British Journal of Nutrition (2003), **90**, 301–309 DOI: 10.1079/BJN2003903

Carbohydrates digestibility and faecal nitrogen excretion in rats fed raw or germinated faba bean (*Vicia faba*)- and chickpea (*Cicer arietinum*)-based diets

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(Received 23 October 2002 – Revised 14 March 2003 – Accepted 3 April 2003)

Raw or germinated faba bean (*Vicia faba* minor var. Alameda) and chickpea *(Cicer arietinum* kabuli var. Athenas) seed meals were incorporated in essential amino acid-supplemented and energy-equalized diets for growing (65 (SD 1) g) male Wistar rats as the only sources of dietary protein. A lactalbumin-based diet was used as the control. Faecal dry weight and N excretion of animals fed legume-containing diets were greater ($P < 0{\cdot}01$) and DM digestibility lower ($P < 0{\cdot}01$) than controls. Apparent faecal digestibilities of amino acids were found to be not different or lower ($P < 0{\cdot}01$) than controls in rats fed diets containing legume seeds, either germinated or not. Total diaminopimelic acid (DAPA) and purine bases excretion were significantly ($P < 0{\cdot}01$) higher than controls in rats fed both legume seed meals. Faecal bacterial N calculated according to DAPA or purine bases values was similar and significantly higher ($P < 0{\cdot}01$) than controls in rats fed legume seed meals. Bacterial N accounted for 50 to 80 % of total faecal N in rats fed legume diets. Apparent faecal N digestibility values (53–65 %) of rats fed legume-based diets were lower ($P < 0{\cdot}01$) than controls, but became substantially higher (85–92 %) when calculated taking into account bacterial N values. Faecal NSP digestibility values for legume diets were 40–57 g/100 g ingested. Germination decreased starch faecal excretion ($P < 0{\cdot}05$) and increased ($P < 0{\cdot}05$) faecal starch and NSP digestibilities of faba bean seeds.

Faba beans: Chickpeas: Germination: Carbohydrates and amino acids digestibility: Nitrogen excretion

Legume seeds such as faba beans (*Vicia faba*) and chickpeas (*Cicer arietinum*) are important protein sources for both human nutrition and animal production. While faba beans are mainly devoted to animal feeding, chickpeas are used for human consumption. Also, chickpea seeds, particularly those from the kabuli types (light-coloured), have been reported to contain relatively low amounts of anti-nutritional factors (Grant *et al.* 1983, 1995; Chavan *et al.* 1986). They appear to be well tolerated by single-stomached animals (Batterham *et al.* 1990; Savage & Thompson, 1993) and constitute a staple food in great areas of the world including, for example, Mediterranean countries. Previous studies with legumes such as faba beans, chickpeas and lupins (*Lupinus angustifolius*) suggest that their low nutritional value is due to the inefficient utilization of their proteins by test animals. This appears to be due to lower nutritive quality of the major reserve proteins rather than to the presence of any known anti-nutritional factors (Rubio *et al.* 1991, 1995, 1998). Thus, in the rat, *in vivo* intestinal digestibility of isolated globulin preparations from faba bean, lupin and soyabean (*Glycine max*) was in fact higher than that of lactalbumin (Rubio *et al.* 1994), which is frequently used as a control protein. Furthermore, since not only isolated protein fractions, but also individual amino acids, have shown ileal digestibility values comparable with control proteins (Rubio *et al.* 2002), the low utilization of some legume seed proteins is probably the result of other factors (Rubio, 2000).

Even though ileal digestibility of legume proteins appears to be high, faecal N excretion is usually higher than in controls. However, not only protein itself but also carbohydrate fractions in the diet have an effect on N digestibility and excretion, particularly through the metabolism of intestinal microbiota (Mathers, 1991). Thus, most of the N excreted through the faeces in man and animals fed diets rich in dietary fibre (NSP + lignin) is considered to come from bacterial fermentation in the large intestine (Cummings, 1984). In these circumstances, if faecal N excretion were not corrected for bacterial N, faecal digestibility of dietary proteins would be underestimated. Bacterial N in faeces can be estimated by using diaminopimelic acid (DAPA) (Goodlad & Mathers, 1990; Rubio, 2003*a*) or purine bases (Surra *et al.* 1997)

Abbreviations: DAPA, diaminopimelic acid; NPU, net protein utilization.

