The bioavailability and postprandial utilisation of sweet lupin (*Lupinus albus*)-flour protein is similar to that of purified soyabean protein in human subjects: a study using intrinsically ^{15}N-labelled proteins

François Mariotti*, Maria E. Pueyo, Daniel Tomé and Sylvain Mahé

UMR INRA-INAPG de Physiologie de la Nutrition et du Comportement Alimentaire, Institut, National Agronomique Paris-Grignon, 16 rue Claude Bernard, 75231 Paris cedex 05

Sweet lupin (*Lupinus albus*), a protein-rich legume devoid of anti-nutritional factors, is considered to have a high potential for protein nutrition in man. Results concerning the nutritional value of lupin protein are, however, conflicting in animals and very scarce in human subjects. Furthermore, where fibre-rich protein sources are concerned, the long-term nutritional results are often obscured, particularly since fibre-promoted colonic fermentation may bias the energy supply and redistribute N flux. We therefore studied, during the postprandial phase, the bioavailability and utilisation of lupin-flour protein in nine healthy men who had ingested a mixed meal containing intrinsically ^{15}N-labelled lupin flour as the protein source (Expt 1). The real ileal digestibility (RID) and ileal endogenous N losses (IENL) were assessed using a perfusion technique at the terminal ileum, and the N content and ^{15}N enrichment of ileal samples. Lupin flour exhibited a high RID of 91 (SD 3) % and low IENL (5·4 (SD 1·3) mmol N/h). Postprandial dietary deamination was also assessed from body dietary urea and urinary dietary N excretion, and compared with results in nine healthy men following an iso-energetic meal containing a ^{15}N-soyabean-protein isolate with a similar RID, as a control (Expt 2). Postprandial dietary deamination was similar after lupin and soyabean meals (17 (SD 2) and 18 (SD 4) % ingested N respectively). We therefore conclude that lupin protein is highly bioavailable, even if included in fibre-rich flour, and that it can be used with the same efficiency as soyabean protein to achieve postprandial protein gain in healthy human subjects.

Soyabean protein: Lupin protein: Ileal digestibility: Postprandial retention: Nitrogen isotopes

Since the considerable success of soyabean as a plant protein source for human nutrition, attention has been paid to other plants that are rich in protein and could be grown at low cost in some areas, as an alternative or complement to existing systems of culture. Recent breakthroughs achieved in breeding have renewed interest in different lupin varieties (Huyghe, 1997; van Barneveld, 1999). Sweet lupin (*Lupinus albus*) is considered to have significant potential as a protein source for both animals and man, in particular because of its extremely low content of anti-nutritional factors when compared with other pulses (Grant *et al.* 1995; van Barneveld, 1999). Furthermore, some new white lupin (*Lupinus albus* L.) lines have been developed that are virtually free of alkaloids (<0·2 g/kg), thus making

them a target for human nutrition. Despite considerable interest in new plant protein sources, almost no direct information is available on the nutritional value of lupin protein in man. Studies in animals have failed to provide a clear picture of the nutritional value of lupins, possibly because the chemistry of lupins differ according to species, cultivars and agronomic conditions (Eggum *et al.* 1993; Gdala *et al.* 1996; van Barneveld, 1999), but principally because experiments were relative to the species or age of the test animal (Batterham, 1992; van Barneveld, 1999). The aim of the present study was therefore to determine the nutritional value of protein from *Lupinus albus* cultivar Arès compared with soyabean protein during a direct investigation in human subjects.

For this purpose, intrinsically ^{15}N-labelled lupin flour was used to assess the real ileal digestibility (RID) and postprandial metabolic utilisation of lupin protein in healthy men. The latter variable was compared with the results of a second experiment in which a ^{15}N-labelled soyabean-protein isolate with previously determined digestibility was given in a similar meal (Mariotti *et al.* 2000). Assessing the body retention of dietary N during the postprandial phase is considered to be an appropriate method as it specifically addresses the fate of dietary protein during the sensitive phase of protein repletion (Garlick *et al.* 1980; Millward & Pacy, 1995; Tomé & Bos, 2000). The results are discussed on the basis of comparison using the amino-acid scoring (AAS) procedure.

