JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY

Biological Activity of α-Galactoside Preparations from *Lupinus angustifolius* L. and *Pisum sativum* L. Seeds

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Biological activity tests were performed on α-galactoside preparations obtained from *Lupinus angustifolius* L. cv. Mirela (alkaloid-rich) and *Pisum sativum* L. cv. Opal seeds. The studies included the following tests: acute toxicity, cytotoxic test, delayed type hypersensitivity (DTH), plaque-forming cell number (IgM-PFC), and influence on the growth of bifidobacteia and coliform presence in rat colon. Results of these studies showed that α-galactosides from lupin and pea seeds were essentially nontoxic. Their acute toxicity (LD_{50}) in mice was >4000 mg kg^{-1} of body weight. α-Galactoside preparations were not cytotoxic for mouse thymocytes in vitro. The in vitro test shows that oligosaccharides from lupin and pea are utilized by selected beneficial colon bacterium strains. The in vivo experiment demonstrated that α-galactosides from legume significantly influenced the growth of bifidobacteria in rats colon. Simultaneously, the decrease of the coliform presence was observed. The chemical composition of the tested preparations had no significant effect on their biological activity.

KEYWORDS: α-Galactosides; lupin; pea; toxicity; immunotropic activity; bifidobacteria

INTRODUCTION

α-Galactosides, also called raffinose family oligosaccharides (RFOs), are widespread compounds in the plant kingdom. They are α-(1→6)-galactosides linked to carbon C-6 of the glucose moiety of sucrose. RFOs are low molecular weight, nonreducing sugars soluble in water (*1*, *2*). An especially large amount of these saccharides occurs in generative parts of plants belonging to the Leguminoseae family (*3*), where they perform protective physiological functions (*4*−*6*).

From the nutritional point of view, RFOs are considered as antinutritional factors, because they are not hydrolyzed by mucosal enzymes in the small intestine of monogastric animals**.** They are fermented in the lower gut with the liberation of gas (*7*−*9*). In recent years, these compounds have been an object of growing interest of nutritionists as prebiotics due to the fact that they modify the composition of the colon bacterial microflora (*10*−*14*). In this respect, fructooligosaccharides are the best known and most commonly applied oligosaccharides (*14*−*17*).

Extracts obtained during the debittering process of alkaloid-rich lupin seeds are a rich source of α-galactosides (*18*). They are byproducts, containing ∼40% RFOs. Also, the byproducts of protein isolate production from pea seeds are a rich source of galactooligosaccharides (*19*). Recently, a simple and fast method of isolation and purification of these oligosaccharides on a large scale was published by Gulewicz et al. (*20*). This method allowed high-purity RFO preparations, which differ from each other in their chemical composition, to be obtained from legume seeds.

The main objective of these studies was the investigation of the relationship between the chemical composition of RFO preparations and their toxicity, immunotropic activity, and the effect on the growth of selected beneficial strains of bacteria in the colon. The results of these studies are important when the utilization of galactooligosaccharides as health ingredients (prebiotics) in the human diet is considered.

MATERIALS AND METHODS

Samples and Chemicals. Seeds of *Lupinus angustifolius* L. var. Mirela were supplied by Dr. S. Stawiński from the Plant Breeding and Acclimatization Station at Przebędowo near Poznań, Poland. Seeds of pea *Pisum sativum* L. cv. Opal were purchased from a local market (Poland). Diatomaceous earth, charcoal, and naphthoresorcinol were purchased from Sigma. Dowex 50WX8, 100−200 mesh was purchased

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10.1021/jf010973y CCC: $22.00
Published on Web 12/19/2001

from Serva. Ethanol, 2-propanol, acetic acid, glucose, potassium phosphatate, dibasic ammonium citrate, magnesium sulfate × $7H_2O$, manganese sulfate × $4H_2O$, and sodium acetate × $3H_2O$ were supplied by POCh-Gliwice. Acetonitrile (HPLC grade) was purchased from Acros Organic Belgium. Silica gel 60 F_{254} TLC plates, raffinose, and stachyose were from Merck, Darmstadt, Germany. Millipore FH (0.45 μm) membranes were supplied by Millipore, Bedford, MA. Beef extract and Pepton Tryptone were supplied by Difco (Detroit, MI). Yeast extract, medium with bile, and brilliant green were purchased from BTL.

