Applied and Environmental Microbiology, Aug. 2008, p. 5244–5249 Vol. 74, No. 16
0099-2240/08/$08.00+0 doi:10.1128/AEM.00514-08
Copyright © 2008, American Society for Microbiology. All Rights Reserved.

Probiotic *Lactobacillus plantarum* 299v Does Not Counteract Unfavorable Phytohemagglutinin-Induced Changes in the Rat Intestinal Microbiota[▽]

Gabriele Gross,[1,2]† Jessica Wildner,[1]‡ Arjan Schonewille,[1] Jan L. W. Rademaker,[1] Roelof van der Meer,[1] and Johannes Snel[1]*

Department Health and Safety, NIZO food research, P.O. Box 20, 6710 BA Ede, The Netherlands,[1] and Animal Breeding and Genomics Centre, Animal Sciences Group of Wageningen UR, P.O. Box 65, 8200 AB Lelystad, The Netherlands[2]

Received 3 March 2008/Accepted 24 June 2008

Application of phytohemagglutinin (PHA) in weaning feed has been suggested to stimulate intestinal epithelium maturation. In this study, PHA strongly affected the fecal bacterial population structure of rats. *Escherichia coli* overgrowth was not prevented by probiotic mannose-adhering *Lactobacillus plantarum* 299v. Therefore, use of PHA in weaning feed deserves careful evaluation.

Plant lectins such as kidney bean-derived phytohemagglutinin (PHA) have gained renewed interest for potential use in animal feed and infant nutrition since a positive impact on weaning adaptation and gut health has been suggested based on stimulation of the functional maturation of the gastrointestinal tract of suckling rats and piglets (24, 25, 34, 39). In contrast, several detrimental effects of PHA on gut microbiota and physiology such as reduced growth rates, loss of body weight, diarrhea, and epithelial hyperplasia have been demonstrated in several species (4, 5, 12, 33).

Previously, PHA has been reported to dose dependently induce bacterial overgrowth in the intestine, which is associated with weight loss, malabsorption, and villus damage (2, 3). It has been suggested that PHA increases the turnover of epithelial cells, promoting the expression of mannosylated receptor glycans on the gut surface and thus leading to increased adhesion and subsequent proliferation of mannose-sensitive type 1-fimbriated *Escherichia coli* (31, 32). One would assume that based on this mechanism multiple bacterial strains besides *E. coli* that adhere to these additional receptor sites are stimulated by PHA. Therefore, the first aim of the present study was to investigate the influence of PHA on overall composition and dynamics of the intestinal and fecal microbiota of young adult rats. To this end, fragment length polymorphism analysis of the amplified 16S ribosomal terminal restriction fragment (T-RFLP) was performed and the 16S-23S ribosomal intergenic spacer (IGS) was evaluated, in addition to bacterial plate counts and real-time PCR quantification of exemplary bacteria.

In addition to *E. coli*, mannose-specific adhesion has been demonstrated for *Lactobacillus plantarum*, e.g., the probiotic strain 299v (1, 29). Addition of mannose-adhering probiotics may prevent disturbances of the intestinal microbiota like overgrowth of *E. coli* and could therefore contribute to an improved introduction of PHA in weaning feeds. Therefore, the second objective of the present study was to assess whether an orally administered mannose-adhering *L. plantarum* strain could prevent *E. coli* stimulation in the PHA-affected rat intestine by mannose-specific competitive exclusion and thereby counteract the adverse effects of PHA.

Crude red kidney bean extract containing PHA was purchased from the Laboratory of Biochemistry and Glycobiology at the University of Ghent, Belgium. It was isolated from *Phaseolus vulgaris* basically according to the method of Pusztai et al. (32).

Specific-pathogen-free male Wistar rats (WU, Harlan, Horst, The Netherlands), eight per group, 8 weeks old, were housed individually in metabolic cages as described earlier (38). All animals were supplied ad libitum with demineralized drinking water and a purified standard diet basically according to the work of Reeves et al. (36) with a high fat (200 g/kg of body weight) and calcium (17.2 g/kg $CaHPO_4 \cdot 2H_2O$) content. Food intake and body weight were recorded daily. The experimental protocol was approved by the animal welfare committee of Wageningen University, The Netherlands.

Animals were acclimated to housing and dietary conditions for 8 days. Starting from day 9, animals were daily additionally fed with 3 g vanilla custard mix containing two-thirds-weight vanilla custard (vanilla vla; De Zaanse Hoeve, Albert Heijn, Zaandam, The Netherlands) and either one-third-weight DeMan-Rogosa-Sharpe broth (MRSB) (Merck, Amsterdam, The Netherlands) or one-third-weight MRSB containing approximately 10^{10} CFU/ml *L. plantarum* 299v (NIZO B2260; bacterial counts were confirmed by plating), grown at 37°C overnight in MRSB under anaerobic conditions.