Subjects and methods

Meals

Lupin plants (*Lupinus albus* cultivar Arès) were grown under controlled conditions and sprayed with NH_3NO_3 on the day of sowing and with $^{15}NH_3{}^{15}NO_3$ (Eurisotop, Saint-Aubin, France) 8 d before blossoming (Station d'amélioration des plantes fourragères, INRA, Luzignan, France). Labelled lupin seeds were then dehulled and turned into a flour according to patent FR914007141 (*Farine de Lupin: Son Procédé d'Obtention et ses Applications*), the final grinding using a laboratory mill (Laboratory Mill 3100; Perten Instruments, Huddinge, Sweden) with a 0·8 mm sieve. Soyabean plants (*Glycine max* cultivar Chandor) were grown under controlled conditions and sprayed with $K^{15}NO_3$ on the days of sowing and blossoming and at the R5 stage (Unité de formation et de recherche Génétique et amélioration des plantes, INRA, Montpellier, France). A soyabean-protein isolate was kindly purified by the Nestec Research Centre (Lausanne, Switzerland) from intrinsically and uniformly ^{15}N-labelled soyabean seeds. Extraction and separation steps were carried out on a laboratory scale. First, the beans were coarsely milled, and the native soyabean flour was then deoiled with hexane. The proteins were extracted from defatted flour with water at a neutral pH. After centrifugation (3500 *g* for 30 min at 0°C), the supernatant fraction was immediately acidified to pH 5·2 to precipitate the protein fraction. The protein coagulum was washed, neutralised (NaOH), sterilised (140°C for 40 s) and freeze-dried.

The uniformity of ^{15}N-labelling (i.e. all amino acids having the same enrichment) has been checked on soyabean protein from the same batch. It has not been checked directly for lupin protein. However, pea protein that was prepared according to the same labelling procedure ($^{15}NH_3{}^{15}NO_3$, 8 d before blossoming) as that used for lupin labelling in the present study proved to be uniformly labelled (F Mariotti, unpublished results; Mariotti *et al.* 2001). Under these conditions, uniform labelling is a general feature resulting from N metabolism during rapid protein deposition in plants. We are therefore very confident that the two dietary proteins were uniformly labelled.

Two experimental meals were prepared. The first meal contained 74 g lupin flour (321 mmol N) (LF). The second meal contained 30 g soyabean-protein isolate (316 mmol N) (SI). Sucrose (20 g) was added to each meal and they were then adjusted with maltodextrin (Glucidex IT19; Roquette, Lille, France) and sunflower oil to attain a final content of 120 g carbohydrate and 15 g lipid. PEG (15 g) (PEG4000; Prolabo, Paris, France) was also added to the meals as a non-absorbable water-soluble marker.

Subjects

Healthy men (*n* 18, nine for each experiment) participated in the study. The subjects were in good health, determined by a thorough medical examination. The results of two subjects from Expt 1 were excluded from the study, one with the intestinal tube positioned at the proximal ileum and the other with the intestinal tube which passed the ileocaecal valve during the experiment. The subjects in both groups were of very similar age, height, weight and BMI (28 (SD 7) *v.* 29 (SD 5) years; 1·81 (SD 0·05) *v.* 1·80 (SD 0·06) m; 79·4 (SD 11·7) *v.* 78·9 (SD 13) kg; 24·2 (SD 2·6) *v.* 24·1 (SD 2·8) kg/m^2, in Expt 1 (*n* 7) and Expt 2 (*n* 9) respectively).

Clinical protocol

Experiment 1

The volunteers were admitted to hospital in the morning of the day before the study day. They underwent bioimpedance analysis (Analycor 5w, Eugedia, Spengler; Cachan, France) to determine their total body water. An intestinal tube was passed through the nose and allowed to descend to the digestive tract, as described previously (Mahé *et al.* 1992). The intestinal tube had two functions: (1) to perfuse Phenol Red, a non-absorbable intestinal marker, into the ileum; and (2) to collect intestinal samples by continuous suction 20 cm distally from the perfusion site. Volunteers ate their dinner at 16.00 hours and then fasted overnight. On the morning of the study, after the positioning of the intestinal tube at the terminal ileum had been checked by X-ray, a catheter was inserted into a superficial forearm vein for blood sampling and another catheter in the contralateral forearm for saline infusion. The subjects then ingested the LF meal within 5 min, and the postprandial sampling period lasted for 8 h. The study was performed while the subjects were resting and they were not allowed to ingest food or fluids until the end of the study period, although 500 ml sterile saline was infused from 2 h to 8 h postprandially. Intestinal aspirates were collected for a period of 8 h, over ice, pooled by 30 min intervals and frozen immediately, the first collection before the meal acting as the baseline. A sample of the ileal effluents was kept for PEG and Phenol Red determination and the remainder was freeze-dried and stored for future analysis. Blood samples were collected hourly during the 8 h period following ingestion of the meal. Plasma was immediately separated from whole blood by centrifugation (1500 *g*, 15 min, 4°C) and frozen at −20°C until analysis. Urine was collected under mineral oil every 2 h (0–2, 2–4, 4–6, 6–8 h) after meal ingestion; thymol crystals were

added as a preservative and the samples were stored at 4°C for later analysis.