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as indirect markers of bacterial growth. These markers have been broadly used in ruminant nutrition where the relevance of bacterial metabolism in the intestinal tract has been always recognized. In the present work, both were used: first, due to the lack of information on these markers in single-stomached animals; second, in order to compare estimations from both methods.

Germination is regarded at present as one of the cheapest and most effective procedures to increase the nutritional value of legumes, supposedly through the breakdown of certain anti-nutritional factors such as phytates, protease inhibitors, lectins and α-galactosides (de la Cuadra *et al.* 1994; Ayet *et al.* 1997; Greiner *et al.* 1998). Also, previous studies (Shekib, 1994; Urbano *et al.* 1995; Chitra *et al.* 1996; Mansour, 1996; Schulze *et al.* 1997) suggested an effect of germination mainly on the carbohydrate fraction of the seeds, together with higher digestibility values for both carbohydrates and proteins. Since most of these studies were conducted *in vitro*, it was considered appropriate to study the effects of germination on both N and carbohydrate digestibilities *in vivo*.

Accordingly, the aims of the present work were to study, in rats fed raw or germinated faba bean or chickpea diets: (a) the ileal and faecal digestibilities of carbohydrate fractions and amino acids; (b) the origin of the N excreted through the faeces.

Materials and methods

Chemical analysis

Faba bean (*Vicia faba* minor var. Alameda) and chickpea (*Cicer arietinum* var. Athenas) seeds were obtained from Centro de Investigación y Desarrollo Agrario (Córdoba, Spain). Seeds were screened for imperfections, placed on trays with sand–water (5:1, w/v) and allowed to germinate for 72 h at 20°C in a constant environment chamber with a photoperiod of 8 h light/d. Seeds were then collected, freeze-dried and ground to pass a 1 mm sieve. Lactalbumin, DL-α ε-DAPA, purine bases (adenine and guanine) and amino acids standards were obtained from Sigma (Poole, Dorset, UK).

Amino acids in feedstuffs and faeces were determined after hydrolysis of samples in boiling HCl (6 mol/l; 2 ml/5 mg protein) for 18 h by using the Pico Tag method in HPLC equipment (Waters, Milford, MA, USA) (Fernández-Fígares *et al.* 1997). Carbohydrate (starch and NSP) analyses in feedstuffs and biological samples were carried out by GLC following the method of Englyst *et al.* (1992). DAPA in faecal samples was determined by reverse-phase HPLC (Rubio, 2003*a*). Briefly, freeze-dried faecal samples (50–100 mg) were incubated in screw-capped tubes at 110°C for 24 h in 6 M-HCl for hydrolysis. After cooling, samples were diluted with water, internal standard (α-aminoadipic acid) was added to samples or standard solutions and dried under vacuum for 2 h. Samples and standards were then re-dried for 1 h and derivatizing solution (phenylisothiocyanate) added, the tubes allowed to stand at room temperature for 15 min and vacuum-dried again. Samples were re-dissolved in 150 μl Pico Tag sample diluent (Waters) for free amino acid analysis, vortex-mixed and 10 μl solution injected for HPLC analysis in a 3·9 × 300 mm Nova-Pak C18 column from Waters. Purine bases were also determined by reverse-phase HPLC (Balcells *et al.* 1992) using two Spherisorb (5 μm) C18 ODS-2 (4·6 × 250 mm) columns. Dried faecal samples (100 mg) were added to 1 ml allopurinol solution (550 μmol allopurinol/l) and hydrolysed for 1 h at 110°C with $HClO_4$ (4 mol/l), followed by neutralization with NaOH (4 mol/l), centrifugation at 2000 ***g*** for 15 min and filtration of the supernatant fraction through a 0·2 μm filter. N was determined by the Kjeldhal method (Association of Analytical Chemists, 1984). Chromium oxide in diets and ileal samples was determined as in Aguilera *et al.* (1988).

Animals, diets and feeding regimen

Six male weaned Wistar rats matched by weight (65 (SD 1) g) were used for each experimental treatment. They were housed individually in metabolism cages under controlled conditions of temperature (25°C), relative humidity (50 %) and lighting (12 h light–dark cycles). All animals were pre-adapted to experimental conditions for 5 d before the experiment and fed the lactalbumin control diet. Feed was withheld overnight before the start of the experiment, which lasted for 9 d. Feed allowance was restricted for all rats to 12 g feed/d, which is close to the *ad libitum* feed intake of control animals of this age and weight. There were no feed refusals, and all animals readily ate all the feed offered to them. Water was freely available at all times. Rats were weighed daily. All management and experimental procedures carried out in the present study were done in strict accordance with the appropriate practices for management of laboratory animals in Spain (Act no. 223/88 of 14 March 1989) by staff trained to carry out such procedures.