Bifidobacterium bifidum NCFB No. 1453 and *Bifidobacterium longum* NCFB No. 2259 were from the National Collection of Food Bacteria. Preparations "Lakcid" (Bio Med, Lublin, Poland) containing *Lactobacillus acidophilus* and "Trilac" (Pharmacia, Uppsala, Sweden) containing *B. bifidum*, *L. acidophilus*, and *Lactobacillus delbrueckii* ssp. *bulgaricus* were commercial products.

Mice of the 129/Ao Boy Jjw strain were obtained from the Breeding Centre of the Institute of Immunology and Experimental Therapy, Polish Academy of Sciences, Wrocław, Poland. Rats of the Wistar strain were purchased from the Breeding Station in Brwinów near Warsaw, Poland.

Isolation and Purification of RFOs. α-Galactosides were isolated from *L. angustifolius* L. cv. Mirela and *P. sativum* L. cv. Opal seeds according to the method described by Gulewicz et al. (*20*).

HPLC Analysis of RFOs. The analysis of separation and quantification of RFOs from legume extracts was carried out by high-performance liquid chromatography using a refraction index detector (HPLC-RI) (*21*). The analysis was performed on an HPLC chromatograph (Waters Associates, Milford, MA) equipped with a Waters 510 pump, a Rheodyne model 7000 sample injector, and a reflection type differential refractometer detector model R410 (Waters Associates). The chromatographic system was controlled by a PC with Maxima HPLC system controller software (Waters Associates). A precolumn (3.2 mm i.d. × 4.0 cm) packed with C18 Porasil B and a μ-Bondapak/carbohydrate column (3.9 mm i.d. × 30 cm) (Waters Associates) were employed. The mobile phase was acetonitrile/distilled water (75:25 v/v) (HPLC grade), and the flow rate was 2.0 mL/min. Solvents were filtered through a Millipore FH (0.45 μm) membrane and degassed under helium. Injection volume was 100 μL.

Standard Solution Preparation. Different amounts of raffinose and stachyose were dissolved in distilled water. Acetonitrile was added to each solution to obtain a composition similar to that of the mobile phase (75:25 v/v).

Legume Extract Preparation and Quantification. Different amounts of pea and lupin extracts were dissolved in distilled water. Acetonitrile was added to each solution to obtain a composition similar to that of the mobile phase (volume ratio of 75:25 v/v). Both the legume extracts and standard solutions were filtered through a Millipore FH (0.45 μm) membrane before injection. Quantification of each sugar was accomplished by comparing the peak areas of the samples with those of the standard solutions. A commercial verbascose standard was not available; therefore, verbascose (pentasaccharide) was identified on the basis of standards obtained in our laboratories according to the previous work (*21*, *22*) and by its retention time and was quantified using raffinose (trisaccharide) and stachyose (tetrasaccharide) as standards. A standard curve was plotted for each sugar and adjusted using a method of least squares. The regression coefficients of the curves for raffinose and stachyose were always >0.990.

Acute Toxicity. The experiments were carried out on inbred male and females 129/Ao Boy Jiw mice, weighing 20−22 g. The oligosaccharide preparations were administered through a stomach tube. After observation over a period of 7 days, the LD_{50} was calculated using the method of probit analysis according to ref *23*.

Cytotoxic Test. Thymocytes obtained from inbred C57B1 mice were used. Thymus cells (4×10^6 mL^{-1}) suspended in RPMI medium with 10% fetal calf serum were incubated for 20 h at 37 °C in 5% CO_2 at 95% humidity with 10, 50, and 100 μg mL^{-1} of RFO preparations. Cell viability was checked using trypan blue (*24*).

