The role of cell-wall polysaccharides and alpha-galactosides in the flatus induced by the comsumption of a legume seed (lupin) in the rat

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THE ROLE OF CELL WALL POLYSACCHARIDES AND α-GALACTOSIDES IN THE FLATUS INDUCED BY THE CONSUMPTION OF A LEGUME SEED (LUPIN) IN THE RAT

RÔLE DES POLYSACCHARIDES PARIÉTAUX ET DES α-GALACTOSIDES DANS LES FLATULENCES INDUITES PAR LA CONSOMMATION D'UNE GRAINE DE LÉGUMINEUSE (LUPIN) CHEZ LE RAT

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SUMMARY

Most of the legume seeds induce flatus which is usually attributed to the presence of α-galactosides. However these legumes contain high levels of fibres which can also be the substrates of caeco-colic fermentations.

In order to dissociate the fermentations due to the α-galactosides from those due to the fibres, a purified fibre fraction of a lupin oil meal was prepared. This fibre fraction was completly free of α-galactosides. Three semi-synthetic diets containing the lupin meal (with most of the α-galactosides of the seed), the fibres isolated from the meal or a mixture of both, were compared to a diet with no lupin fibre during an experiment with rats adapted to the experimental diets. The excretion of gases by the rats was determined using a respiration chamber.

Hydrogen excretion by the rats was much larger with diets containing meal or lupin fibres than with the one which contained only pure cellulose as a fibre source. None of these diets induced a large excretion of methane. There were no statistical differences in amounts of hydrogen excretion by the rats fed diets containing lupin meal, lupin fibres or a mixture of both ingredients.

Key-words: *flatus, α-galactosides, cell wall polysaccharides, lupin, rat.*

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RÉSUMÉ

La plupart des graines de légumineuses provoquent chez l'homme et l'animal des flatulences fréquemment reliées à la présence d'α-galactosides. Toutefois, ces graines contiennent des quantités non négligeables de fibres qui peuvent également être à l'origine de fermentations intestinales.

Afin de dissocier les productions fermentaires dues aux α-galactosides de celles dues aux fibres, un fractionnement a été réalisé à partir de tourteau de lupin. Deux fractions dépourvues d' α-galactosides ont été préparées : un isolat protéique et des fibres de lupin. La mesure d'excrétion de gaz par des rats a été effectuée, en chambre respiratoire, avec trois régimes semi-synthétiques contenant du tourteau de lupin (renfermant la presque totalité des α-galactosides de la graine), des fibres isolées du tourteau ou un mélange des deux. Un régime sans fibres de lupin, ne contenant que 1 % de cellulose Colmacel comme seule source de fibre, a en outre été testé afin de déterminer l'excrétion basale.

Les productions d'hydrogène sont beaucoup plus importantes avec les régimes contenant du tourteau ou des fibres de lupin qu'avec le régime témoin. Aucun de ces régimes ne conduit à une excrétion de méthane importante. Les régimes "tourteau de lupin" et "fibres de lupin" ou leur mélange conduisent à des excrétions d'hydrogène non statistiquement différentes.

Mots clés : *flatulence, α-galactosides, glucides pariétaux, lupin, rat.*

1 - INTRODUCTION

Legume seeds are widely used in human and animal nutrition. They are known to induce flatus which preclude their intensive use in pig feeding. Many workers have attempted to identify the factors in legume seeds which are responsible for these flatulences. By fractionation studies on various legume seeds, α-galactosides were shown to be partially responsible for the production of flatus, although other carbohydrates have also been implicated (FLEMING, 1981a). All the legume seeds contain large amounts of cell wall polysaccharides which are fermented by the large intestine and can produce gases. The possibility of removing part ot the α-galactosides or the fibres by technological treatments or plant breeding requires the knowledge of the respective responsibility of these two components of the seeds in the fermentations.

Hydrogen producing potencies of whole or fractionated legume seeds have been determined using several apparatus and methods for collecting and measuring total hydrogen production in rats (WAGNER *et al.*, 1976; FLEMING, 1980). They have been shown to be good predictive bioassays of flatulence activity for man (WAGNER *et al.*, 1977).

In order to dissociate the roles of the α-galactosides and the fibres, a defatted lupin meal has been fractionated into a fiber fraction and a protein isolate. These fractions were used as ingredients of a "α-galactoside-free lupin meal" containing most of the fibres of the lupin meal but no α-galactosides.

It was compared, after incorporation in a balanced diet, to a diet containing the defatted lupin meal, to a mixture (50/50) of these two diets containing the same amount of fibres that the meal and half of its α-galactosides and to a "fibre and α-galactosides-free diet" containing only 1% fibre (pure cellulose) and no α-galactosides. Rats were fed with these four diets; their hydrogen and methane excretion were measured using a respiration chamber.