On days 11 and 12, 0.5 ml physiological salt solution with red kidney bean extract containing 33 mg PHA was orally administered to all animals by gastric gavage twice daily. A smaller group of animals ($n = 3$) that received 0.5 ml physiological salt solution without PHA and custard mix without *L. plantarum* 299v supplementation was included for reference values. On day 13, animals were sacrificed by carbon dioxide inhalation, and samples from jejunum and ileum were excised for micro-

* Corresponding author. Mailing address: NIZO food research, P.O. Box 20, 6710 BA Ede, The Netherlands. Phone: 31-318-659549. Fax: 31-318-650400. E-mail: Hans.Snel@nizo.nl.
† Present address: LabMET, Ghent University, Ghent, Belgium.
‡ Present address: ttz, Bremerhaven, Germany.
▽ Published ahead of print on 7 July 2008.

biological analyses of lactobacillus and *E. coli* counts. Serial dilutions of fresh, homogenized fecal or intestinal samples in physiological salt solution were plated on Rogosa (Oxoid, Basingstoke, Hampshire, United Kingdom) and eosin methylene blue-Levine (Merck) agar plates. CFU counts were determined after incubation at 37°C for 24 (eosin methylene blue) or 48 (Rogosa; under anaerobic conditions) hours, respectively.

Fecal samples were collected quantitatively 2 days before and during PHA administration, pooled per 2 days, freeze-dried, and ground. DNA extraction from these samples was performed using the QIAamp DNA stool minikit (Westburg, Leusden, The Netherlands) preceded by bead beating (40). DNA was subsequently subjected to DNA fingerprinting methods and quantitative real-time PCR. T-RFLP analysis of the 16S ribosomal gene was carried out essentially as described earlier (35). Briefly, *Taq* PCRs were performed with DNA extracts from rat feces and bacterial reference strains as a template and the labeled eubacterial primers 8-27F and SD-BACT-0926. After gel electrophoresis, the amplified fluorescently labeled fragments were purified, digested overnight with HaeIII and HhaI, purified again, and mixed with Hi-Di-formamide (Applied Biosystems) and rhodamine X-labeled DNA Map Marker 1000 (Bioventures, Murfreesboro, TN). Subsequently, DNA was denatured, cooled to 4°C, and subjected to electrophoresis using an ABI Prism 310 Genetic Analyzer equipped with a capillary filled with Performance Optimized Polymer 4 matrix (Applied Biosystems). Peak profiles were collected using GeneScan 6.7 software (Applied Biosystems) and analyzed using BioNumerics 4.00 (Applied Maths, Sint-Martens-Latem, Belgium). Forward and reverse terminal fragment lengths of the pure reference strains were predicted using the web-based TAP T-RFLP tool provided by the Ribosomal Database Project II, release 8.1 (26). Additionally, the copy number of rRNA operons of 16S rRNA was ascertained with the online database described by Klappenbach et al. (23).

Fluorescent 16S-23S ribosomal IGS analysis of DNA samples was performed using universal eubacterial primers FgPL132 and FgPS1490 (28), with FgPL132 5′ end labeled with VIC (Applied Biosystems) in the initial PCR. Purification of PCR products, preparation of samples, and further analysis were mainly performed as described above for T-RFLP.

Interpretation of the population fingerprint profiles was facilitated by comparison with peak profiles of bacterial reference strains selected according to the main interest of this study. *L. plantarum* 299v (NIZO B2260), *Lactobacillus acetotolerans* (NIZO B1529), *Lactobacillus casei* subsp. *paracasei* (NIZO B1125), *Lactobacillus reuteri* (NIZO B1534), *Lactobacillus acidophilus* (NIZO B1532), and *Lactobacillus animalis* (NIZO B1530), all isolated from human intestine or rat feces, were obtained from the NIZO culture collection. Four bacterial isolates (lactic acid bacterium [NIZO B2380], *E. coli* [NIZO B2384], and *Enterobacteriaceae* strains [NIZO B2383 and NIZO B2386]) with distinct morphologies were recovered from plating of rat fecal samples from this study as described above and stored as frozen glycerol stocks. Lactic acid bacteria were cultured in MRSB or skim milk (BD Difco, Alphen aan den Rijn, The Netherlands), and *Enterobacteriaceae* strains were grown in brain heart infusion broth (Merck), all at 37°C. DNA of the bacterial reference strains was extracted using InstaGene Matrix (Bio-Rad Laboratories, Hercules, CA). The predicted and observed terminal restriction fragment (TRF) sizes of the reference strains confirmed the identity of these strains, although fragment lengths differed for up to 5 bp in most cases (data not shown). However, comparable variations have also been reported in other studies (7).