Plaque-Forming Cell Number (IgM-PFC). The primary immune response to sheep red blood cells (SRBC) in vivo was estimated according to the method of Mishell and Dutton (*25*). Mice of the 129/Ao Boy Jiw strain were administered lupin and pea oligosaccharides in the doses of 100, 200, 400, and 4000 mg kg^{-1} per os for five subsequent days beginning on the day preceding the antigen (0.2 mL of 10% SRBC) injection. PFC number in the spleens was determined 4 days after immunization.

Delayed Type Hypersensitivity (DTH). Experiments were performed on mice according to the method of Papadinitriou et al. (*26*). A sensitizing dose—10^6 SRBC, was injected intravenously, and 4 days later a challenging dose (10^8 SRBC) was injected subcutaneously into the foot pad. The intensity of the foot pad swelling was determined on the day before and 24 and 48 h after the administration of the challenging dose. The results were expressed in units of foot pad swelling in the treated mice and nontreated animals. The preparations of RFOs were administered five times, starting on the day preceding the administration of the sensitizing dose of SRBC.

Influence of RFO Preparations on the Growth of Bifidobacteria in in Vitro Tests. The influence of RFO preparations was estimated on the standard medium according to De Man et al. (MRS) (*27*), in which glucose was substituted by RFO preparations. Cultures in a medium with glucose and without any sugar were the control groups.

Influence of RFO Preparations on the Growth of Bifidobacteria and Coliform Bacteria in in Vivo Tests. A solution of RFO preparations in water was administered with a stomach tube to 1-week-old female Wistar rats (50−70 g body weight). The daily doses were 15 mg per 100 g of body weight. The control group was given only water with the stomach tube in the same volume. All groups had unlimited access to feed. The rats' fecal matter was collected for analysis on the 1st, 10th, and 20th days of the experiment into a test tube with 10 mL of sterile 0.85% sodium chloride solution and was stored in CO_2 atmosphere. Decimal dilutions up to 10^{-6} were made from the suspension. From each dilution, 1 mL was applied on a Petri dish covered with MRS medium and to test tubes with a coliform bacterial medium.

Quantitative Estimation of Bifidobacteria. The number of bifidobacteria was estimated on the MRS medium (*27*) with the addition of an inhibitory agent, lithium chloride (*28*). Incubation was carried out in a CO_2-rich atmosphere for 72 h at 37 °C.

Coli Bacteria Tests. To estimate the coliform bacteria presence, a selective medium with bile and green brilliant (BTL) was used. For determination of the total coliform presence in 1 g of test sample, incubation was carried out for 48 h, at a temperature of 37 °C, and for the fecal coliform test samples were incubated for 48 h, at 44 °C (*29*).

Evaluation of Bacterial Growth by Measurement of Gas Production and pH. Utilization of glucose and galactooligosaccharides by the tested bacteria in MRS medium was determined by production of gas in Durham tubes and measurement of pH medium according to the method described by Burbianka et al. (*27*).

Statistical Analysis. The obtained results were elaborated statistically with the use of the Student *t* test at the significance levels of 0.05 and 0.01.

Table 1. Average Content (Percent) of the Particular α-Galactosides and Sucrose in RFO Preparations Obtained from Lupin and Pea Seeds

sugar	lupin RFO preparation	pea RFO preparation
sucrose	9.10	20.30
raffinose	30.20	4.00
stachyose	46.97	51.50
verbascose	10.76	19.50
total	97.03	95.30

RESULTS AND DISCUSSION

The chemical compositions of lupin and pea RFO preparations are presented in Table 1. Both preparations differed in their chemical composition. In the lupin preparations, the main oligosaccharides were stachyose (46.97%) and raffinose (30.20%). In the pea preparations, the main oligosaccharide was stachyose (51.50%), but the content of raffinose was on a low level (4.00%). The verbascose was on the level of 10.76 and 19.50%

