the food intake, and of the body weight of the experimental animals (Eggum, 1973b). The efficiency of protein for growth and maintenance of rats could be estimated with NPR (Cheftel et al., 1985), which includes the weight loss of a protein-free diet group.

In order to replace these long and expensive methods, other bioassays were tested for estimating protein nutritional quality, such as a 28-h method (Samman et al., 1993; Samman and Farias, 1993) that considers a weight loss of rats during 24 h. But until now, the results of this method are not entirely satisfactory. Among the enzymatic assays, the most widely known are the pepsin digest residue index (Sheffner et al., 1956), the pepsin pancreatin index method (Akenson and Stahmann, 1964), the Ford and Stalter's gel filtration method (1966), the pepsin pancreatin digest dialysat index (Mauron, 1973), the multidigestion method (Satterlee et al., 1979), and the pepsin pancreatin digestion (Gauthier et al., 1982). The *in vitro* results combined with EAA composition give the calculated-protein efficiency ratio (C-PER) for 72 h (Satterlee et al., 1979) or PDCAAS (Dillon, 1992; Madl, 1993).

Protein intake of heated coagulated lupine and of heated coagulated soy protein samples (HLP and HSP) are compared to those of non-heated proteins (LP and SP). Heating involves modifications of protein structure, protein solubility (Adrian and Frangne, 1991), and variation of the diet palatability. Basically, heating involves a better nutritional protein value because hydrolysis sites of denaturated proteins are more accessible for digestion enzymes (Adrian and Frangne, 1991). The results presented here suggest that another modification may be obtained via heating when proteins are coagulated by alginates. The nutritional value of gelatin given by PER is low, because essential amino acids such as tryptophan, tyrosine, and cysteine occur in very low amounts or are absent, whereas glycine and histidine levels are high (Swan and Torley, 1991). Because of their well-equilibrated EAA composition, rapeseed meals have a good PER. A major criticism of PER is its inability to properly credit protein used for maintenance purposes. A protein can be poor for growth and have a PER near zero, but it can be adequate for maintenance. The PER values of proteins differing in quality are not proportional. A protein with a PER of 2.0 cannot be assumed twice as good as a protein with a PER of 1.0. PER should be replaced by a more appropriate and precise method (FAO/WHO, 1991). Related NPR and PER develop in a similar way, but NPR is more precise because of taking into account the weight loss of a protein-free diet group of rats.

AD and TD depend on the high/low nitrogen intake and high/low nitrogen loss between 18 and 26 days. Nitrogen intakes of all samples are similar except for lupine and gelatin proteins. Lowest nitrogen intake of lupine samples is related to the deficiency of EAA composition. Results obtained with gelatin are not surprising because of its EAA deficiency. Regarding TD, the heating process has no effect on protein digestibility. However AD results discriminate the effect of heating according to protein source. Amino acids of lupine proteins are the most sensitive to heat treatment. Damage to lysine can occur from reaction with reducing sugars through a Maillard reaction as in the drying of proteins (Anderson et al., 1984; Friedman, 1996b,c). Dietary fibers influence *in vivo* digestion in several ways, depending on the nature of the fiber. Fibers can reduce enzyme activity in the lumen but can probably also protect the enzymes against degradation. Furthermore, anatomical changes of certain parts of the digestive tract can be observed. Fibers are a substrate for microbial activity in the digestive tract after long-term intake of fibers and can reduce the transit time of the digesta (Boisen and Eggum, 1991). RM and HRM contained 30% of the fibers, which included 18.4% of insoluble fibers as cellulose and lignin (Rozan et al., 1996). A negative effect of these fibers can be related to low AD and TD values. RM and HRM have an equilibrated EAA composition leading to a similar nitrogen intake as compared to casein, but TD is inferior to 30% of that of sodium caseinate. In this case, fibers could be directly involved. Only 50% of nitrogen intake of gelatin diet is utilized: the amino acid utilization depends on the absorption of total amino acids versus limiting amino acid. Complex interactions occur during intestinal transit; excess or lack of a given amino acid can modify competition for absorption sites. Amino acids of unbalanced diets are mainly utilized as energy sources rather than for growth or maintenance of rats (Cheftel et al., 1985).

In vivo determination of nutritional protein quality depends on several parameters being time-consuming and expensive, comparison of animal results with human requirements, and composition and bioavailability of EAA. Two *in vitro* methods were tested in this study to avoid these disadvantages.