Experiment 2

Since the RID of the soyabean-protein isolate from the same batch had been determined previously (Mariotti *et al.* 2000), the volunteers were not equipped with ileal tubes, but underwent exactly the same protocol with the SI meal. The results were used to compare the systemic availability and postprandial deamination of dietary N with that of lupin protein. As far as we know, the only potential effect of the presence of an ileal tube might be a change to the gastric emptying kinetics, and, hence to the kinetics of dietary amino acid inflow. This modification would not alter the digestibility of soyabean protein. The results in the literature on the effect of an intestinal tube on the gastric emptying of a mixed liquid meal in human subjects are scarce and conflicting, reporting either a slight delay in gastric emptying using an indirect method (Medhus *et al.* 1999) or no effect using a more direct method (Muller-Lissner *et al.* 1982). The latter study also reported no effect of a transpyloric tube on the duodeno–gastric reflux after feeding. Our personal results do not support a significant effect of ileal tubes on the kinetics of dietary nitrogen absorption or utilization (F Mariotti, unpublished results). Lastly, given the weak overall kinetic difference between the two groups in the present paper, a tube-induced delay, if any, in gastric emptying in the soyabean group is unlikely to have affected the utilization of dietary amino acids.

Extraction of amino acids, urea and ammonia from plasma and urine

Urea and NH_3 were isolated using the method described by Preston & McMillan (1988). Urinary NH_3 was first extracted by mixing 5 ml urine with 2 ml cation exchange resin (Na/K form, Dowex AG-50X8, Mesh 100–200; Bio-Rad, Marne la Coquette, France) for 15 min on a low-speed rotator. For urea extraction, the supernatant fraction was collected and 2 ml were mixed with 3 ml water, 2 ml resin and 20 μl urease (680 U/ml; Sigma, Saint-Quentin-Fallavier, France) and placed in a water-bath for 2 h at 30°C with regular shaking. For amino acid and urea extraction, 4 ml plasma was first pelleted by mixing with 200 mg solid 5-sulfosalicylic acid (Prolabo) and kept for 1 h at 4°C. After centrifugation (2400 *g*, 25 min, 4°C), the supernatant fraction was collected and buffered to pH 7 with NaOH and NaH_2PO_4. For urea extraction, 2 ml supernatant fraction was mixed with 1 ml resin and 8 μl urease (680 U/ml; Sigma) and placed in a water-bath for 2 h at 30°C with regular shaking. The supernatant fraction, considered to be the plasma amino acid fraction, was then freeze-dried and stored in a cool place. After extraction, the resins were washed five times with distilled water and stored at 4°C.

Immediately before isotopic analysis, NH_3 and urea-derived NH_3 were eluted from the resins by adding 2·5 M-KH_2SO_4.

Analytical methods

The total N content in samples was determined using an elemental N analyser according to the Dumas method (NA 1500 series 2; Micromass, Manchester, UK). Urea was assayed in both plasma and urine by an enzymatic method (urease–glutamate dehydrogenase) using a clinical analyser (Dimension automate; Dupont de Nemours, Les Ulis, France). NH_3 was measured in urine by an enzymatic method using a clinical analyser (Kone automate; Kone, Evry, France). Glucose was measured in plasma using a glucose oxidase method (glucose kit GOD-DP; Kone). Insulin was measured in plasma using a radioimmuno assay method (INSIK-5 Diasorin; Antony, France). The concentration of PEG 4000 in the digesta was measured by a turbidimetric method, and that of Phenol Red by colorimetry, as described previously (Mahé *et al.* 1996). Isotopic N_2 enrichments (^{15}N:^{14}N) were determined by isotopic ratio MS. An aliquot was burned in an elemental analyser (NA 1500 series 2; Micromass) at 1020°C interfaced with an isotope ratio MS (Optima; Micromass). The ^{15}N:^{14}N ratios (m/z 28: m/z 29: m/z 30) were measured with reference to a calibrated ^{15}N: ^{14}N N tank.

For amino acid analysis (except for tryptophan and sulfur amino acids), protein samples were first hydrolysed in vacuum tubes at 110°C for 24 h with 6 M-HCl. The hydrolysates were then analysed by ion-exchange chromatography with post-column ninhydrin detection (Amino-System 2500; BIO-TEK, Saint-Quentin en Yvelines, France). For the analysis of sulfur amino acids, samples were oxidised with performic acid prior to hydrolysis in vacuum tubes at 110°C for 24 h with 6 M-HCl and analysis by ion-exchange chromatography. For tryptophan analysis, samples were hydrolysed in vacuum tubes at 110°C for 16 h with 5 M-NaOH before analysis by ion-exchange chromatography.

Calculations

Dietary and endogenous nitrogen flux at the terminal ileum. The assessments of Phenol Red dilutions between the perfusion solution and samples collected using the ileal perfusion technique enabled the calculation of flow rates in the ileum (calculation of the mean flux per 30 min period), as previously described (Gausserès *et al.* 1996). The fraction of dietary and endogenous N in ileal samples was calculated from both total N and isotopic ^{15}N enrichment. Dietary N ($N_{diet-ileal}$, mmol N) and endogenous N ($N_{endo-ileal}$, mmol N) transiting the terminal ileum were thus calculated using the equations:

$$N_{diet-ileal}(t) = N_{tot-ileal}(t) \times (E(t) - E(0))/(E_{meal} - E(0)),$$

and

$$N_{endo-ileal}(t) = N_{tot-ileal}(t) \times (E_{meal} - E(t))/(E_{meal} - E(0)),$$

where $N_{tot-ileal}(t)$ is the total N transiting through the terminal ileum at time t, $E(t)$ the ^{15}N enrichment (expressed as atom percent) in the ileal sample at time t and E_{meal} the ^{15}N enrichment (expressed as atom percent) in the meal.