Table 2. Effect of Different RFO Preparations on the Viability of Mouse Thymocytes

preparation	concn of preparation (μg mL^{-1})	% of living cells	% of control
control		87	100.0
lupin	10	84	98.8
	50	85	97.0
	100	83	95.4
pea	10	87	100
	50	86	98.8
	100	86	98.8

Table 3. Influence of Lupin and Pea RFO Preparations on the DTH Reaction

		foot pad thickens after					
		24 h			48 h		
preparation	dose (mg/kg)	*a*	±SE	*p* Student test	*a*	±SE	*p* Student test
lupin	400	21.2	2.2	NS[b]	14.8	1.3	NS
	200	18.2	2.2	NS	10.0	0.7	NS
	100	19.4	0.8	NS	11.2	0.6	NS
	50	19.2	0.7	NS	12.6	1.2	NS
pea	400	17.6	0.9	NS	13.2	0.6	NS
	200	15.2	0.5	NS	11.2	0.7	NS
	100	17.2	1.2	NS	12.2	0.6	NS
	50	17.8	0.9	NS	13.0	0.8	NS
control		17.6	1.6		11.6		

[a] Results are expressed as mean value in units ± SE of five mice. [b] Nonsignificant.

Table 4. Influence of Legume RFO Preparations on the Number of PFC in the Spleen

preparation	dose (mg/kg)	no. of PFC/10^6 spleen cells[a]	±SE	*p* Student test
lupin	400	1711	59.7	<0.01
	200	1618	70.9	<0.01
	100	1527	101.5	<0.01
control		1956	64.6	
pea	400	1656.0	156.6	NS[b]
	200	2216	67.8	<0.01
	100	1613	127.2	NS
control		1761	151.5	

[a] Results are expressed as a mean ± SE of five mice. [b] Nonsignificant.

for lupin and pea preparations, respectively. The purity of α-galactoside preparations for lupin and pea was 97.75 and 95.30%, respectively.

Acute toxicity expressed as LD_{50} in the mice was >4000 mg kg^{-1} of body weight for both RFO preparations, which means that they are essentially nontoxic.

Also, the RFO preparations were not cytotoxic when tested in vitro on mouse thymocytes. The viability of the cells was not affected by concentrations ranging from 10 to 100 μg/mL (Table 2).

The lupin and pea RFO preparations did not influence the cellular response to SRBC (Table 3). Changes in foot pad thickness observed in the animals receiving different doses of RFO preparations were not statistically significant.

Th effect of lupin and pea preparations on the number of IgM-PFC is presented in Table 4. In the case of lupin RFO preparations, all doses resulted in a decreased number of PFC cells in spleen cells (78.0%) in comparison with the control value. In the case of the pea RFO preparations, only doses of 200 mg/kg caused a statistically significant increase in the number of PFC cells in the spleen.

The effect of RFO preparations on the number of bacterial cells grown on the MRS medium is shown in Table 5. Both lupin and pea preparations stimulated growth of all tested bacterium strains in comparison with the control group (MRS medium without addition of any sugars). Stimulation caused by the pea preparation was clearly higher than that caused by lupin RFOs. Only in the case Trilac were no major differences between the effects of the two preparations observed.

Table 6 shows the effect of RFO preparation on the dry matter of cells in MRS media. Both RFO preparations caused an increase of dry matter of the tested bacterium strains in comparison with the control without sugars, although these increases were not the same for lupin and pea oligosaccharides. The biggest increase of dry matter was observed for *B. longum* and the Trilac preparation (1.76 and 1.77 mg mL^{-1}, respectively) in the case of pea preparation. In the case of lupin preparation, the biggest increase of dry matter (1.49 mg mL^{-1}) was noted for *B. bifidum*.