The diets (Table 1) were based on raw (non-germinated) or germinated faba bean or chickpea seed meals and were equalized in digestible energy (15·5 kJ/g) and protein (lactalbumin in controls or bean proteins plus synthetic amino acids, which were added as extra protein in the experimental diets). Total calculated amounts were (g/kg): lactalbumin control, 100; faba bean and germinated faba bean, 106·3; chickpea and germinated chickpea, 104·8. Crude protein was calculated as N × 6·25 for lactalbumin and N × 5·5 for bean protein (Mossé, 1990). Appropriate amounts of synthetic essential amino acids were added to the legume-based diets taking into account their amino acid composition to reach or exceed control (lactalbumin) values (Table 1) where legume protein values were under requirements for diets containing 100 g protein/kg. Even though it is well established that added amino acids are not absorbed in the same way as those incorporated in a protein structure (Rérat *et al.* 1987), supplementation was carried out in order to rule out as much as possible the negative effects of amino acid imbalance on protein utilization. Due to the effects of caecal fermentation, the effects of supplementation on faecal amino acid excretion (as determined in the present study) are lower compared with those in ileal flows. The diets were also supplemented with vitamins and minerals to meet requirements (Grant *et al.* 1993). Chromium oxide (2 g/kg diet) was added to

Table 1. Composition (g/kg) of the diets

Diet*...	Lactalbumin	Faba bean and germinated faba bean	Chickpea and germinated chickpea
Maize starch	422·6	121·3	170·8
Potato starch	150	–	–
Oil (maize)	50	110	56
Glucose	150	150	150
Vitamin+mineral mix†	100	100	100
Silicic acid	0·4	0·4	0·4
Chromium oxide	2	2	2
Lactalbumin	125	–	–
Faba bean	–	510	–
Chickpea	–	–	516
Essential amino acids			
Histidine	–	0·3	–
Isoleucine	–	1·4	0·3
Methionine	–	2·1	2·4
Phenylalanine	–	0·6	–
Tryptophan	–	0·6	0·7
Tyrosine	–	0·2	0·8
Valine	–	1·1	0·6
Composition‡			
Digestible energy (kJ/g)§	15·5	15·5	15·4
Protein (g/kg)‖	100	106·3	104·8
Fibre	75	84·7	60·3
Fat	50	116·6	74·2

* For details of the feeding regimen, see p. 302. The compositions of diets based on germinated faba bean or chickpea seeds were equal to those for raw materials.

† As in Grant *et al.* (1993).

‡ Calculated according to analysed composition of ingredients.

§ Calculated digestible energy (kJ/g) of ingredients according to composition: chickpea meal 14·7; faba bean meal 12·4.

‖ N × 6·25 for lactalbumin and N × 5·4 + synthetic amino acids for legumes (see p. 302).

the diets as an indigestible marker. Potato and maize starch were the same as used in previous studies (Sigma catalogue nos. S4251 and S4126 respectively) (for example, see Rubio *et al.* 1995, 1998).

Amino acids apparent ileal digestibilities were calculated by using the expression:

$$((AA_f/Cr_2O_{3f})-(AA_i/Cr_2O_{3i}))/(AA_f/Cr_2O_{3f}),$$

where AA_f and AA_i are amino acid contents (g/100 g) and Cr_2O_{3f} and Cr_2O_{3i} are chromium oxide concentrations (g/100 g) in feed and ileal contents respectively. Starch and NSP apparent ileal digestibilities were calculated in the same manner. Apparent faecal digestibilities of amino acids, starch and NSP were calculated by difference between amounts ingested and excreted. Bacterial N in faeces was calculated by using DAPA and purine bases values according to the following expressions:

$$\text{Bacterial } N_D \text{ (mg)} = 19{\cdot}45 \times \text{DAPA (mg)} + 0{\cdot}297$$
(Ahrens & Kaufmann, 1985);

$$\text{Bacterial } N_P\text{(mg)} = 1{\cdot}05 \times \text{purine bases } (\mu M)$$
(Surra *et al.* 1997).

Corrected apparent faecal N digestibilities based on DAPA or purine bases in faeces (Table 2) were then calculated by subtracting bacterial N values from total faecal N excretion. Ileal and faecal legume starch digestibilities in legume diets were calculated assuming a 100 % digestibility for maize starch as previously determined by others (Mathers *et al.* 1997) and by Rubio *et al.* (1995) in the control lactalbumin diets.