The pH-stat method gives information about the ability of a protein to be degradated by a multi-enzyme system during exactly 10 min. Sodium caseinate has a high DH because of its balanced EAA composition and the absence of other components. The low result obtained for RM could be explained, on one hand, by the presence of fibers, such as lignin, whose phenol units can be complexed with proteins and, on the other hand, by relative insolubility of RM proteins in water and in neutral salt solutions of low ionic strength. As a consequence, enzyme hydrolysis is restrained. The decrease of DH of HSP and HRM as compared to SP and RM seems related to heating, which could modify the soy protein structures and the position of the enzyme site fixations. The HRM result could be explained by the disappearance of glucosinolates and polyphenols and the decrease of fiber interactions with proteins facilitating access to the action site of enzymes. DH of lupine samples are similar but their PERs differ significantly. These results indicate that heating could have different consequences on protein nutritional value, depending on the nature of proteins and their environment. EAA deficiencies of gelatin explain the low DH obtained, because trypsin action preferentially occurs near a basic amino acid (i.e., arginine or lysine)

binding a carboxylic group. Peptidase plus pancreatin is an enzyme cocktail that has diverse action sites according to absence or deficiency of tryptophan and phenylalanine. The digestion decrease of HLP as compared to LP and the similar results of HSP and SP digestions illustrate the various effects of heating on digestibility. Nitrogen release of RM and HRM is very low because of difficulties of protein solubilization and presence of insoluble fibers.

In vitro methods use enzymatic systems that are well-known, whereas the *in vivo* system is a complex, evolving with the age of animals. The most serious problem of *in vivo* assays is the higher requirements of rats for sulfur-containing amino acids as compared to humans.

In theory, the most logical approach for evaluating protein quality is to compare amino acid content of a food with human amino acid requirements, taking bioavailability into account. PDCAAS procedure directly reflects the essential amino acid content, true digestibility of the protein, and bioavailability of the amino acids in the food. To improve the accuracy of scoring procedures, chemically determined amino acid content may have to be corrected for digestibility or biological availability (FAO/WHO, 1991). AAS_1 were obtained from the same EAA for samples, heated or not, and were very low for samples that had a great EAA deficiency (lupine and gelatin). Gelatin contains no tryptophan, as a consequence AAS and PDCAAS equal zero. In the case of proteins of very poor quality, the amino acid scoring approach has been criticized for non-agreement between amino acid scores and estimates or protein quality based on biological assays (FAO/WHO, 1991). Gelatin completely lacking tryptophan has a TD equal to 56.3%, due to different needs for growth and maintenance and to the capability of an organism to adapt to low intakes of tryptophan. The results of AAS_1 suggest that the single ratio of the first limiting amino acid may not describe accurately enough dietary nutritional protein quality. AAS_1 value of HLP concentrates is lower to that of gelatin, meanwhile its PER is better than that of gelatin. Consideration of the nutritional role of each amino acid does not involve the role of limiting ones but depends on the major ratio of EAA. Skamoto et al. (1992) suggest that total ratio of the three EAA (Lys, Met+Cys, Trp) in protein has a major significance for protein nutritive value rather than the single value of the first limiting amino acid. This agrees notably with the present results in all protein samples. Gelatin has the lowest AAS_1 value (0.0), moreover its amino acids content (Trp, Lys,Tyr, and Arg) is poor. Lupine protein concentrate is especially deficient in sulfur amino acids with 0.1 AAS_1 value. In the same way, AAS_1 is not affected by moderate heating of plant protein. The PER values difference between heated and freeze-dried lupine is not explained by either AAS_1 nor TD. It is assumed that one or many non-protein-soluble components of lupine seed, retained with protein during the coagulation process, are modified by heating.