Utilization of both RFO preparations by the tested bacteria caused the decrease of pH culture. In the case of the pea preparation, the mean of pH after culture of all tested bacteria was on the level 4.6; in the case of lupin, it was ∼4.9 (Table 7).

The RFO preparations used did not result in gas production by the tested bifidobacteria. A very small amount of gases was observed only in the case of *L. acidophilus* and the Trilac preparation.

The effect of lupin and pea RFO preparations on the number of bifidobacteria and coliform presence in 1 g of sample in the in vivo tests is shown in Table 8. In the groups of rats receiving RFO preparations, an increased amount of bifidobacteria was observed. Simultaneously, a decrease of fecal and total coliform presence in comparison with the control group was noted. The amount of bifidobacteria in the sample depended on the ingestion time of oligosaccharides by rats, and it was highest after the 20th day (3.5×10^5 and 3.2×10^5 for lupin and pea preparations, respectively). A significant difference in the number of bifidobacteria in the lupin and pea groups was observed only on the 10th day of ingestion (2.0×10^5 and 1.1×10^5 for lupin and pea preparations, respectively). As can be seen, this difference disappeared on the 20th day of α-galactoside ingestion. In contrast, the total and fecal coliform presence levels were not dependent on the time of ingestion and were established after the 10th day at the level of 10^3.

As shown in the literature data, legume plants, and in particular their generative parts, that is, seeds, are a rich source of α-galactosides (*3*, *18*). The oligosaccharides of different legumes differ in their qualitative and quantitative composition. Also, a different chemical composition of RFO preparations is obtained during the isolation and purification process of different legume seeds. This is clearly evident in the example of lupin and pea RFO preparations (Table 1). Whether these differences influence their biological activity or not is a question for consideration. The toxicity and immunotropic activity of α-galactosides preparations are very important from the nutritional point of view, as toxic preparations may not be used in nutrition. Therefore, our findings in that regard are fundamental. The determination of the effect of both preparations on the growth of selected strains of beneficial colon bacteria also has a great value.

Many tests were performed to determine the toxicity and immunotropic activity. These tests showed that lupin and pea

Table 5. Influence of Lupin and Pea RFO Preparations on the Number of Bacteria

medium	*B. bifidum*		*B. longum*		*L. acidophilus*		Trilac	
	a	±SE	*a*	±SE	*a*	±SE	*a*	±SE
MRS	5.2×10^7 a	2.2×10^6	8.0×10^8 a	4.2×10^7	4.56×10^7 a	2.7×10^6	1.2×10^8 a	5.2×10^6
MRS with glucose	3.44×10^9 b	5.1×10^7	1.60×10^9 b	1.6×10^7	1.89×10^9 b	2.8×10^7	8.33×10^8 b	1.9×10^7
MRS with lupin RFO preparation	6.00×10^8 c	5.4×10^6	8.57×10^8 c	1.7×10^7	9.71×10^8 c	2.1×10^7	2.86×10^8 c	1.2×10^7 c
MRS with pea RFO preparation	1.8×10^9 d	1.2×10^8	1.31×10^9 d	3.28×10^7	1.31×10^9 d	2.75×10^8	3.13×10^8 d	5.3×10^7 d

[a] Results are expressed as mean value ± SE of five repetitions. Letters a–d following entries indicate statistical significance at $p < 0.05$ and $p < 0.01$.

Table 6. Effect of Different Legume RFO Preparations on Dry Matter of Bacteria

medium	*B. bifidum* (mg mL^{-1})		*B. longum* (mg mL^{-1})		*L. acidophilus* (mg mL^{-1})		Trilac (mg mL^{-1})	
	a	±SE	*a*	±SE	*a*	±SE	*a*	±SE
MRS	0.49 a	0.012	0.71 a	0.012	0.32 a	0.056	0.30 a	0.015
MRS with glucose	2.14 b	0.042	2.42 b	0.011	2.40 b	0.021	2.15 b	0.019
MRS with lupin RFO preparation	1.49 c	0.010	0.89 c	0.014	0.51 c	0.022	0.44 c	0.016
MRS with pea RFO preparation	1.52 d	0.009	1.76 d	0.013	1.68 d	0.035	1.77 d	0.010

[a] Results are expressed as mean value ± SE of five repetitions. Letters a–d following entries indicate statistical significance at $p < 0.05$ and $p < 0.01$.