Sampling procedures

Rats were killed under pentobarbital sodium (40 mg/kg body weight) anaesthesia on day 10, exactly 2 h after giving them 5 g feed. The abdominal and thoracic cavities were opened, and ileal (0–250 mm up from the ileocaecal junction) contents for digestibility measurements were washed out with ice-cold water, collected in plastic vials, immediately frozen (−20°C), and subsequently freeze-dried. Faecal samples were collected daily throughout the study, stored at −20°C until required, freeze-dried and ground in a mortar.

Statistical analysis

The results were subjected to a factorial (2 × 2) two-way ANOVA. The Tukey multiple comparison test was used to determine differences between means (Minitab Statistical Software Package; Minitab, New York, NY, USA).

Results

Analysed starch and NSP composition of raw and germinated legume seeds are described in Table 3. The carbohydrate composition of germinated faba bean and chickpea seeds was similar to those of non-germinated materials. Starch content in both legume seed meals was 405–429 mg/g, while chickpea seeds contained lower amounts (97–103 mg/g) of total NSP than faba beans (160–164 mg/g). That difference was mainly due to lower glucose (cellulose) and uronic acid (pectins) amounts in chickpea seeds.

Table 2. Excretion of total and bacterial nitrogen through the faeces, and calculated apparent faecal nitrogen digestibility values, in rats fed raw or germinated faba bean- or chickpea-based diets for 9 d†

(Mean values and pooled standard deviations)

							Probability		
	Lactalbumin	Faba bean	Germinated faba bean	Chickpea	Germinated chickpea	Pooled SD	S	T	S × T
Dry weight (g)	3·9^{a}	15·1^{b}	12·1^{c}	9·3^{d}	9·1^{d}	1·1	**	**	**
Total N (g)	0·14^{a}	0·89^{b}	0·80^{c}	0·68^{d}	0·71^{d}	0·50	**	NS	*
App. N dig (%)	92·0^{a}	53·6^{b}	58·3^{c}	65·1^{d}	63·9^{d}	2·5	**	NS	*
DM digestibility (%)	96·3^{a}	85·8^{b}	88·7^{c}	91·4^{d}	91·5^{d}	0·9	**	**	**
DAPA (mg/g)	1·94	2·43	1·81	1·90	2·45	0·71	NS	NS	NS
Total DAPA (mg)	7·3^{a}	36·4^{b}	21·7^{c}	17·5^{c}	21·8^{c}	7·5	*	NS	*
Bacterial N_D (mg)	141·3^{a}	707·5^{b}	422·4^{c}	340·4^{c}	424·5^{c}	145·3	*	NS	*
App. N dig_D (%)	99·7^{a}	90·5^{b}	82·8^{b}	82·9^{b}	85·3^{b}	8·0	NS	NS	NS
Purine bases (μM/g)	28·6^{a}	37·4^{ab}	42·4^{b}	55·2^{c}	56·1^{c}	7·2	**	NS	NS
Total purines (μM)	111·9^{a}	558·6^{b}	511·7^{b}	507·2^{b}	518·9^{b}	84·4	NS	NS	NS
Bacterial N_P (mg)	117·4^{a}	586·6^{b}	537·2^{b}	532·5^{b}	544·8^{b}	88·6	NS	NS	NS
App. N dig_P (%)	94·9^{a}	84·7^{b}	88·0^{ab}	92·4^{a}	91·5^{a}	4·1	**	NS	NS

S, seed (faba bean or chickpea); T, treatment (germinated or not germinated); App. N dig, apparent N digestibility; DAPA, diaminopimelic acid; Bacterial N_D, bacterial N in faeces calculated from DAPA values; App. N dig_D, apparent faecal N digestibility corrected for bacterial N according to faecal DAPA values; Bacterial N_p, bacterial N in faeces calculated from purine bases values; App. N dig_p, apparent faecal N digestibility corrected for bacterial N according to faecal purine bases values; NS, not significant ($P > 0·05$).

[a,b,c,d]Mean values within a row with unlike superscript letters were significantly different ($P < 0·01$).

*$P < 0·05$, **$P < 0·01$.

† For composition of the diets see Table 1 and p. 302.