Numbers of *E. coli* and *L. plantarum* bacteria in freeze-dried fecal samples were also quantified using real-time PCR with specific 16S rRNA-targeted primers, mainly as described by Huijsdens et al. (16) and Bron et al. (6), respectively, using diluted DNA extracts from rat fecal samples. Standard curves were prepared from DNA isolated from liquid cultures of *E. coli* strain ATCC JM109 and *L. plantarum* 299v, respectively.

To illustrate the degree of diarrhea by determination of percent dry weight, lyophilized fecal material was reconstituted with distilled water to 20% dry weight. After homogenization, samples were incubated for 1 h at 37°C, with mixing every 15 min, followed by centrifugation at 13,500 $\times$ g at 37°C for 20 min. The supernatant was centrifuged for 2 min, and osmolarity was measured (Osmomat 030-D; Gonotec, Berlin, Germany). Assuming an osmolarity of intestinal contents of 300 mosmol/liter, even in the diarrheal state (11), the dry weight of fecal samples was calculated.

Results of measurements of physiological parameters and of bacterial counts obtained by microbiological and real-time PCR analyses are expressed as mean values $\pm$ standard errors of the means. Differences between the results of control and treatment groups were tested for significance using Student's t test (two sided, considered statistically significant when $P < 0.05$).

PHA-induced shifts in rat intestinal microbial populations. The present study proves the applicability of T-RFLP and 16S-23S IGS analyses to characterize microbial communities in rat feces. These molecular fingerprint analyses showed clearly that PHA administration induced changes in intestinal bacterial population structure (Fig. 1 and 2) and resulted in an increased diversity of the predominant intestinal microbiota (Fig. 1 and 2, arrows). In other studies, the effect of kidney beans or PHA on *E. coli* was most pronounced, but also increases of *Streptococcus* sp., bifidobacteria, and, not consistently, *Lactobacillus* have been reported in rats (3, 5, 10, 32).

Among animals within a diet group, compositions of the microbiota were found to be similar. Increased numbers of fecal *E. coli*, lactic acid bacteria, and other *Enterobacteriaceae* were observed during PHA administration for almost all animals (Fig. 1 and 2, circles). The stimulation of *E. coli* indicated by the population profiles is in line with previously described effects of PHA (32) and was confirmed by *E. coli*-specific real-time PCR (Table 1; $P = 0.00005$). Furthermore, increased bacterial counts of *E. coli* in jejunal samples and of lactobacilli in ileal samples were determined during PHA administration compared to those of animals that had not received PHA (Table 2).

The comparison of the two fingerprinting methods revealed that the general effects of PHA on intestinal microbial populations could clearly be visualized by 16S-23S IGS fragment length analysis but that the more complex peak profiles of T-RFLP were more powerful in the identification of particular species.

Effects of *L. plantarum* supplementation on PHA-affected rat intestinal microbial populations. The HhaI forward-TRF fingerprints support the presence of *L. plantarum* in the samples