The PEG flux for each time period was also assessed

using calculated flow rates and the concentration of PEG in the sample. The total amount of PEG passed at the terminal ileum was then calculated in order to check both the completeness of the passage of the liquid phase and the overall accuracy of flow rate estimates by comparison with the total amount ingested with the meal.

Systemic availability of dietary amino acids. At time t, the proportions of plasma free amino acids containing dietary N $(AAdiet/AAtot)(t)$ were calculated using the equation:

$$(AAdiet/AAtot)(t) = (E(t) - E(0))/(E_{meal} - E(0)),$$

where $E(t)$ is the ^{15}N enrichment (expressed as atom percent) in the amino acid fraction at time t, and E_{meal} the ^{15}N enrichment (expressed as atom percent) in the meal.

Dietary and endogenous nitrogen in the body urea pool. Total body urea N $(N_{tot-urea})$ was calculated as the product of the plasma urea concentration and its volume of distribution on the assumption that urea was distributed throughout the total body water:

$$N_{tot-urea} = ([Nurea]/0.92) \times TBW,$$

where $[Nurea]$ is the plasma urea N concentration (mmol N/l), 0·92 the corrective factor for the proportion of water in plasma, and TBW the total body water (litres).

Dietary and endogenous N incorporated in the body urea pool ($N_{diet-urea}$ and $N_{endo-urea}$, mmol N) were calculated using the formulas:

$$N_{diet-urea}(t) =$$
$$N_{tot-urea}(t) \times (E_{urea}(t) - E_{urea}(0))/(E_{meal} - E_{urea}(0)),$$

and

$$N_{endo-urea}(t) =$$
$$N_{tot-urea}(t) \times (E_{meal} - E_{urea}(t))/(E_{meal} - E_{urea}(0)),$$

where $E_{urea}(t)$ is the ^{15}N enrichment (expressed as atom percent) in the plasma urea at time t.

Urinary dietary and endogenous nitrogen. Dietary and endogenous N incorporated in urinary N ($N_{diet-urin}$ and $N_{endo-urin}$, mmol N) were calculated using the formulas:

$$N_{diet-urinary}(t) =$$
$$N_{tot-urinary}(t) \times (E_{urin}(t) - E_{urin}(0))/(E_{meal} - E_{urin}(0)),$$

and

$$N_{endo-urinary}(t) =$$
$$N_{tot-urinary}(t) \times (E_{meal} - E_{urin}(t))/(E_{meal} - E_{urin}(0)),$$

where $N_{tot-urinary}(t)$ is the quantity of urinary N (in the form of either total-, urea- or NH_3-N) at time t, and $E_{urin}(t)$ the ^{15}N: ^{14}N ratio (expressed as atom percent) in urinary N (in the form of either total-, urea- or NH_3-N) at time t.

Dietary and endogenous deamination. The dietary (and endogenous) deamination fluxes were computed every 2 h for the mean periods 0–2, 2–4, 4–6 and 6–8 h, and over the 8 h period, by summing the urinary dietary (or endogenous) N excreted during the period and variations in dietary (or endogenous) body urea N. The postprandial biological value was calculated as the relative amount of absorbed N that was not deaminated during the 8 h following the meal.

Curve fittings and other curve estimates. Different model curves were used during the postprandial period to fit the experimental quantity of: (1) dietary N excreted in the urine as urea or NH_3; (2) dietary urea present in the body; and (3) dietary plasma amino acids.

For (1) the curve took the form: $y = a(1 - e^{-bt})^c$, where t is time, and a, b and c are regression estimated constants, while for (2) and (3), the curve took the form: $y = a \times e^{-(1/2)(\ln(t/t_0)/b)^2}$, where t is time, and a, b and t_0 are regression estimated constants. Curve fittings were performed using Sigma Plot 5·0 (SPSS Inc., Erkrath, Germany).

Statistical analysis

Results were expressed as means values and standard deviations. The overall effects of meal, time and interactive effect (meal × time) were assessed using an ANOVA procedure with time as a repeated factor (GLM procedure, SAS/STAT version 6.03; Statistical Analysis Systems Institute, Cary, NC, USA). For those measures for which there was a significant interaction, *post hoc* testing of differences between meals at each time point was performed with Student's t tests, with the P value adjusted by Bonferroni correction. Single planned comparisons between the two meals (namely postprandial deamination or postprandial biological values at 8 h after meals) were performed using two-sided Student's t tests (SAS/STAT version 6.03; Statistical Analysis Systems Institute). A probability of $P < 0.05$ was considered as significant.