Performance and N balance indices of rats fed these diets have been presented elsewhere (Rubio *et al.* 2002). Briefly, weight gains, gain:feed values, N retention and net protein utilization (NPU) values of rats fed diets containing raw or germinated legume seed meals as the only source of protein were lower ($P < 0·01$) than controls. Rats fed germinated faba bean diets had higher ($P < 0·01$) NPU values than those fed raw seed meal, while there was no difference between raw and germinated chickpea seeds with regard to rat performance and N balance.

Apparent faecal digestibilities of amino acids (Table 4) were found to be not different or lower ($P < 0·01$) than controls in rats fed diets based on legume seeds, either germinated or not.

Faecal dry weight and N excretion by animals fed legumes were greater ($P < 0·01$) and DM digestibility lower ($P < 0·01$) than controls (Table 2). On the other hand, although faecal DAPA concentration values (1·81–2·45 mg/g) were not different among treatments, total DAPA excretion was significantly ($P < 0·01$) higher than controls in rats fed legume seed meals. Purine bases concentrations and total faecal excretions were higher ($P < 0·01$) than controls in rats fed legume-based diets. Faecal bacterial N calculated according to DAPA or purine bases values was significantly higher ($P < 0·01$) than controls in rats fed legume seed meals. Apparent faecal N digestibility values ranged between 53 and 65 % and were significantly lower ($P < 0·01$) than controls for rats fed legume seeds, either germinated or not. However, apparent faecal N digestibility values were substantially higher (85–92 %) when calculated taking into account bacterial N values (see p. 303 and Table 2). Apparent N digestibility values calculated according to purine bases in the faeces were not different from controls in germinated faba bean- and both chickpea-based diets. Bacterial N accounted for 50–80 % of total faecal N in rats fed legume diets. Even though values were similar, bacterial N values estimated from purine bases values tended to be higher than those estimated from DAPA.

Germination of faba beans decreased ($P < 0·05$) faecal excretion of starch (Table 5), tended to increase ileal starch and NSP digestibilities and significantly ($P < 0·05$) improved faecal starch and NSP digestibilities, while no effect was observed with germinated compared with raw

Table 3. Carbohydrates (starch + non-starch polysaccharides) composition (mg/g dry matter) of raw and germinated faba bean and chickpea seed meals

	Faba bean	Germinated faba bean	Chickpea	Germinated chickpea
Starch	421	429	405	415
Rhamnose	3·0	3·3	2·3	3·2
Arabinose	18·6	21·7	24·9	28·9
Xylose	12·2	12·3	5·5	5·1
Mannose	4·0	4·0	4·8	4·8
Galactose	9·7	9·4	8·7	10·0
Glucose	75·2	66·3	27·9	28·2
Uronic acids	41·5	43·3	23·8	23·0
Total NSP	164·2	160·3	97·9	103·2

Table 4. Apparent faecal digestibility (g/100 g ingested) of amino acids in rats fed diets containing raw or germinated faba bean or chickpea seeds as the only protein source†

(Mean values and pooled standard errors)

	Lactalbumin	Faba bean	Germinated faba bean	Chickpea	Germinated chickpea	Pooled SE	Probability S	Probability T	Probability S × T
Essential									
Arginine	74	68	74	82	80	2	NS	NS	NS
Histidine	79^{a}	57^{b}	58^{b}	68^{ab}	55^{b}	2	NS	*	*
Isoleucine	87^{a}	58^{b}	56^{b}	53^{b}	36^{c}	2	*	*	*
Leucine	92^{a}	61^{b}	59^{b}	60^{b}	46^{c}	2	*	*	NS
Lysine	87^{a}	49^{b}	57^{b}	58^{b}	57^{b}	2	NS	NS	*
Phenylalanine	83^{a}	56^{b}	59^{b}	69^{ab}	46^{b}	2	NS	NS	*
Threonine	79^{a}	55^{b}	51^{b}	67^{ab}	54^{b}	2	NS	*	NS
Tyrosine	87^{a}	57^{b}	57^{b}	71^{c}	55^{b}	2	NS	*	*
Valine	85^{a}	58^{bc}	55^{bc}	61^{b}	43^{c}	2	NS	*	*
Non essential									
Alanine	81^{a}	46^{b}	41^{b}	54^{b}	43^{b}	3	NS	NS	NS
Aspartate	88^{a}	73^{b}	85^{a}	83^{a}	84^{a}	1	NS	*	NS
Glutamate	89^{a}	70^{b}	78^{b}	82^{ab}	78^{b}	1	*	NS	*
Glycine	61^{a}	51^{ab}	53^{ab}	71^{b}	56^{ab}	2	NS	NS	*
Proline	81^{a}	49^{b}	55^{b}	73^{ab}	53^{b}	2	NS	NS	*
Serine	77^{a}	60^{b}	65^{ab}	75^{a}	66^{b}	2	NS	NS	*

S, seed (faba bean or chickpea); T, treatment (germinated or not germinated); NS not significant ($P > 0{\cdot}05$).