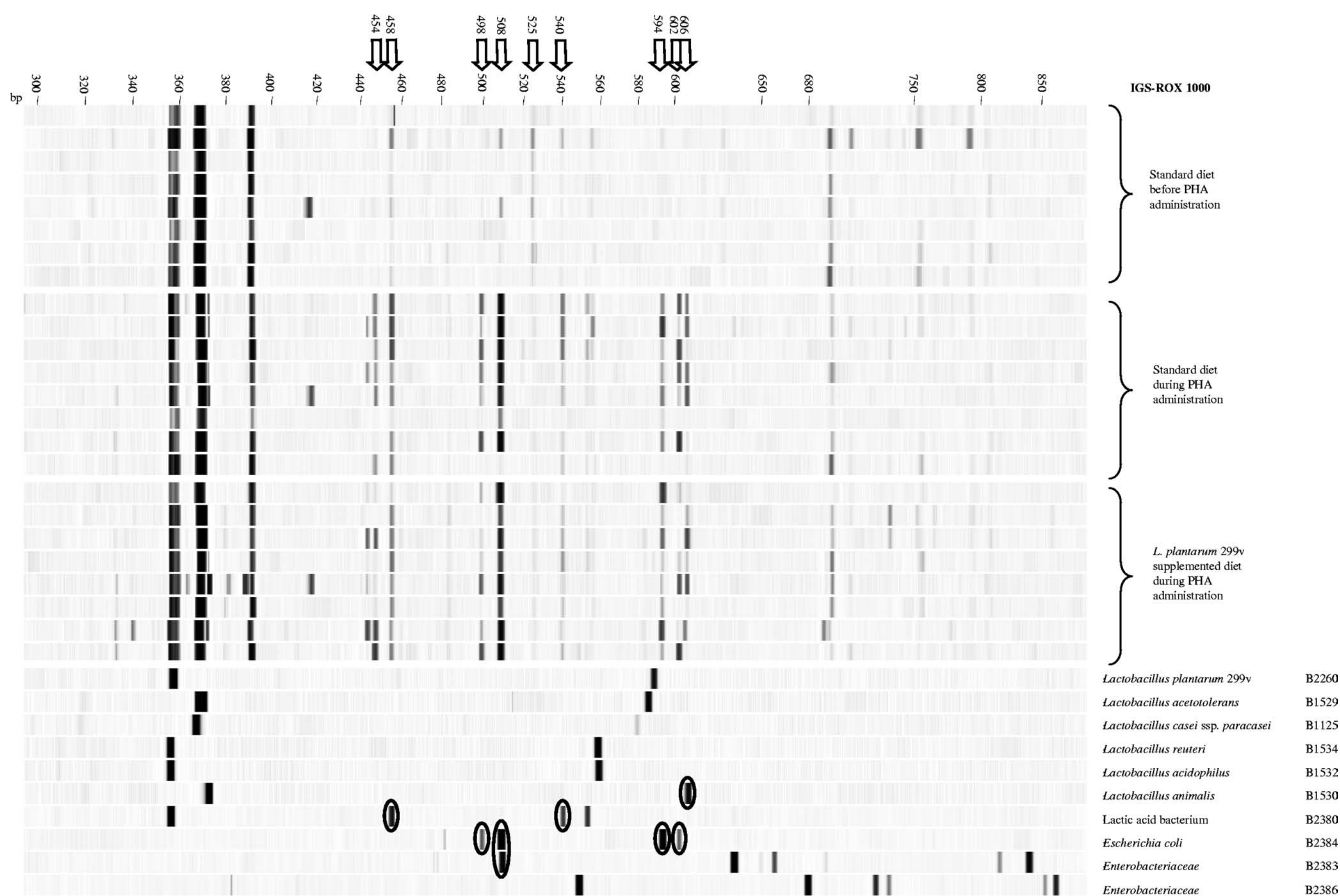

FIG. 1. 16S-23S ribosomal IGS analysis from individual animals (rows). Profiles are grouped according to experimental conditions and illustrate the effect of PHA on rat fecal microbial community structure. Amplified spacer fragments that were found to be specifically affected by PHA treatment are indicated with white arrows. Bands of the reference strains matching those found to be different among the rat fecal profiles are circled. ROX, rhodamine X.

of animals that received the *L. plantarum*-supplemented diet, since fragments could be detected that matched with corresponding *L. plantarum*-specific bands from the reference profile (Fig. 2), which was confirmed by macroscopically identical colony morphologies of the two isolates. Plating of fecal samples collected on day 11, before PHA administration, indicated that lactobacillus counts were higher in animals receiving *L. plantarum* (9.9 ± 0.1 $\log_{10}$ CFU/g) than in the control group (7.2 ± 0.4 $\log_{10}$ CFU/g) ($P = 0.00001$). (*E. coli* counts were 7.7 ± 0.2 and 7.9 ± 0.1 $\log_{10}$ CFU/g for the groups fed the standard diet and the *L. plantarum*-supplemented diet, respectively. There were eight samples in each group.) Furthermore, increased numbers of *L. plantarum* in feces of animals fed with this strain during PHA administration were detected by real-time PCR (Table 1, $P = 2.6 \times 10^{-18}$). These findings indicate that *L. plantarum* 299v is able to survive gastrointestinal passage as described earlier (13, 19) and to become part of the predominant fecal microbiota. Also in other studies, probiotic *Lactobacillus* strains could be tracked in T-RFLP analyses (9, 17, 22). Here, *L. plantarum* was also detected in fecal samples of control rats, although in lower numbers. This suggests that *L. plantarum* is a natural inhabitant of the intestinal tract not only of humans (27) but also of rats.

Despite the abundant presence of *L. plantarum* 299v in the fecal microbiota, no relevant inhibition could be observed of the PHA-induced increase of *E. coli* or other PHA-affected bacterial species according to the population fingerprint profiles. Basal fecal *E. coli* counts and the PHA-induced increase in numbers of *E. coli* bacteria compared to those in rats receiving the standard diet were not altered by *L. plantarum* supplementation (see above). PHA administration led to increased bacterial counts of both *E. coli* and lactobacilli in intestinal samples also in animals that received *L. plantarum* supplementation (Table 2). Interestingly, the number of *E. coli* bacteria in ileal samples after PHA administration tended to be lower in animals fed *L. plantarum* than in the control group ($P = 0.13$); the number detected was comparable to that of animals without PHA administration. However, these results contradict the findings of De Waard (8), who reported that 3 or 10 days of oral *L. plantarum* 299v administration prior to ingestion of the same or even a higher dose of PHA for 3 days reduced bacterial overgrowth of coliforms and loss of body weight in rats. This discrepancy might have been caused by differences in diet resulting in a higher level of *E. coli* in the current study, which was subsequently multiplied by PHA treatment. The results presented here support studies showing that consumption of *L. plantarum* 299v and other probiotic strains hardly influenced human intestinal microbiota compo-