The validity of the PDCAAS method is limited by lack of standardized and reproducible procedures for determining tryptophan and sulfur amino acids, by insufficient data on digestibility of amino acids in foods, and by uncertainty about human requirements to be used for the scoring pattern. Significant developments have been done, and they have made easier the use of an amino acid scoring procedure adjusted for digestibility. This is a better predictor of protein quality for humans than the rat growth method which is, in many cases, the only convenient in vivo approach (FAO/WHO, 1991). A criticism of PDCAAS method includes its inability to take into account the possible adverse effect of disproportionate amounts of EAA on the utilization of the most limiting amino acid. Excessive levels of non-essential amino acids and non-protein nitrogen may also influence the overall utilization of a dietary protein. However, the possible occurrence of amino acid imbalance in mixed or properly amino acid-supplemented human diets does not appear to be of any major practical significance. Utilization of AAS_1 and *in vitro* methods have permitted the calculation of DHCAAS and NDCAAS, taking into account DH and ND results, respectively. Evolution of results obtained for the PDCAAS, NDCAAS, and DHCAAS is similar for each sample source. These two new parameters could involve the elimination of a time-consuming *in vivo* method used for PDCAAS.

To replace *in vivo* methods, two *in vitro* procedures (cell digestion and pH-stat) were proved to correlate with TD determination. PER is not correlated with these methods, and weight gain (g/d) and protein intake (g/d) of rats have been verified. They are not correlated with TD, DH, and ND (data not shown).

It would be beneficial to perform correlations between PDCAAS and DHCAAS or NDCAAS methods because of the time-consuming nature of the *in vivo* method that was used usually for determination of PDCAAS. Better relationships than correlations between TD and *in vitro* methods would be obtained.

The excellent correlation obtained between PDCAAS and NDCAAS to determine plant protein quality could suggest the use of NDCAAS as a means of evaluation of the nutritional quality of plant proteins.

LITERATURE CITED

Adrian, J.; Frangne, R. Influence des traitements physiques et chimiques sur la structure des protéines (en particulier les protéines laitières). *Med. Nutr.* **1991**, *XXVII* (3), 119−128.

Akenson, W. R.; Stahmann, M. A. A pepsin pancreatin digest of protein quality evaluation. *J. Nutr.* **1964**, *83*, 257−264.

Alder-Nissen, J. *Enzymic hydrolysis of food proteins*; Elsevier Applied Science: London; 1986.

Anderson, P. A.; Sneed, S. M.; Skurray, G. R.; Carpenter, K. J. Measurement of lysine damage in proteins heated with gossypol. *J. Agric. Food Chem.* **1984**, *32* (5), 1048−1053.

AOAC. *Official methods of analysis*, 14th ed. Association of Official Analytical Chemists: Arlington, VA, 1984.

Ballaster, D.; Yanez, E.; Garcia, R.; Erazo, S.; Lopez, F.; Harrdt, E.; Corne, J. S. Chemical composition, nutritive value and toxicological evaluation of two species of sweet lupine (*Lupinus albus* and *Lupinus luteus*). *J. Agric. Food Chem.* **1980**, *28*, 402−405.

Block, R. J.; Mitchell, H. H. The correlation of the amino-acid composition of proteins with their nutritive value. *Nutr. Abstr. Rev.* **1946**, *16* (2), 249−278.

Boisen, S.; Eggum, B. O. Critical evaluation of *in vitro* methods for estimating digestibility in simple-stomach animals. *Nutr. Res. Rev.* **1991**, *4*, 141−162.

Campos, A. A.; Arêas, J. A. G. Protein nutritional value of extrusion-cooking defatted lung flour. *J. Food Chem.* **1993**, *47*, 61−66.

Chang, H. I.; Catignani, G. L.; Swaisgood, H. E. Effects of sucrose, starch and oil on the *in vitro* determination of protein digestibility using the immobilized digestive enzyme assay (idea) system. *J. Food Biochem.* **1992**, *16*, 133−140.

Chango, A.; Villaume, C.; Bau, H. M.; Nicolas, J. P.; Méjean, L. Process of lupine seeds debittering, producing fibrious

protein concentrate and alkaloids: nutritional evaluation of the extract protein. *J. Sci. Food Agric.* **1993**, *63*, 195−200.

Cheftel, J. C.; Cuq, J. L.; Lorient, D. Modification des protéines alimentaires au cours des traitements technologiques et de l'entreposage. In *Protéines alimentaires: biochimie-propriétés fonctionnelles- valeur nutritionnelle- modifications chimiques*; Lavoisier Tec & Doc: Paris, 1985; pp 1−309.

Dillon, J. C. Les méthodes d'évaluation de la valeur nutritive des protéines en alimentation humaine. La méthode récemment recommandée par OMS/FAO. *Cah. Nutr. Diet.* **1992**, *XXVII* (1), 54−58.