^{a,b,c}Mean values within a row with unlike superscript letters were significantly different ($P < 0{\cdot}01$).

$*P < 0{\cdot}05$, $**P < 0{\cdot}01$.

†For composition of the diets, see Table 1 and p. 302.

chickpea seeds (Table 6). Ileal starch digestibility in rats fed legume seed meals was 41–54 g/100 g ingested, while in faeces values were 94–97 g/100 g ingested. Faecal NSP digestibility values for legume diets were 40–57 g/100 g ingested. Among individual sugars, higher digestibility values were determined for uronic acids, arabinose and mannose in both faba bean and chickpea meals.

Discussion

The inclusion of faba bean or chickpea seed meals in the diet resulted in lower performance (weight gain, gain:feed) and N retention and NPU values than lactalbumin controls. Germination of chickpea seeds brought about no significant improvement in these parameters as compared with raw materials, which is in keeping with previously published data (Khaleque *et al.* 1985). On the contrary, germination of faba beans resulted in higher N retention and NPU values compared with non-germinated seeds, even though values did not reach those of controls (Rubio *et al.* 2002). Previously reported experiments with germinated materials (Shekib, 1994; Urbano *et al.* 1995; Chitra *et al.* 1996; Mansour, 1996; Schulze *et al.* 1997) suggested an effect of germination mainly on the carbohydrate fraction of the seeds, together with higher digestibility values for both carbohydrates and proteins. Although most of these data referred to are from *in vitro* studies, and the agreement of *in vivo* with *in vitro* results is usually difficult

Table 5. Faecal starch and non-starch polysaccharides excretion (mg/g dry matter) of rats fed diets containing raw or germinated faba bean or chickpea seeds as the only protein source†

(Mean values and pooled standard deviations)

	Faba bean	Germinated faba bean	Chickpea	Germinated chickpea	Pooled SD	Probability S	Probability T	Probability S × T
Starch	89^{a}	61^{b}	68^{b}	60^{b}	12	*	**	*
Rhamnose	$5{\cdot}6^{a}$	$6{\cdot}0^{a}$	$9{\cdot}6^{b}$	$9{\cdot}0^{b}$	1·7	**	NS	NS
Arabinose	26·6	24·4	64·2	37·3	27·8	*	NS	NS
Xylose	$46{\cdot}2^{a}$	$42{\cdot}5^{a}$	$22{\cdot}5^{b}$	$19{\cdot}1^{b}$	6·6	**	NS	NS
Mannose	$7{\cdot}3^{a}$	$7{\cdot}6^{a}$	$14{\cdot}0^{b}$	$13{\cdot}6^{b}$	4·5	*	NS	NS
Galactose	29·3	34·2	39·2	42·5	8·4	*	NS	NS
Glucose	$179{\cdot}5^{a}$	$179{\cdot}0^{a}$	$99{\cdot}9^{b}$	$107{\cdot}1^{b}$	33·0	**	NS	NS
Uronic acids	40·2	45·5	47·6	33·8	9·5	NS	NS	*
Total NSP	$355{\cdot}8^{a}$	$327{\cdot}2^{ab}$	$277{\cdot}9^{b}$	$287{\cdot}1^{b}$	42·7	*	NS	NS

S, seed (faba bean or chickpea); T, treatment (germinated or not germinated); NS, not significant ($P > 0{\cdot}05$).

^{a,b,c}Mean values within a row with unlike superscript letters were significantly different ($P < 0{\cdot}05$).

$*P < 0{\cdot}05$, $**P < 0{\cdot}01$.

† For composition of the diets, see Table 1 and p. 302.