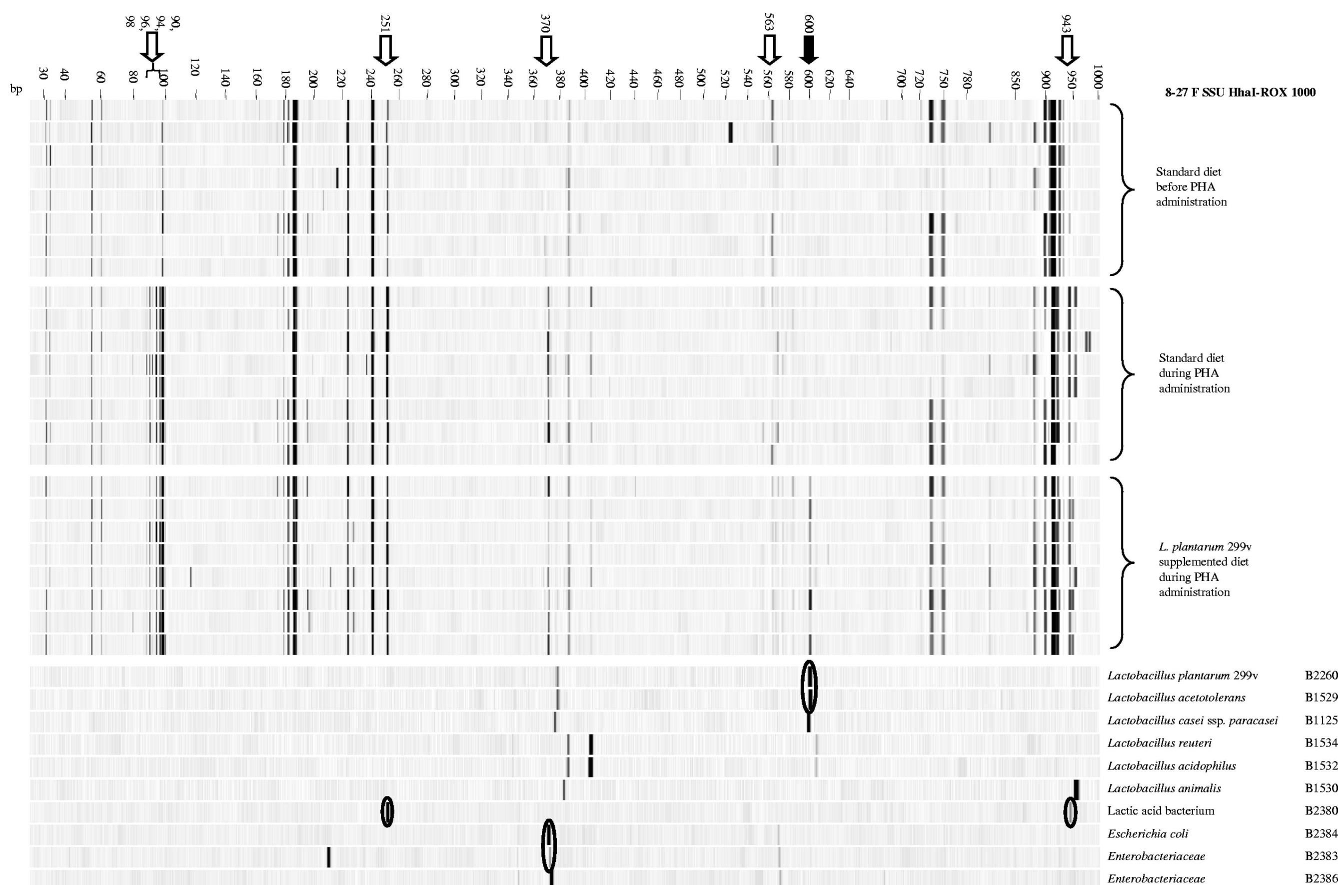

FIG. 2. HhaI forward T-RFLP analysis from individual animals (rows). Profiles are grouped according to experimental conditions. Bands from *L. plantarum* 299v and *E. coli* reference profiles can be recognized in the fecal profiles. TRFs that were found to be specifically affected by PHA treatment are indicated with white arrows; fragments specifically found after *L. plantarum* supplementation are marked with a black arrow. Bands of the reference profiles matching those found to be different among the rat fecal profiles are circled. ROX, rhodamine X.

sition (9, 14, 37). Contrasting findings indicating effects of *L. plantarum* 299v on other bacterial species in intestinal or fecal samples might be explained by mechanisms other than competition for adhesion sites or the use of gnotobiotic animals lacking endogenous microbiota (15, 18). Since supplementation with *L. plantarum* did not increase lactobacillus counts in intestinal samples (Table 2), it can be concluded that *L. plantarum* did not colonize the intestinal mucosa and therefore could not prevent *E. coli* overgrowth based on competitive exclusion.