Ethical considerations

The protocol had previously been approved by the Institutional Review Board for Saint-Germain-en-Laye Hospital (78100 Saint-Germain-en-Laye, France). All subjects gave their full, written consent to participation in the study after the experimental protocol had been explained to them in detail.

Results

Real ileal nitrogen digestibility

Dietary and endogenous N fluxes were determined at the terminal ileum for 8 h after the ingestion of [^{15}N]-labelled lupin flour (Expt 1). The total quantity of PEG passing the terminal ileum over the 8 h period was in very close agreement with the amount mixed into the LF meal (14·9 (SD 1·1) estimated *v.* 15 g ingested), suggesting that the mean flow rates were correctly assessed.

The ileal endogenous N flux did not vary with time and was 5·4 (SD 1·3) mmol/h over the study period. The total amount of dietary N that escaped digestion in the small intestine was 28·9 (SD 9·7) mmol and thus, by comparison

with the amount ingested, the RID of LF protein was 91·0 (SD 3·0) %.

These results were compared (Table 1) with those previously obtained with a soyabean-protein isolate from the same batch as that used in the present study (Expt 2). Both ileal endogenous losses and RID values were very similar with lupin and soyabean.

Kinetics of plasma dietary amino acids, glucose and insulin

The kinetics of dietary N transfer to the plasma free amino acid pool after LF or SI meals is shown in Fig. 1. Over the whole 8 h period, there was a significant meal × time interaction on the plasma dietary amino acid concentration, but no significant difference between meals was found at individual time points. Plasma glucose concentrations (Fig. 2) did not differ between LF and SI, but there was a trend $(0·05 < P < 0·1)$ towards a meal × time interaction. Plasma glucose had returned to baseline after 5 h and 4 h following LF and SI respectively $(P > 0·05)$ and did not differ between the two meals at individual time points. The type of meal had no main or interactive effect on plasma insulin (Fig. 2). Insulin had returned to baseline after 6 h and 5 h following LF and SI, respectively $(P > 0·05)$.

Dietary and endogenous postprandial deamination

Figure 3 shows the different products of dietary amino acid deamination: body urea, urinary urea and NH_3. Dietary urea concentrations and kinetics did not differ between meals over the whole period. The type of meal modulated significantly the kinetics of dietary N and urea excretion in urine (meal × time interaction: $P < 0·05$). The same effect appeared with borderline significance $(0·05 < P < 0·1)$ for urinary dietary NH_3. Total (8 h) dietary deamination (as the sum of dietary body urea and urinary dietary N) was 54·2 (SD 6·2) and 57·3 (SD 12·0) mmol N for LF and SI respectively (NS). The total (8 h) postprandial recovery of endogenous N in the form of deaminated metabolites differed significantly between meals (215·3 (SD 41·7) and 260·7 (SD 40·5) mmol N for LF and SI respectively).

Postprandial biological values and amino acid scores

The amino acid compositions and postprandial biological values for lupin and soyabean protein are shown in Table 2. AAS, calculated using the FAO/WHO amino acid reference pattern (Food and Agriculture Organization/World Health Organization 1990), were higher for soyabean (calculated from sulfur AAS) than for lupin (calculated from the lysine score). In contrast, no difference was observed between the two proteins with regards to postprandial biological values.

Discussion

During the present study, the RID of lupin-flour protein was shown to be 91 %, which did not differ from that of soyabean-protein isolate (Mariotti et al. 2000). The digestibility of lupin protein may even be higher if they are extracted, since the high-fibre content in lupin flour might have acutely impaired the digestion and absorption of dietary N by reducing enzyme activity, obstructing their access, or adsorbing amino acids (Dunaif & Schneeman, 1981; Isaksson et al. 1983). However, the extent of these potential effects on oro−ileal dietary protein digestion remains unclear, particularly because it may vary as a function of the type of fibre and the animal tested (Sauer et al. 1991; Schulze et al. 1994; Lenis et al. 1996; Lien et al. 1996; Muir et al. 1996; Grala et al. 1998). Fine grinding of the lupin flour may have benefited dietary protein hydrolysis and weakened the effect of fibre, but the extremely low content of anti-nutritional factors is probably the principal reason for the high digestibility of lupin-flour protein.