2 - MATERIAL AND METHODS

Fractionation of the lupin meal. Two fractions (protein isolate and cell wall material) were prepared, using the wet process described by DAVIN and BRILLOUET (1986), from a lupin meal (dehulled and defatted lupin seeds).

Diets. The composition of the diets is described in table 1. Three diets were formulated to contain (per kg dry matter) around 110 g total dietary fibres and 190 g (N x 5.7) both from lupin seed origin. The control diet had the same amount of lupin proteins but contained only 1% pure cellulose as the fibre source; it was used as a "lupin fibre and α-galactosides free" diet.

Table 1
Composition of the diets (% dry matter)

Diet	Meal	Meal-fibres	Fibres	Control
Lupin meal	42.2	21.1	-	-
Lupin fibre	-	7.2	14.4	-
Lupin isolate	-	9.9	19.9	21.3
Corn starch	48.4	51.9	55.4	67.4
Corn oil	4.0	4.5	4.9	4.9
Cellulose Colmacel	-	-	-	1.0
Minerals (1)	4.5	4.5	4.5	4.5
Vitamins (1)	0.5	0.5	0.5	0.5
DL methionnine	0.4	0.4	0.4	0.4

(1) CHAMP et al. *(1989).*

Animals. Four rats, about ten weeks old at the beginning of the experiment, were fed the three experimental diets and the control diet during four consecutive 12-days periods.

Experiment. The rats were maintained in individual cages, at 22°C with light from 8:00 a.m. to 8:00 p.m. During the first five days, the rats were fed a commercial diet; then they were adapted to one of the experimental diet for a period of nine days. During this adaptation period, the animals were fed *ad libitum*, with a daily control of the intake. After the end of this adaptation period,

Table 2
Composition of the lupin materials ($g \cdot kg^{-1}$ dry matter) (1)

	Meal	Fibres	Isolate
Rhamnose (2)	3.1	7.1	0
Arabinose	32.2	91.7	0
Xylose	12.5	38.2	0
Mannose	6.8	0	7.3
Galactose	171.6	431.7	2.7
Glucose	32.5	66.3	0
Uronic acids	25	57	1
α -galactosides	88	0	0
Protein (nitrogen x 5.7)	438	83.6	917.5
Lipids	21	nd	26
Ash	47	nd	nd
Total	877.7	782.7	954.5

(1) Previously more fully described by CHAMP et al. *(1990).*
(2) The monosaccharides are reported as anhydro-sugars.
nd Not determined.

Table 3
Gases excretion in the respiration chamber (mean ± SEM) (1)

	Meal	Meal-fibres	Fibres	Control
Food ingestion g DM/day	22.6 (0.2) (2) a	21.2 (0.2) b	20.8 (0.8) b	21.6 (0.7) ab
Fibers ingestion g NS+UA (3) /day	2.70 (0.03) a	2.35 (0.02) b	2.11 (0.08) c	0.27 (0.01) d
α -gal ingestion g/day	0.84 (0.01) a	0.40 (0.00) b	0.00 (0.00) c	0.00 (0.00) c
Gases production cm^3/day				
H_2	32.2 (6.4) a	57.5 (9.8) a	34.6 (6.7) a	0.77 (0.18) b
CH_4	0.89 (0.55) (4) a	1.62 (0.82) (4) a	1.24 (0.07) (4) a	0.19 (0.05) (4) a
mmoles gases excreted/mole of ose ingested				
H_2	67 (14) a	153 (27) a	117 (20) a	21 (5) b
CH_4	0 (4) a	1 (4) a	2 (4) a	3 (4) a

(1) Standard error of the mean.
(2) Values on the same line sharing a common letter are not significantly different ($P < 0.05$).
(3) Neutral sugars + uronic acids.
(4) Means calculated from two observations (two rats).

gases (hydrogen and methane) excretion was measured for 72 hours in a respiration chamber built according to the model described by LECOZ *et al.* (1989) slightly modified [membran and valve pump CM 400 (Piot et Tirouflet, Mennecy, France), butyl tubes (Normagaz-NF), no bacterial filters]. The volume of the chamber was 33.6 liters and the gas flow was 105.4 l/hour. The rats received an amount of food corresponding to four days of mean daily food intake during the adaptation period. Gases samples were collected twice a day. After each period of measurement, the animals were immediately fed another experimental diet.

Analysis. The gases (hydrogen and methane) were analysed, after appropriate dilution, with a Microlyzer® Quintron gas chromatograph. Gases excretion were expressed 1) as the volume (cm^3) of hydrogen and methane excreted per day and 2) in moles of gases per mole of ose ingested. The lupin meal, isolated fractions and diets were analyzed by usual methods previously described by CHAMP *et al.* (1989).