Effect of PHA on physiological parameters and influence of *L. plantarum* on PHA-induced physiological changes. After administration of PHA, collection of fresh fecal samples was no longer possible because samples were too watery. Calculated percentages of dry weight in feces, food intake, and

TABLE 1. Bacterial numbers assessed in this study in fecal samples before (days 9 and 10) and during (days 11 and 12) PHA administration, as determined by *E. coli*- and *L. plantarum*-specific real-time PCR[c]

Diet and timing	Log_{10} bacteria/g	
	L. plantarum	*E. coli*
Standard diet		
Before PHA administration	Not determined	7.2 ± 0.2
During PHA administration	7.0 ± 0.1	8.7 ± 0.2[b]
L. plantarum-supplemented diet during PHA administration	10.6 ± 0.1[a]	8.7 ± 0.1[b]

[a] $P < 0.05$ versus standard diet during PHA administration.
[b] $P < 0.05$ versus standard diet before PHA administration.
[c] The number of samples was eight for all groups.

TABLE 2. Bacterial numbers assessed in this study in intestinal samples on day 13, after 2 days of PHA administration, as determined by plating of serial dilutions on appropriate agar plates

Diet (no. of samples)	Intestinal sample	Log_{10} CFU/g	
		Lactobacillus	*E. coli*
Standard diet			
Without PHA administration (3)	Jejunum	5.7 ± 0.1	3.9 ± 0.1
	Ileum	5.5 ± 0.4	4.6 ± 0.3
With PHA administration (8)	Jejunum	6.1 ± 0.1	4.8 ± 0.3[b]
	Ileum	6.9 ± 0.2[a]	5.6 ± 0.3
L. plantarum-supplemented diet with PHA administration (8)	Jejunum	6.0 ± 0.2	4.8 ± 0.3[b]
	Ileum	6.7 ± 0.3[b]	4.9 ± 0.4

[a] $P < 0.05$ versus standard diet without PHA administration.
[b] $P < 0.1$ versus standard diet without PHA administration.

TABLE 3. Physiological parameters before (days 9 and 10) and during (days 11 and 12) PHA administration[d]

Diet and timing	Dry wt in feces (%)[a]	Food intake (g)	Body wt (g)[b]
Standard diet			
Before PHA administration	35.4 ± 1.7	18 ± 1	346 ± 5
During PHA administration	22.0 ± 1.1[c]	6 ± 1[c]	341 ± 6
L. plantarum-supplemented diet during PHA administration	21.3 ± 0.9[c]	6 ± 1[c]	336 ± 4

[a] Calculated based on osmolarity measurements.
[b] Starting with 307 ± 2 g for all animals on day 0.
[c] $P < 0.05$ versus standard diet before PHA administration.
[d] The number of samples was eight for all groups.

accordingly body weight of the animals decreased rapidly (Table 3). Reference animals without PHA administration did not display a corresponding reduction of food intake and body weight (data not shown). *L. plantarum* supplementation did not have a significant effect on any of these physiological changes caused by PHA.

Dietary application of PHA is of interest not only in the context of weaning adaptation but also with respect to cancer treatment and the prevention of gastrointestinal atrophy during parenteral nutrition (20, 21, 30). A combination of PHA and dietary factors that prevent the adverse effects of PHA might be beneficial. Alternatively to probiotics, other dietary means could be selected since, e.g., GNA, a lectin from snowdrop (*Galanthus nivalis*) has been shown to suppress PHA-induced *E. coli* overgrowth in rat small intestine via competition for mannose-binding sites in vivo (32). Notably, in the current study a comparably high dosage of PHA was applied to maximize the effect of PHA on the intestinal microbiota and to create a wide range for probiotic intervention.

In conclusion, the present study demonstrates that the effects of dietary PHA extend beyond a specific stimulation of *E. coli*. The adverse effects of PHA on gut physiology could not be counteracted by mannose-adhering *L. plantarum* 299v. Its impact on the composition of the intestinal microbiota indicates that a dose of PHA comparable with that used in this study is not advisable for nutritional applications.

This work has been supported by the Centre for Human Nutrigenomics, The Netherlands.

We thank Jan Hoolwerf (NIZO food research) for excellent technical advice on molecular fingerprinting techniques.