Table 7. pH Value of Supernatant after 48 h of Proliferation in MRS Medium with Addition of Different α-Galactoside Preparations

medium	*B. bifidum*		*B. longum*		*L. acidophilus*		Trilac	
	a	±SE	*a*	±SE	*a*	±SE	*a*	±SE
MRS	5.41a	0.034	5.32 a	0.011	6.28 a	0.046	6.41 a	0.032
MRS with glucose	3.66 b	0.031	3.9 b	0.016	4.15 b	0.023	4.07 b	0.028
MRS with lupin RFO preparation	4.93 c	0.023	4.95 c	0.058	5.51 c	0.021	5.14 c	0.025
MRS with pea RFO preparation	4.61 d	0.026	4.68 d	0.036	4.62 d	0.015	4.62 d	0.022

[a] Results are expressed as mean value ± SE of five repetitions. Letters a–d indicate statistical significance at $p < 0.05$ and $p < 0.01$.

Table 8. Influence of Legume RFO Preparations on the Number of Bifidobacteria and Coliform Presence in the in Vivo Test[a]

day of sampling	presence of coliform bacteria in 1 g of sample						total number of bifidobacteria in 1 g of sample		
	total			fecal matter					
	c	l	p	c	l	p	c	l	p
0	$>10^7$	$>10^7$	$>10^7$	$>10^7$	$>10^7$	$>10^7$	$3.3 \times 10^3 \pm$ SE 61.82	$3.3 \times 10^3 \pm$ SE 77.74	$3.3 \times 10^3 \pm$ SE 36.51
10	10^4	10^3	10^3	10^3	10^3	10^3	$6.8 \times 10^3 \pm$ SE 138.88	$2.0 \times 10^5 \pm$ SE 3786.52	$1.1 \times 10^5 \pm$ SE 1699.67
20	10^4	10^3	10^3	10^3	10^3	10^3	$7.7 \times 10^3 \pm$ SE 168.12	$3.5 \times 10^5 \pm$ SE 2017.699	$3.2 \times 10^5 \pm$ SE 1943.65

[a] c, control; l, lupin RFO preparation; p, pea RFO preparation.

RFO preparations were essentially nontoxic. The RFO preparations from lupin and pea seeds administered to mice in doses of up to 4000 mg kg^{-1} did not result in any deaths; therefore, doses >4000 mg kg^{-1} are accepted as the LD_{50} value. These results are in line with the findings for soybean galactooligosaccharides (*10*). The acute toxicity for these oligosaccharides tested on rats is LD_{50} > 15000 mg kg^{-1}. Nonacute, chronic toxicity tests on rats showed that continuous daily oligosaccharide doses of 4.000 mg/kg for 35 and 180 days were not toxic.

None of the RFO preparations were cytotoxic for mouse thymocytes cultured in vitro. Neither preparation showed immunotropic activity. These data in the case of lupin RFO preparations indirectly correspond with our earlier studies on the toxicity and immunotropic activity of extracts and their fractions from *L. angustifolius* and *L. albus* seeds (*30*, *31*). Studies carried out by Bednarczyk et al. and Uziebło et al. (*32*, *33*) also showed that the crude sugar fraction of *L. angustifolius* extract did not have any negative influence for hatchability of chicken and mortality of broilers.

The beneficial influence of oligosaccharides, mainly fructooligosaccharides, on monogastric organisms has been investigated by many scientists (*10*, *14*). In this regard, relatively little is known about legume oligosaccharides, including those from pea and lupin. Up to now, only soybean oligosaccharides have been an object of interest of a few papers (*34*−*36*). A GRAS (generally regarded as safe) affirmation petition for soybean oligosaccharides was accepted for filing in 1990 by the U.S. Food and Drug Administration.

