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Effect of germination of legume seeds on chemical composition and on protein and energy utilization in rats

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Chemical composition of soybeans, lupin seeds and black beans, and protein utilization and energy digestibility of soybeans and lupin seeds determined in a rat model, were compared before and after a 48-h germination. Black beans had a much higher starch content and lower levels of low-molecular-weight (LMW) sugars than soybeans and lupin seeds. Lupin seeds had about twice as much non-starch polysaccharides than the other legumes and higher insoluble dietary fiber. Soluble dietary fiber represented over one third of total dietary fiber in all legumes. After germination, LMW α-galactosides decreased in all seeds, particularly in lupins. Sucrose levels increased in lupins and black beans but decreased in soybeans. Biological indices were significantly higher in lupin seeds than in soybeans. Germination produced a small increase in protein utilization in both legumes. These results indicate that the main advantage of a 48-h germination of legume seeds is the reduction in the α-galactosides.

INTRODUCTION

Legume seeds are important sources of energy and protein in many parts of the world, both for animal and human nutrition. However, their nutritional value may be limited in part by the presence of undesirable components known as antinutritional factors. These factors include protease inhibitors, lectins, phenolic compounds, phytates and indigestible carbohydrates of the raffinose family (Deshpande *et al.*, 1984). The content of these components may vary for different legumes, and this difference may be reflected in the efficiency of nutrient utilization.

Germination is considered a suitable procedure to improve the nutritional value of legume seeds by reducing levels of antinutritional factors (Chen *et al.*, 1975; Vanderstoep, 1981; Jood *et al.*, 1986). Germination of *Phaseolus vulgaris* promoted a substantial decrease (almost 80% after 3 days) in the α-galactoside content and proved to be very efficient in reducing intestinal fermentation, as measured by breath-H_2 levels, in human volunteers after consumption of the seeds (Trugo *et al.*, 1990). A similar effect in the α-galactoside content after germination was observed with lupin seeds (Trugo *et al.*, 1993*a*) also associated with a substantial, although more variable, reduction in breath-H_2 levels in humans. Differences in the extent of the biological response between black beans and lupins may be due to differences in other carbohydrate components in these seeds.

Information regarding the effect of germination on detailed carbohydrate composition of different legumes is scarce, particularly when related to the *in-vivo* study of nutrient utilization, such as protein and energy. The purpose of this study was to compare the chemical composition, especially carbohydrate, of different legumes (soybeans, lupin seeds and black beans), and the protein utilization and energy digestibility (soybeans and lupin seeds) measured in the rat model before and after a 48-h germination. Legumes were tested uncooked in order to evaluate, specifically, the effect of germination. Since the microbial intestinal activity may contribute to the overall protein and energy utilization and mask small differences in bioutilization related to germination, groups of rats with and without treatment with Nebacetin, an antibiotic that reduces microbial activity in the large intestine (Eggum *et al.*, 1985), were compared.

MATERIALS AND METHODS

Samples

Seeds of a sweet variety of lupin (*Lupinus albus* cv. *mul-*

tolupa) were supplied by the Instituto Agronomico do Parana (Ponta Grossa, Brazil), and black beans (*Phaseolus vulgaris*) and soybeans (*Glycine max*) were from EMBRAPA, (Rio de Janeiro, Brazil). Uncooked, non-germinated and freeze-dried germinated seeds (see below) were milled to pass a 0·75 mm sieve and kept frozen until analyses and formulation of diets for rat bioassay.

Germination

Germination was performed by a modification of the method of Khaleque *et al.* (1985). Seeds of the legumes were initially washed in distilled water and soaked for 10 min in 70% ethanol containing 3% $CaCl_2$. Afterwards, the seeds were thoroughly rinsed in distilled water and soaked in water for 4 h (seed/water proportion of 1 : 2, w/v). Extraction of low-molecular-weight (LMW) carbohydrates by this procedure was negligible. The seeds were then allowed to germinate in the dark at 28°C for 48 h and finally freeze-dried.

Analytical methods

Proximate analyses were performed by standardized AOAC (1984) methods. Starch and non-starch polysaccharides (NSP), including cellulose and non-cellulosic polysaccharides (NCP), were determined as described by Bach Knudsen *et al.* (1987); LMW sugars were measured according to Bach Knudsen and Li (1991), and dietary fiber, including soluble, insoluble and total (TDF), based on Asp *et al.* (1983).

Zn, Fe, Cu and Mn were measured by flame absorption spectrophotometry and Ca and P by colorimetry, as previously described (Trugo *et al.*, 1993*b*).