Statistical procedure. All the results were expressed as the mean ± the standard error of the mean (SEM). Differences between groups were statistically tested with Student's t-test.

3 - RESULTS

The composition of the lupin fractions *(table 2)* has been previously described (CHAMP *et al.*, 1990). Total fibres content of the meal, the fibre and the protein isolate fractions were respectively 28.4, 69.2 and 1.1%. The α-galactosides concentration in the lupin meal represented 8.8% of the dry matter and nil in the fibre and protein isolate fractions. Protein (N x 5.7) level of the lupin meal was much higher (43.8%) than that of the fibre fraction (8.4%). Lupin isolate contained almost 92% proteins.

Hydrogen and methane excretion figures are shown in table 3. The intake of the meal diet appeared to be slightly but significantly higher than that of both meal + fibres and fibres diets. The plots for accumulation of hydrogen and methane versus time indicated a constant rate of production of both gases over the 72 hours period of measurement. The rate of hydrogen excretion was very slow when the animals were fed the control diet. It was significantly ($p < 0.05$) higher with the meal and fibre diets. A mixture 50/50 of those two diets almost doubled the rate of the hydrogen excretion; however the differences between the three diets containing lupin fibres were not statistically significant.

Methane which had been quantified in only half of the animals was always excreted in very low amount; it presented a slow excretion rate for the control diet and a more rapid rate of excretion with the "meal-fibres" diet.

4 - DISCUSSION AND CONCLUSION

Flatus production in presence of legume seeds or of some of their fractions have been studied on rats by using respiration chambers. In most of the studies, H_2 accumulation has been measured during less than 24 hours after one single meal distributed at time 0 of the experiment (WAGNER *et al.*, 1976 and 1977; FLEMING, 1981a, b; PHILLIPS *et al.*, 1988). It appeared that the plot for the accumulation of H_2 versus time comprised three distinct phases whereas "CH_4 plot" only presented one single constant slope which was found to be unrelated to the diet. In the present study, the period of measurement lasting 72 hours and the food being available *ad libitum*, there was a simple linear relationship between both H_2 and CH_4 excretions and time which made easier the expression of the results.

The very low hydrogen excretion observed in the "control" group of rats fed a semi-synthetic diet containing 1% pure cellulose as a source of fibre agrees with the data obtained by CHAMP *et al.* (1990) on caecal VFA concentrations in rats fed the same diets. The three groups fed lupin meal fibres (diets "fibres", "meal-fibres" or "meal") had large and non significantly different hydrogen excretions. From these results, it could be concluded that fibres are the main source of flatus from lupin seeds or meal. However, if the flatulence activities were very similar with "fibres" and "meal", a (non significantly) larger excretion of hydrogen was observed with the intermediate "meal-fibre" diet.

The role of α-galactosides and fibres in flatus has already been investigated, in rats and human studies, for several legume seeds, using seed fractionation (FLEMING, 1981a and b; WAGNER *et al.*, 1976 and 1977). From these studies, it seems that α-galactosides and mainly stachyose may explained half or more of the flatulence activity of the legume seeds. Stachyose being the main α-galactoside of the lupin meal, at least part of the flatus could have been expected to be due to the fermentation of the lupin α-galactosides. The similarities, in the present experiment, between production rates of H_2 for "meal" and "fibre" diets may be explained by an improvement of the fermentability of the cell wall polysaccharides after extraction. The higher gases production rate (though non significant) for "meal + fibres" diet could be explained by a synergic effect between the α-galactosides of the meal and the purified fibres. In fact, OLSON *et al.* (1975) suggested that an α-galactoside free bean residue (hexane and 70% ethanol extraction) may even have a synergetic effect with α-galactosides in the formation of gases. More recently, DUFOUR (1989) also showed that adding dextrans to a diet containing easily fermentable (beet pulp) fibres increased gases excretion by the rats. This author indicated that dextrans could promote fermentation of the beet pulp fibres as activators of the bacteria responsible of these degradations.

In conclusion, fractionation, which seemed to be the easiest way to identify the legume seed components responsible for flatulence, does not allow to determine which of the fibres or the α-galactosides is mainly responsible of the flatus induced by lupin seeds consumption. Extraction procedure (wet process, at least) appears to increase the rate of gases production from the fibre part of the lupin meal. The present experiment had to be conducted with a small number of

animals and did not show any significant differences between the diets. However, it seems to confirm various hypotheses found in the litterature, such as the higher fermentability of the purified fibres or the promoting effect of easily fermentable sugars on the degradation of some fibres.

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