Table 6. Ileal and faecal starch and non-starch polysaccharides digestibilities (g/100 g ingested) of diets containing raw or germinated faba bean or chickpea seeds as the only protein source in rats†

(Mean values and pooled standard deviations)

	Faba bean	Germinated faba bean	Chickpea	Germinated chickpea	Pooled SD	Probability		
						S	T	S × T
Ileal digestibility								
Starch	41·2	47·0	54·4	43·8	8·6	NS	NS	*
NSP	10·7	16·8	–‡	–‡	8·8	–	–	–
Faecal digestibility								
Starch	94·1^{a}	96·7^{b}	97·2^{b}	97·5^{b}	0·9	**	**	**
Rhamnose	48·2	62·9	29·7	52·4	15·6	NS	*	NS
Arabinose	58·4	80·5	66·2	75·3	12·2	NS	*	NS
Xylose	1·1^{a}	27·7^{b}	37·2^{b}	24·1^{b}	12·4	**	*	**
Mannose	38·8	60·2	52·4	42·4	13·9	NS	NS	NS
Galactose	14·8	24·2	27·0	33·7	16·8	NS	NS	NS
Glucose	28·1	42·2	41·9	27·8	13·8	NS	NS	*
Uronic acids	71·3	79·0	66·3	74·0	8·9	NS	NS	NS
Total NSP	39·5^{a}	56·1^{b}	52·7^{ab}	53·3^{ab}	8·9	NS	*	*

S, seed (faba bean or chickpea); T, treatment (germinated or not germinated); NS, not significant ($P > 0·05$).

^{a,b,c} Mean values within a row with unlike superscript letters were significantly different ($P < 0·05$).

*$P < 0·05$, **$P < 0·01$.

† For composition of the diets, see Table 1 and p. 302.

‡ Excessive variability of data.

to establish, faecal starch and NSP digestibility values in the present work (Table 6) were higher for germinated compared with non-germinated faba beans. Also, higher protein efficiency ratio, protein digestibility, biological value and ileal amino acid digestibilities have been previously reported in germinated as compared with raw faba beans (Khalil, 2001).

A number of explanations for the lower nutritional value of legume-based diets for growing single-stomached animals have been reported in the last few decades. Among those explanations, some deal with an alleged lower digestibility of their constituent fractions, particularly proteins. However, most data referred to are from *in vitro* studies or apparent faecal digestibility values, and less information exists on ileal *in vivo* digestibility values. Values recently reported (Rubio *et al.* 2002) indicate high apparent ileal digestibility of amino acids, which would suggest that legume proteins are well digested and absorbed within the small intestine. Nevertheless, apparent faecal amino acid digestibilities decreased (Table 4) and total faecal N excretion increased (Table 2) in legume-fed rats, which would apparently contradict the previous observation. However, it is important to keep in mind that high N excretion values are usually linked in single-stomached animals and man to high NSP dietary contents (Cummings, 1984; Mason, 1984; Bach Knudsen *et al.* 1991; Goodlad & Mathers, 1992), as occurs in faba bean- and chickpea-based diets. Thus, raw faba bean and chickpea seed meals contain 164 and 97 g NSP/kg (Table 3), which resulted in 83 and 50 g NSP/kg diet for faba bean and chickpea diets respectively. The main reason for that higher faecal N excretion is that intestinal, and particularly lower gut, microflora increases its growth at the expense of undigested carbohydrates coming from the small intestine and urea supplied mainly through the large-intestinal wall. Goodlad & Mathers (1990) found faecal N excretion values similar to those here reported in rats fed diets based on pea seed meal as the main protein source. The amounts of DAPA determined in that work by a different method increased by 3-fold compared with controls, which agrees with the DAPA values determined in the present study (Table 2). Furthermore, faecal bacterial N calculated from purine bases excretion, which is determined independently from DAPA, and is also used as an indicator of bacterial growth (Surra *et al.* 1997), gave similar results. According to DAPA and purine bases excretion in faeces, bacterial N accounts for 50–80 % of the total faecal N in faba bean- or chickpea-fed rats. When bacterial N was subtracted from total faecal N, much higher apparent N digestibility values (84–92 %) were obtained. On the other hand, the inclusion in the diet of faba bean, chickpea and lupin isolated carbohydrate fractions, which contain most of the starch and NSP present in the whole seed meals, was previously found to give rise to total faecal N excretion values (234–314 mg) higher than controls but significantly lower than those of rats fed diets containing whole legume seed meal. In addition, the inclusion of legume carbohydrate fractions in rat control diets had no effect on final NPU values, which were not different from controls (Rubio *et al.* 1991, 1995, 1998). Therefore, it seems to be not only the presence of energy-yielding substrates (usually undigested carbohydrates), but other factors in the meal that bring about higher N excretion in legume-fed growing rats. The reason is probably that the intestinal flora overgrowth is also dependent on the presence of N sources, such as urea in plasma, which is taken from the intestinal wall (Mason, 1984), as previously suggested by others. Thus, Beames & Eggum (1981) in rats and Mosenthin *et al.* (1992) in pigs showed that the presence of undigested (potato) starch allowed the assimilation by the intestinal flora of nitrogenous materials arising from the blood. Also, in diets differing in protein quality, not only the amount of N excreted in faeces but also in urine is affected by the type and fermentability of carbohydrates (Pastuszewska *et al.* 2000). This has also led to the suggestion that fermentation of a large amount of carbohydrates in the large intestine (which enhances the faecal excretion of bacterial N) could be of value to minimize urea excretion by the kidneys when renal function is impaired (Rémésy & Demigné, 1989). On the other hand, as both faba bean and chickpea diets raised blood urea concentrations, an effect also detected in rats fed purified legume proteins (Rubio *et al.* 1999), intestinal bacterial overgrowth and consequently increased faecal N excretion might depend indirectly at least in part on protein quality. Although the reasons for the lower protein utilization and increased urea production and N excretion in growing rats fed legume or legume protein-based diets compared with controls are still unclear, the explanation might be related to differences in the digestion–absorption process, as suggested by studies in Caco-2 cell cultures. Thus, Rubio & Seiquer (2002) observed that the rate of transport of amino acids from apical to basolateral chambers of Caco-2 cells grown in Transwell bicameral plates was slower for legume proteins hydrolysates compared with casein. That would be in keeping with lower amounts of amino acids afferent per unit of time from the intestine to the liver recently determined in rats fed legume-based diets compared with controls (Rubio, 2003*b*), which in turn might explain the observed effects on liver and whole-body N metabolism (Rubio *et al.* 1999) because the mix of amino acids available for protein synthesis in the tissues at a given moment could be imbalanced. This imbalance can lower the rate of protein synthesis, resulting in an increase in amino acid catabolism, lower protein retention values and increased N excretion in the form of urea. Therefore, lower apparent faecal amino acid digestibilities in rats fed legume-based diets are probably not mainly due to lower legume protein digestibility in the small intestine but to changes in protein metabolism leading to increased urea production (Rubio, 2000) and bacterial overgrowth in the large intestine at the expense of undigested starch and NSP present in the meal. Thus, according to results in Tables 3 and 6, substantial amounts of faba bean and chickpea starch (220–233 and 180–227 g respectively), and 58–75 g faba bean NSP were digested in the lower gut of the rats fed legume-containing diets, either germinated or not. This means that starch was actually the major source of carbohydrate delivered to the caecum of rats fed faba bean or chickpea diets.