REFERENCES

1. **Adlerberth, I., S. Ahrné, M. L. Johansson, G. Molin, L. Å. Hanson, and A. E. Wold.** 1996. A mannose-specific adherence mechanism in *Lactobacillus plantarum* conferring binding to the human colonic cell line HT-29. Appl. Environ. Microbiol. **62:**2244–2251.
2. **Banwell, J. G., D. H. Boldt, J. Meyers, and F. L. Weber, Jr.** 1983. Phytohemagglutinin derived from red kidney bean (*Phaseolus vulgaris*): a cause for intestinal malabsorption associated with bacterial overgrowth in the rat. Gastroenterology **84:**506–515.
3. **Banwell, J. G., R. Howard, D. Cooper, and J. W. Costerton.** 1985. Intestinal microbial flora after feeding phytohemagglutinin lectins (*Phaseolus vulgaris*) to rats. Appl. Environ. Microbiol. **50:**68–80.
4. **Banwell, J. G., R. Howard, I. Kabir, T. E. Adrian, R. H. Diamond, and C. Abramowsky.** 1993. Small intestinal growth caused by feeding red kidney bean phytohemagglutinin lectin to rats. Gastroenterology. **104:**1669–1677.
5. **Banwell, J. G., R. Howard, I. Kabir, and J. W. Costerton.** 1988. Bacterial overgrowth by indigenous microflora in the phytohemagglutinin-fed rat. Can. J. Microbiol. **34:**1009–1013.
6. **Bron, P. A., M. Marco, S. M. Hoffer, E. Van Mullekom, W. M. de Vos, and M. Kleerebezem.** 2004. Genetic characterization of the bile salt response in *Lactobacillus plantarum* and analysis of responsive promoters in vitro and in situ in the gastrointestinal tract. J. Bacteriol. **186:**7829–7835.
7. **Clement, B. G., L. E. Kehl, K. L. DeBord, and C. L. Kitts.** 1998. Terminal restriction fragment patterns (TRFPs), a rapid, PCR-based method for the comparison of complex bacterial communities. J. Microbiol. Methods **31:**135–142.
8. **De Waard, R.** 2002. Lactobacilli and host resistance. Ph.D. thesis. Utrecht University, Utrecht, The Netherlands.
9. **Engelbrektson, A. L., J. R. Korzenik, M. E. Sanders, B. G. Clement, G. Leyer, T. R. Klaenhammer, and C. L. Kitts.** 2006. Analysis of treatment effects on the microbial ecology of the human intestine. FEMS Microbiol. Ecol. **57:**239–250.
10. **Fanedl, L., F. V. Nekrep, and G. Avgustin.** 1998. Random amplified polymorphic DNA analysis and demonstration of genetic variability among bifidobacteria isolated from rats fed with raw kidney beans. Can. J. Microbiol. **44:**1094–1101.
11. **Fine, K. D., G. J. Krejs, and J. S. Fordtran.** 1993. Diarrhea, p. 1043–1072. *In* M. H. Sleisenger and J. S. Fordtran (ed.), Gastrointestinal disease. W. B. Saunders Company, London, England.
12. **Freed, D. L.** 1999. Do dietary lectins cause disease? BMJ **318:**1023–1024.
13. **Goossens, D., D. Jonkers, M. Russel, E. Stobberingh, A. Van Den Bogaard, and R. Stockbrügger.** 2003. The effect of *Lactobacillus plantarum* 299v on the bacterial composition and metabolic activity in faeces of healthy volunteers: a placebo-controlled study on the onset and duration of effects. Aliment. Pharmacol. Ther. **18:**495–505.
14. **Goossens, D. A., D. M. Jonkers, M. G. Russel, E. E. Stobberingh, and R. W. Stockbrügger.** 2006. The effect of a probiotic drink with *Lactobacillus plantarum* 299v on the bacterial composition in faeces and mucosal biopsies of rectum and ascending colon. Aliment. Pharmacol. Ther. **23:**255–263.
15. **Herias, M. V., C. Hessle, E. Telemo, T. Midtvedt, L. Å. Hanson, and A. E. Wold.** 1999. Immunomodulatory effects of *Lactobacillus plantarum* colonizing the intestine of gnotobiotic rats. Clin. Exp. Immunol. **116:**283–290.
16. **Huijsdens, X. W., R. K. Linskens, M. Mak, S. G. Meuwissen, C. M. Vandenbroucke-Grauls, and P. H. Savelkoul.** 2002. Quantification of bacteria adherent to gastrointestinal mucosa by real-time PCR. J. Clin. Microbiol. **40:**4423–4427.
17. **Jernberg, C., A. Sullivan, C. Edlund, and J. K. Jansson.** 2005. Monitoring of antibiotic-induced alterations in the human intestinal microflora and detection of probiotic strains by use of terminal restriction fragment length polymorphism. Appl. Environ. Microbiol. **71:**501–506.
18. **Johansson, M. L., G. Molin, B. Jeppsson, S. Nobaek, S. Ahrné, and S. Bengmark.** 1993. Administration of different *Lactobacillus* strains in fermented oatmeal soup: in vivo colonization of human intestinal mucosa and effect on the indigenous flora. Appl. Environ. Microbiol. **59:**15–20.
19. **Johansson, M. L., S. Nobaek, A. Berggren, M. Nyman, I. Björck, S. Ahrné, B. Jeppsson, and G. Molin.** 1998. Survival of *Lactobacillus plantarum* DSM 9843 (299v), and effect on the short-chain fatty acid content of faeces after ingestion of a rose-hip drink with fermented oats. Int. J. Food Microbiol. **42:**29–38.
20. **Jordinson, M., J. Calam, and M. Pignatelli.** 1998. Lectins: from basic science to clinical application in cancer prevention. Expert Opin. Investig. Drugs **7:**1389–1403.
21. **Jordinson, M., R. A. Goodlad, A. Brynes, P. Bliss, M. A. Ghatei, S. R. Bloom, A. Fitzgerald, G. Grant, S. Bardocz, A. Pusztai, M. Pignatelli, and J. Calam.** 1999. Gastrointestinal responses to a panel of lectins in rats maintained on total parenteral nutrition. Am. J. Physiol. **276:**G1235–G1242.
22. **Kaplan, C. W., J. C. Astaire, M. E. Sanders, B. S. Reddy, and C. L. Kitts.** 2001. 16S ribosomal DNA terminal restriction fragment pattern analysis of bacterial communities in feces of rats fed *Lactobacillus acidophilus* NCFM. Appl. Environ. Microbiol. **67:**1935–1939.
23. **Klappenbach, J. A., P. R. Saxman, J. R. Cole, and T. M. Schmidt.** 2001. rrndb: the Ribosomal RNA Operon Copy Number Database. Nucleic Acids Res. **29:**181–184.
24. **Kruszewska, D., P. Kiela, Å. Ljungh, K. H. Erlwanger, B. R. Weström, A. Linderoth, and S. G. Pierzynowski.** 2003. Enteral crude red kidney bean (*Phaseolus vulgaris*) lectin—phytohemagglutinin—induces maturational changes in the enterocyte membrane proteins of suckling rats. Biol. Neonate **84:**152–158.
25. **Linderoth, A., O. Prykhod'ko, S. G. Pierzynowski, and B. R. Weström.** 2006. Enterally but not parenterally administered *Phaseolus vulgaris* lectin induces growth and precocious maturation of the gut in suckling rats. Biol. Neonate **89:**60–68.
26. **Marsh, T. L., P. Saxman, J. Cole, and J. Tiedje.** 2000. Terminal restriction fragment length polymorphism analysis program, a web-based research tool for microbial community analysis. Appl. Environ. Microbiol. **66:**3616–3620.
27. **Molin, G., B. Jeppsson, M. L. Johansson, S. Ahrné, S. Nobaek, M. Stahl, and S. Bengmark.** 1993. Numerical taxonomy of *Lactobacillus* spp. associated with healthy and diseased mucosa of the human intestines. J. Appl. Bacteriol. **74:**314–323.