Phytic acid was determined by the method of Harland and Oberleas (1977). Trypsin inhibitor activity was measured according to Kakade *et al.* (1974). Results were expressed as trypsin inhibitory units per mg of dried sample.

All results are given as means of duplicate analyses.

Rat bioassay

The experimental procedures for digestibility trials and nitrogen balance have been described by Eggum (1973). A total of eight dietary treatments were tested in a 2 × 2 × 2 factorial design with two types of legume (soybeans and lupin seeds) tested and with germination and use of Nebacetin as factors. The diets were composed of a N-free mixture (starch, 80·7%; sucrose, 8·9%; cellulose, 5·2% and peanut oil, 5·2%), vitamins (1·6%), minerals (4%) and the legume seed, either non-germinated or germinated, added to provide 15 g N/kg dry weight. Each diet was fed either with or without 7 g Nebacetin (neomycin sulphate–bacitracin; 1 : 2 w/w)/kg dry matter.

Groups of five rats per treatment (initial weight *c.* 70 g) were used, with a preliminary feeding period of 4 days and a balance period of 5 days. Each animal received 10 g dry matter (150 mg N) of diet daily. True protein digestibility (TD), biological value (BV), net protein utilization (NPU) and digestible energy (DE) were used as biological indices and were determined as already described, including appropriate correction factors (Eggum, 1973).

Statistical analyses

Evaluation of the effects of germination and use of Nebacetin on the biological indices for each legume type was done by ANOVA. Comparisons of results between legumes were done by Student's *t*-test.

RESULTS AND DISCUSSION

Effect of germination on the chemical composition of legumes

Germination during 48 h did not modify the proximate composition of the legumes on a dry matter basis (Table 1) except for a small decrease in total carbohydrates in soybeans associated with a similar small increase in crude protein. Levels of individual minerals were also mostly unaffected by germination, although small decreases of Fe and Cu contents were observed, probably related to losses in the washing and soaking of the seeds prior to germination. The high Mn level of *L. albus* found in this study has already been observed (Trugo *et al.*, 1993*b*; Gladstones & Drover, 1962), and it seems characteristic of this species.

No decrease in phytic acid content was observed in the legume seeds after 2 days of germination. This period of germination is not sufficient to activate phytases to degrade phytic acid to a significant extent. This is in agreement with previous results in lupin seeds showing that only after 4 days of germination was a significant decrease (30–40%) of phytic acid observed (Souza *et al.*, 1988). However, it is interesting to note that *L. albus* presents a much lower phytic acid content compared to soy or black beans. Therefore, phytate : mineral molar ratios, particularly phytate : zinc ratios with values of 7–8, 19–22 and 27–30 for lupin, soybeans and black beans, respectively, are more favorable in lupins, indicating a potentially better mineral bioavailability in these seeds (Ellis *et al.*, 1987).

The effect of 48-h germination on trypsin inhibitory activity was a 20 and 25% reduction for black beans and soybeans, respectively, in agreement with previous observations (Trugo *et al.*, 1990; Bates *et al.*, 1977). No trypsin inhibitory activity was detected in the lupin seeds, as previously observed (Eggum *et al.*, 1993), suggesting that the natural protective mechanisms against insect infestation of the seeds may differ between legumes.

Marked differences in the detailed carbohydrate composition of the legumes were observed (Table 2). Considering the non-germinated seeds, black beans had a much higher starch content (41% compared to less than 1% in soybeans and lupins) but lower total levels

Table 1. Chemical composition (dry matter basis) of germinated and non-germinated legume seeds (mean, *n* = 2)

Composition	*L. albus* cv. *multolupa*		Soybeans		Black beans	
	NG[a]	G[a]	NG	G	NG	G
Proximate composition (g%)						
Protein (N × 6·25)	38·1	38·4	41·1	45·6	20·7	21·2
Fat	7·4	7·6	21·1	22·4	1·4	1·8
Carbohydrates	46·9	49·2	24·8	20·6	65·0	63·0
Lignin	0·7	0·8	0·9	0·9	3·5	3·4
Ash	3·5	3·6	5·4	4·8	4·2	4·6
Mineral composition						
Calcium (mg/g)	2·02	2·32	2·50	2·80	1·49	1·40
Phosphorus (mg/g)	2·90	3·07	4·37	4·81	—	—
Zinc (μg/g)	51	51	46	45	48	46
Iron (μg/g)	86	63	95	47	85	79
Copper (μg/g)	5·2	3·6	5·3	3·9	10·2	10·6
Manganese (μg/g)	1900	1980	16	15	21	21
Phytic acid (g%)	0·4	0·4	0·9	1·0	1·3	1·4
Trypsin inhibitor (Tiu/mg)[b]	ND[c]	ND[c]	130	97	122	98