In contrast to soybean, RFO preparations from other legume species (for example, lupin, pea, and lentil) contain verbascose and, in the case of lentil, additionally ciceritol—galactosylcyclitol (*3*, *20*, *37*). So far, the galactooligosaccharides from legumes have been an object of particular interest mainly as antinutritional factors in the diet. The attitude toward α-galactosides has been changing over recent years together with the search for new functional food ingredients. The beneficial effect of oligosaccharides in the diet depends on the increase of the bifidobacterium population in the colon, which in turn contributes to human health in many ways (*14*). In this respect, an important step of our studies was to check how these preparations influence the colon bacterium profiles. We showed that lupin and pea galactooligosaccharides were a very good source of carbon for bifidobacteria that colonize the large intestine. It is especially visible in the in vivo tests where RFO preparations

were given to rats (Table 8). On the 20th day of sampling in the groups fed with RFO preparations we observed 3.5×10^5 and 3.2×10^5 bifidobacteria in 1 g of sample (for lupin and pea, respectively), which was higher than the result obtained for the control group, 7.7×10^3. It is worth noting that in the case of in vitro tests we observed a different dependence: the increase of the number and biomass of bifidobacteria in the case of pea preparations was much higher in comparison with the lupin one (Tables 5 and 6). That also applies to the decrease of the pH values (Table 7). This discrepancy between the results from the in vivo and in vitro tests is caused by different sucrose contents in the RFO preparations (9.10 and 20.30% for lupin and pea, respectively). Sucrose and glucose in the diet are absorbed in the small intestine of monogastric organisms and are not substrate for colon bacteria. This fully corresponds with the results obtained in the in vivo test (Table 8). The lupin RFO preparation with the sucrose content lower than in the pea preparation better influenced the growth of colon bacteria in the in vivo tests. In this respect, the difference in the verbascose content in both RFO preparations (10.76 and 19.50% for lupin and pea, respectively) seems to be less essential. It is clearly shown that differences between in vitro and in vivo data concerning growth of bifidobacteria were mainly a result of the content of galactooligosaccharides in RFO preparations. According to the literature data bifidobacteria prevent the growth of exogenous pathogenic microbes and the excessive growth of indigenous detrimental microflora through production of short-chain fatty acids, mainly acetic and lactic ones at a 3:2 mole ratio (*10*, *38*). Therefore, production of acids by bifidobacteria results in a decrease of pH of the medium. Our studies not only show that lupin and pea RFO preparations have influenced the increase in the number and biomass of bifidobacteria (Tables 5 and 6) but also indicate that this fact has been directly correlated with the decrease in pH value (Table 7). There are numerous papers showing that bifidobacteria, contrary to others, have not metabolized oligosaccharides to gaseous products (*38*−*40*). The above is confirmed by our studies. This seems to be important from the nutritional point of view because the presence of RFO preparations in the diet has not caused arduous flatulence**.** In our opinion, both of these RFO preparations should be tested on humans as the next step of the study.

Moreover, the continuation of these studies is fully justified from the point of view of the easy availability of the raw material of α-galactosides. Many industrial byproducts are very rich raw materials of raffinose family oligosaccharides (e.g., residue of the debittering process of lupin seeds and byproduct of production of pea seed protein isolates).

ACKNOWLEDGMENT

We thank Dr. Andrzej Kliber from the Department of Physiology and Biochemisty of Animals of the Agricultural University in Poznań for his help in the experiments with rats.

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Received for review July 24, 2001. Revised manuscript received October 25, 2001. Accepted October 25, 2001. This work is the result of a bilateral scientific cooperation joint project between Poland (PAS) and Spain (CSIC). These studies were supported by the State Committee for Scientific Research, Poland, and AGL2001-2301, Spain.

JF010973Y