Eastoe, J. E. ; Leach, A. A. Chemical constitution of gelatin. In *The science and technology of gelatin*; Ward, A. G., Courts, A., Eds.; Academic Press: London, 1977; pp 73−107.

Eggum, B. O. *A study of certain factors influencing protein utilization in rats and pigs*; Publication 406; National Institute of Animal Science: Copenhagen, 1973a.

Eggum, B. O. The influence of dietary protein level on protein utilization. In *A study of certain factors influencing protein utilization in rats and pigs*; Publication 406; National Institute of Animal Science: Copenhagen, 1973b; pp 106−111.

FAO/WHO food and nutrition paper. Protein quality evaluation. Report of joint FAO/WHO expert consultation. *FAO/WHO* **1991**, *51*, 1−66.

Ford, D. E.; Salter, D. N. Analysis of enzymatically digested food protein by sephadex gel filtration. *Br. J. Nutr.* **1966**, *20*, 843−849.

Friedman, M. Nutritional value of proteins from different food sources. A review. *J. Agric. Food Chem.* **1996a**, *44* (1), 6−29.

Friedman, M. Food browning and its prevention: An overview. *J. Agric. Food Chem.* **1996b**, *44* (3), 631−653.

Friedman, M. The impact of the Maillard reaction on the nutritional value of food proteins. In *The Maillard reaction*; Ikan, R., Eds.; Wiler, J., & Sons Ltd: Albany, CA, 1996c.

Gaudard-de Weck, D.; Hischenhuber, C.; Kruseman, J. Protéines-définition, aspects nutritionnels et méthodes d'évaluation: un article de revue. *Trav. Chim. Aliment. Hyg.* **1994**, *85*, 317−339.

Gauthier, S. F.; Vachon, C.; Jones, J. D.; Savoie, L. Assessment of protein digestibility by *in vitro* enzymatic hydrolysis with simultaneous dialysis. *J. Nutr.* **1982**, *112*, 1718−1725.

Hsu, H. W.; Vavak, D. L.; Satterlee, L. D.; Miller, G. A. A multienzyme technique for estimating protein digestibility. *J. Food Sci.* **1977**, *42* (5), 1269−1273.

Kakade, M.; Rackis, J. J.; Mcghee, J. E.; Puski, G. Determination of trypsin inhibitor activity of soy products: a collaborative analysis of an improved procedure. *Cereal Chem.* **1974**, *51*, 376−382.

King, A. H. Brown seaweed extracts (alginates). In *Food hydrocolloids*; Glicksman, M., Ed.; CRC Press, Boca Raton, 1983; pp 115−188.

Kroll, J.; Przybilski, H. Toxicological evaluation of rapeseed protein products by means of a thyroidea stimulating test. *Fat Sci. Technol.* **1991**, *6*, 228−231.

Linder, M.; Fanni, J.; Parmentier, M.; Sergent, M.; Phan-Tan-Luu, R. Protein recovery from veal bones by enzymatic hydrolysis. *J. Food Sci.* **1995**, *60*, 949−952, 958.

Linder, M.; Rozan, P.; Lamghari el Kossori, R.; Fanni, J.; Villaume, C.; Méjean, L.; Parmentier, M. Nutritional value of veal bone hydrolysate. *J. Food Sci.* **1997**, *62* (1), 183−189.

Lopez-Bellido, L.; Fuentes, M. Lupine crop as an alternative source of protein. *Adv. Agron.* **1986**, *38*, 239−296.

Mac Donough, F. E.; Sarwar, G.; Steinke, F. H.; Slump, P.; Garcia, S.; Boisen, S. *In vitro* assay for protein digestibility: interlaboratory study. *J. Assoc. Off. Anal. Chem.* **1990**, *73* (4), 622−625.

Madl, R. L. Evolution of protein quality determination. *CFW.* **1993**, *38* (8), 576−577.

Marshall, H. F.; Wallace, G. W.; Satterlee, L. D. Prediction of protein digestibility by an *in vitro* procedure using human, porcine, and rat pancreatin preparations. *Nutr. Rep. Int.* **1979**, *19* (6), 901−913.

Mauron, J. The digest dialysat index of food proteins, amino acid composition and nutritive value. In *Protein in human nutrition*; Porter, J. W. G., Rolls, B. A., Eds.; Academic Press: London, 1973.