[a]NG, non germinated; G, germinated.
[b]Tiu, trypsin inhibitory units.
[c]ND, not detected.

of LMW sugars (6% compared to 9% in soybeans and lupins). Lupin seeds had about twice as much NSP as the other legumes, including higher levels of cellulose and NCP. This is consistent with the observation of higher insoluble dietary fiber in lupin (24% compared to 11 and 14% in soybeans and black beans, respectively) and also of soluble dietary fiber (14% compared to 8% in black beans and 6% in soybeans). It should be noted that, in general, soluble fiber represented over one third of total fiber in all legumes. This proportion of soluble/total dietary fiber is substantially higher than what has been found in cereals (Nyman *et al.*, 1984).

Combining the results on the carbohydrate composition of the three legumes studied, it is possible to conclude that black beans have the highest level of total digestible carbohydrates (starch plus fructose, glucose and sucrose, 44%), while lupins have the highest levels of total non-digestible carbohydrates (NSP plus LMW α-galactosides 43%), being a source rich in dietary fiber.

Table 2. Carbohydrate composition (dry matter basis) and dietary fiber of the germinated and non-germinated legume seeds (mean, *n* = 2)

Component (g %)	*L. albus* m.		Soybeans		Black beans	
	NG[a]	G[a]	NG	G	NG	G
Starch	0·5	1·7	0·2	0·1	40·6	40·5
NSP[b]						
Cellulose	10·5	12·4	4·4	5·4	3·8	3·3
NCP[c]	26·8	27·9	11·3	12·7	14·6	15·1
Total	37·3	40·3	15·7	18·1	18·4	18·4
LMW sugars						
Fructose	0·1	0·2	0·1	0·1	—	—
Glucose	0·1	0·1	0·1	—	0·1	0·1
Sucrose	3·4	6·6	4·4	0·9	2·8	3·1
Raffinose	0·9	0·1	0·9	0·3	0·5	0·3
Stachyose	4·7	0·2	3·4	1·2	2·6	0·6
Total	9·1	7·2	8·9	2·4	6·0	4·1
Dietary fiber						
Soluble	14·4	14·4	5·7	6·0	7·5	8·1
Insoluble	23·6	26·7	11·0	12·9	14·4	13·7
Total	38·0	41·1	16·6	19·0	21·9	21·8

[a]NG, non-germinated; G, germinated.
[b]NSP = non-starch polysaccharides.
[c]NCP = non-cellulosic polysaccharides.

Germination for 48 h had some differential effects on the carbohydrate composition of the seeds, with major changes occurring in the LMW sugars (Table 2). The content of LMW α-galactosides decreased with germination in all the legumes, in agreement with previous results (Deshpande *et al.*, 1984; Jood *et al.*, 1985; Trugo *et al.*, 1993*a*), with the smallest changes in black beans and the largest changes in lupins. This suggests that, during germination, α-galactosidase is more active in lupins than in other legumes, but in all legumes, hydrolysis of the α-galactosides may contribute to the energy needs of the developing seed in the initial stages of germination. The decrease in raffinose content was 40, 67 and 89% in black beans, soybeans and lupins, respectively. The decrease in stachyose content was of 65, 77 and 96% in soybeans, black beans and lupins, respectively. NSP showed only small proportional increases in content with 48-h germination in lupins and soybeans and no change in black beans, suggesting that they are not used to a significant extent as an energy reserve of the seeds in this period.

Changes, with germination, in available carbohydrate content were also different for each legume. Considering changes in sucrose levels, there was a substantial increase in lupins (107%), a small increase in black beans (11%) and a pronounced decrease in soybeans (80%). Starch content changed with germination only in lupins, remaining, however, at very low levels when compared to black beans. The differential effects of germination on carbohydrate composition suggest that carbohydrate metabolism is different during germination in the lupins, soybeans and black beans.

Effect of germination on protein and energy utilization in rats

Results of the rat bioassay are shown on Table 3. All indices measured, TD, BV, NPU and DE, were significantly and markedly higher in lupin seeds than in soybeans, indicating much better protein and energy utilization in lupins. Published data on the *in-vivo* nutrient utilization of lupins are limited to few studies. It has been shown in humans (Egaña *et al.*, 1992) that NPU of *L. albus* cv. *multolupa* protein is on average 77% that of egg protein. More recently, Eggum *et al.* (1993) studied, in rats, the variation between lupin cultivars in protein and energy utilization and the response to methionine supplementation. It was found that variations occurred mainly in BV (64·7–85·9%) and that methionine supplementation improved BV by 4·6–19·7%. The present results show that *L. albus* cv. *multolupa* compares favorably with soybeans in terms of protein utilization when fed uncooked and non-supplemented with limiting amino acids. Its lack of trypsin inhibitory activity may be a contributing factor to the higher TD. A more adequate essential amino acid pattern may contribute to the higher BV. Also, in spite of the higher level of TD in lupins, DE was also higher compared to soybeans. Therefore, lupins may be a potential alternative to soybean, for instance as animal feed.