The results also showed that endogenous ileal losses of N did not differ from baseline after the lupin-flour meal (mean value 5·4 mmol/h) and were somewhat lower than previous values observed after mixed, fibre-free meals made of purified soyabean or pea proteins (5·8 and 6·5 mmol/h respectively) (Mariotti et al. 2000, 2001); thus lupin flour did not acutely induce any extra endogenous losses of N. The effect of fibre on endogenous ileal losses might also be a partially delayed phenomenon (Corring et al. 1989). More probably, lupin NSP did not impair the digestive and absorptive processes, as suggested by the high dietary protein digestibility, and thus may not have impaired the re-absorption of endogenous N, which has been proposed as the major mechanism behind fibre-mediated increases in endogenous N losses (Nyachoti et al. 2000). Taken together, these findings agree with the high apparent ileal digestibility that has been reported for white lupin or its fractions in rats (Rubio et al. 1994,

Table 1. Real ileal digestibility and endogenous ileal losses of lupin-flour protein and soyabean-protein isolate*

(Mean values and standard deviations)

	Lupin-flour protein*		Soyabean-protein isolate†	
	Mean	SD	Mean	SD
Real ileal digestibility (%)	91·0	3·0	91·5	2·0
Postprandial ileal endogenous nitrogen losses (mmol N/h)	5·4	1·3	5·8	3·8

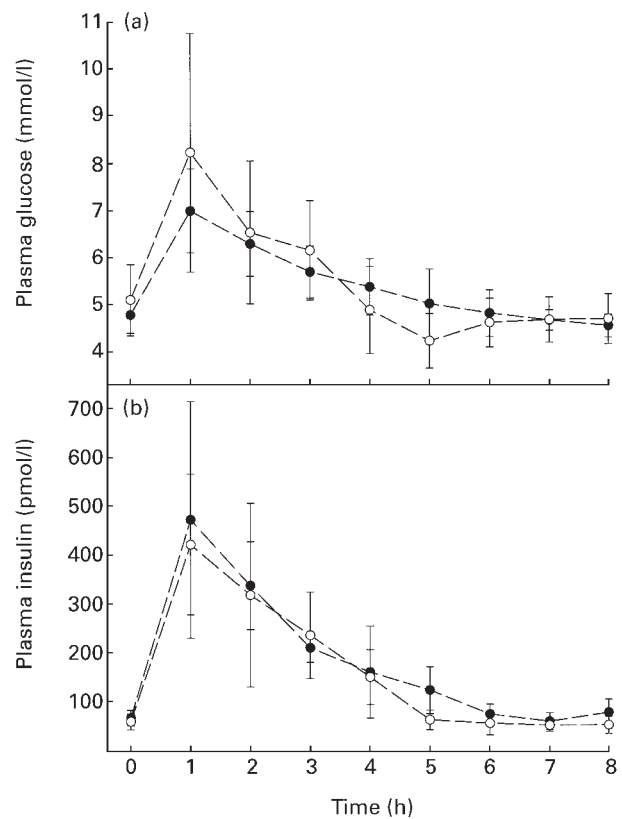* As determined in Expt 1, n 7 (for details of subjects and procedures, see p. 316).
† As reported by Mariotti et al. (2000).
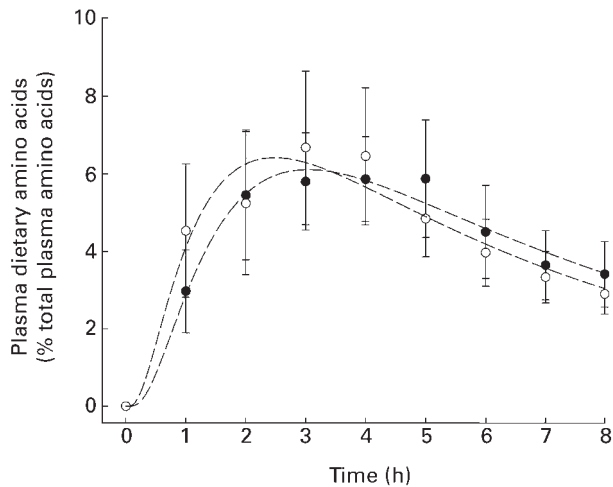
Fig. 1. Dietary nitrogen incorporation in the plasma amino acid nitrogen pool after the ingestion of a mixed meal containing soya-bean-isolate protein (○, *n* 9) or lupin-flour protein (●, *n* 7). There was a significant ($P<0.05$) meal × time interaction, but no meal main effect (repeated measures ANOVA) or difference between meals at each time point (Bonferroni-adjusted *t* tests). For details of subjects and procedures, see p. 316. Values are means with standard deviations shown by vertical bars.

Fig. 2. (a) Plasma glucose and (b) insulin, after the ingestion of a mixed meal containing soyabean-isolate protein (○, *n* 9) or lupin-flour protein (●, *n* 7). There was no significant ($P>0.05$) effect of meal on either plasma glucose or insulin (repeated measures ANOVA). For details of subjects and procedures, see p. 316. Values are means with standard deviations shown by vertical bars.

1995) and pigs (Fernandez & Batterham, 1995) and with the high amino acid availability reported by van Barneveld (cited by van Barneveld (1999)).