Moore, S.; Spackman, D. M.; Stein, N. H. Chromatography of amino acids on sulfonated polystyrene resins. *Anal. Chem.* **1958**, *30*, 1185.

Pedersen, B.; Eggum, B. O. Prediction of protein digestibility by an *in vitro* enzymatic pH-stat procedure. *Z. Tierphysiol. Tierernaehr. Futtermittelkd.* **1983**, *49*, 265−277.

Roe, J. H. The determination of sugar in blood and spinal fluid with anthrone reagent. *J. Biol. Chem.* **1955**, *212*, 335−343.

Rozan, P.; Villaume, C.; Bau, H. M.; Schwertz, A.; Méjean, L.; Nicolas, J. P. Detoxication of rapeseed meal by *Rhizopus oligosporus sp-T3*: a first step towards rapeseed protein concentrate. *Int. J. Food Sci. Technol.* **1996**, *31* (1), 85−90.

Samman, N.; Farias, R. N. A 28-h method for estimating protein nutritional quality. *J. Agric. Food Chem.* **1993**, *41*, 52−56.

Samman, N.; Molina, O. E.; Perotti de Galvez, N. I.; Callieri, D. A. S.; Farias R. N. Application 28-h method for monitoring nutritional protein quality changes during single-cell protein processing. *J. Agric. Food Chem.* **1993**, *41*, 57−60.

Satterlee, L. D.; Marshall, H. F.; Tennyson, J. M. Measuring protein quality. *J. Am. Oil Chem. Soc.* **1979**, *56*, 103−109.

Savoie, L.; Gauthier, S. F. Dialysis cell for the *in vitro* measurement of protein digestibility. *J. Food Sci.* **1986**, *51* (2), 494−498.

Schoeneberger, H.; Gross, R.; Cremer, H. D.; Elmadfa, I. The protein quality of lupines (*lupineus mutabilis*) alone in combination with other protein sources. *Qual. Plant.—Plant Foods Hum. Nutr.* **1983**, *32*, 133−144.

Sheffner, A. L.; Eckffeldt, G. A.; Spector, H. The pepsin-digest-residu (PDR) amino acids index of net protein utilization. *J. Nutr.* **1956**, *60*, 105−110.

Skamoto, K.; Kobayashi, M.; Okuba, H.; Sato, Y. Statistical analysis of relationship between net protein utilization in rat and amino acid compositions in cooked foods. *Int. J. Vit. Nutr. Res.* **1992**, *62*, 269−272.

Slominski, B. A.; Campbell, L. D. Non-starch polysaccharides of canola meal: quantification, digestibility in poultry and potential benefit of dietary enzymz supplementation. *J. Sci. Food Agric.* **1990**, *53*, 175−184.

Slominski, B. A.; Campbell, L. D.; Guenter W. Oligosaccharides in canola meal and their effect on nonstarch polysaccharide digestibility and true metabolizable energy in poultry. *Poultry Sci.* **1994**, *73*, 156−162.

Southgate, D. A. T. Determination of carbohydrates in food. II. Unavailable carbohydrates. *J. Sci. Food Agric.* **1969**, *20*, 331−335.

Spiegel, C.; Bestetti, G. E.; Rossi, G. L.; Blum, J. W. Feeding of rapeseed presscake meal to pigs: effects on thyroid morphology and function and on thyroid hormone blood levels, on liver and on growth performance. *J. Vet. Med. Ser. A* **1993**, *40*, 45−57.

Swaisgood, H. E.; Catignani, G. L. Protein digestibility: *in vitro* methods of assessment. In *Advances in food and nutrition research*; Academic Press, Inc.: New York, 1991; pp 185−236.

Swan, J. E.; Torley, P. J. Collagen: structure, function and uses. *Meat Ind. Res. Inst. N. Z. Rep.* **1991**, *883*, 1−49.

Zarkadas, C. G.; Yu, Z.; Zarkadas, G. C.; Minero-Amador, A. Assessment of the protein quality of beefstock bone isolates for use as an ingredient in meat and poultry products. *J. Agric. Food Chem.* **1995**, *43*, 77−83.

Received for review September 23, 1996. Revised manuscript received February 12, 1997. Accepted February 20, 1997.[⊗]

JF960723V

[⊗] Abstract published in *Advance ACS Abstracts,* April 15, 1997.