Germination during 48 h had no effect on TD, little or no effect on BV and a small increase in NPU, in both legumes. This suggests that, although germination has a positive effect on protein utilization of the legumes seeds, the magnitude of the effect is most probably of little physiological significance. These results are in agreement with previous results observed with other legumes or in cereals. No effect on protein quality was observed by Nattress *et al.* (1987) after a 3-day germination of mung beans and garbanzo beans. A slight improvement of NPU with germination was observed in sorghum and millet, but no effect was evident after 7 days of germination in barley (Pedersen *et al.*, 1989).

Germination had no effect on DE in soybeans but produced a small decrease in DE of lupins. The explanation for this result is not evident, particularly in the case of lupin, since there were no major changes of macro-

Table 3. Effect of germination of legume seeds and antibiotic treatment on protein utilization and energy digestibility in rats[a]

Measurement	Lupin seeds		Soybeans	
	NG[b]	G[b]	NG	G
True digestibility (%)				
without antibiotic	92·8 ± 1·5	93·8 ± 1·0	81·0 ± 1·3	80·8 ± 1·5
with antibiotic	92·1 ± 1·1	90·6 ± 1·1[a,d]	83·4 ± 0·8[d]	84·7 ± 0·5[e]
Biological value (%)				
without antibiotic	69·0 ± 0·8	70·5 ± 1·2	59·4 ± 1·4	62·2 ± 1·0[a]
with antibiotic	70·2 ± 0·8	64·3 ± 0·8[c,f]	56·7 ± 0·8[e]	61·3 ± 0·7[b]
Net protein utilization (%)				
without antibiotic	64·0 ± 1·0	66·1 ± 1·2[a]	48·1 ± 0·5	50·2 ± 1·3[a]
with antibiotic	64·6 ± 1·3	58·3 ± 1·2[c,f]	47·3 ± 0·9	51·9 ± 0·7[b,d]
Energy digestibility (%)				
without antibiotic	89·2 ± 0·9	84·5 ± 0·5[c]	80·9 ± 0·8	80·6 ± 0·8
with antibiotic	70·1 ± 1·8[f]	69·8 ± 0·5[f]	71·9 ± 0·6[f]	66·3 ± 0·5[c,f]

[a]Significantly different from non-germinated: a — $P<0{\cdot}05$; b — $P<0{\cdot}01$; c — $P<0{\cdot}001$. Significantly different from those without antibiotic: d — $P<0{\cdot}05$; e — $P<0{\cdot}01$; f — $P<0{\cdot}001$.
[b]NG, non-germinated; G, germinated.

constituents with germination and there was a slight increase in insoluble dietary fiber (and NSP) in both legumes. Moreover, the decrease in LMW α-galactosides with germination was more pronounced for lupins.

Inhibition of the intestinal fermentation by Nebacetin markedly decreased DE in the two legumes, both germinated and non-germinated. This is an expected result, in agreement with previous studies (Eggum *et al.*, 1984, 1985), since fermentation of residual dietary components by the intestinal microflora may contribute with additional absorbable energy substrates to the host organism. Treatment with Nebacetin caused different responses in protein utilization in the two legumes. With lupin, the treatment produced significant decreases in TD, BV and NPU only in the germinated seeds. With soybeans, Nebacetin treatment produced significant increase in TD, both in germinated and non-germinated seeds, decrease in BV only in the non-germinated legume and increase in NPU only in the germinated legume. These variable responses to treatment with the antibiotic indicate the complexity of events occurring during fermentation in the rat hind gut.

In conclusion, 48-h germination of legume seeds affected the individual carbohydrate components differently, with more pronounced changes in LMW sugars, mainly α-galactosides and sucrose. These changes were quite specific for each legume species. Germination produced only a discrete increase in protein utilization in lupin and soybean; therefore, the main advantage for short-term germination of legume seeds is the substantial reduction in the flatus-producing oligosaccharides. Based on the present results, uncooked lupin may be a better source of protein and energy than uncooked soybeans when used in animal feeding.

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