Low small-intestinal digestibility and fermentation of legume carbohydrates in the lower gut have both health and productive implications. Legumes are at present regarded as low-glycaemic index foods, and high-carbohydrate low-glycaemic-index dietary advice has been found to improve β-cell function and may be useful in the management of impaired glucose tolerance (Wolever & Mehling, 2002). Also, short-chain fatty acids (mainly acetate, propionate and butyrate) are the major products of carbohydrate fermentation in the colon. Among these, butyrate, which has been shown to be produced in higher proportions through fermentation of starch, has been linked to beneficial effects on the ecology of the intestinal flora and to protective effects against colon carcinoma (Williams *et al.* 2001). On the other hand, low small-intestinal digestibility of both starch and NSP in raw or germinated faba beans and chickpeas determined in the present study probably influence energy metabolism and growth. First, the products of carbohydrate fermentation in the large intestine, particularly short-chain fatty acids, are absorbed and utilized in the energy metabolism of the body (Mathers, 1991). Second, intraduodenal infusions of fibre (potato) evoked a tendency for an increase in the volume of secretion of pancreatic juice and a significant increase both in the mean values of the total protein content and total activities of lipase, trypsin and α-amylase probably linked to the presence of short-chain fatty acids (Jakob *et al.* 2000), and exocrine pancreatic secretion and daily weight gain in pigs have been found to be positively correlated (Botermans & Pierzynowski, 1999).

The main conclusions of the present study were that: (a) between 50–80 % of the N found in the faeces of rats fed faba bean- or chickpea-based diets, either germinated or not, was bacterial N; (b) germination improved faecal starch and NSP digestibilities of faba bean seeds but not those of chickpeas.

Acknowledgements

The author is indebted to Mrs E. Colmenero and A. B. Rodriguez, and to Dr L. Lara and Dr D. Yañez for excellent technical assistance. Financial support was from the Spanish M.A.P.A. (project no. SC 97057).

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