Our present results also demonstrated a high postprandial retention of N from lupin protein, which did not differ from that of soyabean protein. This finding is difficult to compare with other results in the literature because the lupin sources, dietary conditions and animal species

Table 2. Amino acid analysis of lupin-flour protein and soyabean-protein isolate (as mg/g protein, N × 5·40 and N × 5·52 respectively*), amino acid score and postprandial biological values

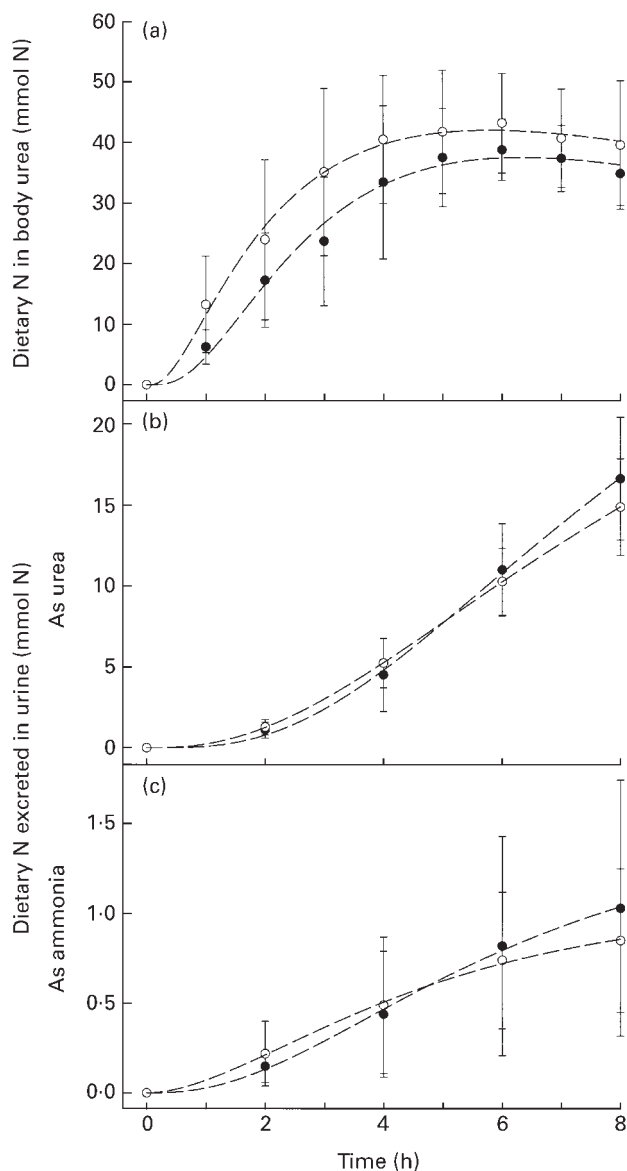|	Lupin-flour protein	Soyabean-protein isolate	FAO/WHO pattern†
Histidine	25·9	29·9	14
Isoleucine	46·3	56·6	28
Leucine	81·3	96·4	66
Lysine	50·6	68·9	58
Methionine	11·9	12·2	25
Cysteine	18·0	14·5	25
Phenylalanine	51·5	63·3	63
Tyrosine	50·9	46·4	63
Threonine	40·5	47·3	34
Tryptophan	9·7	18·4	11
Valine	37·4	56·5	35
Aspartic acid	114·2	144·9	
Serine	57·0	66·9	
Glutamic acid	204·4	238·0	
Proline	42·9	61·6	
Glycine	35·3	53·5	
L-Alanine	29·8	50·0	
Arginine	99·3	57·8	
Amino acid score	0·87	1·07	
Postprandial biological value (%)‡	81·3§ (SD 2·6)	80·1 (SD 4·2)	

* Nitrogen to protein conversion factors as reported by Mossé (1990).
† Food and Agriculture Organization/World Health Organization (1990).
‡ Mean values were calculated using real ileal digestibility (see Table 1) and postprandial dietary deamination 8 h after the meal.
§ Mean value was not significantly different from that of soyabean-protein isolate group (Student's *t* test, $P=0.52$).

Fig. 3. Dietary urea in (a) body water and (b) cumulative urinary excretion of dietary urea and (c) ammonia after the ingestion of a mixed meal containing soyabean-isolate protein (○, n 9) or lupin-flour protein (●, n 7). There was a significant ($P<0.05$) meal × time interaction on urinary dietary urea, but no meal main effect (repeated measures ANOVA) or difference between meals at each time point (Bonferroni-adjusted t tests). For details of subjects and procedures, see p. 316. Values are means with standard deviations shown by vertical bars.

were different. In adult human subjects, the only published N balance study with lupin reported that the net protein utilisation of white lupin was 77 % that of egg protein, mainly because of a reduction in faecal digestibility (Egana et al. 1992). Since the subjects fed lupin meals also received higher levels of NSP for 10 d, this result may now be ascribed to the shift from urinary to faecal losses of N, this being associated with the promotion of bacterial activity in the colon (Younes et al. 1995; Tetens et al. 1996). In that case, the difference in net protein utilisation values may result from lower biological

values rather than lower digestibility. This would then be in line with the low content in indispensable amino acids of the lupin that was used in that study (AAS 0·57). However, during a long-term N balance study, modifications to the entero–hepatic cycle may certainly not be the only mechanism hindering the interpretation of the nutritional value (Young et al. 1981). Other adaptive mechanisms or biases are also set in motion, and particularly the influence of energy on N metabolism, which is indeed biased by NSP supply (Young, 1991). In contrast, focusing on postprandial protein gain following a specific protein input minimises the influence of such factors (Tomé & Bos, 2000).

Some experiments conducted in rats have reported a very inefficient utilisation of protein from *Lupinus angustifolius*, which persisted even when the diets were supplemented with amino acids (Rubio et al. 1995; Rahman et al. 1997). However, this unexplained phenomenon, assumed to originate from an unknown factor associated with protein that would limit its biological value (Rahman et al. 1997), has not been always recorded in rats fed methionine-supplemented lupin diets (Sgarbieri & Galeazzi, 1978; Ballester et al. 1984; Eggum et al. 1993). In the pig, some lower growth rates have been reported when including lupin in the diet, but they were generally consistent with a reduction in feed intake. In the pig, lupin of various species is not therefore clearly associated with a reduction in feed efficiency (Fernandez & Batterham, 1995; Gdala et al. 1996). Indeed, the relative performance or N balance of lupin-fed pigs appears to fluctuate in line with various factors such as inclusion level, age, alkaloid content and lupin species or lines (Fernandez & Batterham, 1995; McNiven & Castell, 1995; Gdala et al. 1996; van Barneveld, 1999). There are also some marked differences between animal species in their ability to utilise certain amino acids, as illustrated by studies on lysine bioavailability (Batterham, 1992). This further supports the argument that the biological value of a protein should better be assessed in the target species, as earlier suggested (Forsum et al. 1982).

The present study also provides a basis to compare the evaluation of lupin and soyabean protein using the AAS method, based on amino acid analysis and the FAO/WHO amino acid reference pattern (Food and Agriculture Organization/World Health Organization, 1990) with postprandial biological values measured in human subjects. Despite the fact that lupin protein had a lower AAS than soyabean protein, there was no difference in postprandial N retention. Indeed, it is generally accepted that lupins are poor source of sulfur amino acids, lysine and tryptophan (Eggum et al. 1993; Molvig et al. 1997; van Barneveld, 1999). In the present experiment, the lupin flour proved to be rich in sulfur amino acids (about 30 mg/g protein), which therefore did not limit the AAS. Previous amino acid analyses had already shown that total content of sulfur amino acids (irrespective of the methionine:cysteine ratio) are limiting or only slightly limiting in human subjects in the case of many lupin cultivars (USDA Nutrient Database for Standard Reference, Release 13 2000, 1999, U.S. Department of Agriculture ARS; van Barneveld, 1999), in as much as 5·40 instead of 6·25 is used as the N to protein conversion factor (Mossé, 1990). With

reference to the Food and Agriculture Organization/World Health Organization (1990) pattern, the two limiting amino acids in the lupin under study were lysine and tryptophan, with individual AAS values of 0·87 and 0·88 respectively. However, recent data on lysine requirements in human subjects suggest that the value of 58 mg/g in the reference pattern is probably too high, and values of 50 mg/g (El-Khoury *et al.* 2000) or even 31–38 mg/g (Millward, 1999; Millward *et al.* 2000) have recently been proposed. Under these conditions, the 50·6 mg lysine/g lupin approximately matched the requirement. Similarly, the 9·7 mg tryptophan/g provided by lupin protein may be close to the required level, because the actual requirement may be 10 or even 6 mg/g (Millward, 1999; Young & Borgonha, 2000). Therefore, the smallest adjustments to lysine and tryptophan requirements would raise the AAS of lupin to 0·97, which is close to that of soyabean (1·07). Lastly, it has also been suggested that some acute accommodative phenomena may compensate for a slightly lower supply of certain amino acids during maintenance (Fuller & Garlick, 1994). In particular, some amino acids, especially lysine, may be recycled from the endogenous free amino acid pool in adult human subjects receiving an adequate protein intake (Millward *et al.* 2000). In this context, which is that of developed countries, the AAS principle may be little relevant when assessing the nutritional value of a protein source.

In conclusion, these results show that sweet white lupin-flour protein, given selectively to healthy human subjects, exhibited a high digestibility and can be used as efficiently as soyabean protein for postprandial protein deposition without promoting specific endogenous N losses either in the ileum or through protein catabolism. These results provide encouragement for the study of the nutritional adequacy of new protein-rich sources for man, as well as analysis of the factors influencing dietary protein utilisation.

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