28. **Navarro, E., P. Simonet, P. Normand, and R. Bardin.** 1992. Characterization of natural populations of *Nitrobacter* spp. using PCR/RFLP analysis of the ribosomal intergenic spacer. Arch. Microbiol. **157:**107–115.
29. **Pretzer, G., J. Snel, D. Molenaar, A. Wiersma, P. A. Bron, J. Lambert, W. M. de Vos, R. van der Meer, M. A. Smits, and M. Kleerebezem.** 2005. Biodiversity-based identification and functional characterization of the mannose-specific adhesin of *Lactobacillus plantarum*. J. Bacteriol. **187:**6128–6136.
30. **Pryme, I. F., and S. Bardocz.** 2001. Anti-cancer therapy: diversion of polyamines in the gut. Eur. J. Gastroenterol. Hepatol. **13:**1041–1046.
31. **Pusztai, A., S. W. Ewen, G. Grant, W. J. Peumans, E. J. Van Damme, M. E. Coates, and S. Bardocz.** 1995. Lectins and also bacteria modify the glycosylation of gut surface receptors in the rat. Glycoconj. J. **12:**22–35.
32. **Pusztai, A., G. Grant, R. J. Spencer, T. J. Duguid, D. S. Brown, S. W. Ewen, W. J. Peumans, E. J. Van Damme, and S. Bardocz.** 1993. Kidney bean lectin-induced *Escherichia coli* overgrowth in the small intestine is blocked by GNA, a mannose-specific lectin. J. Appl. Bacteriol. **75:**360–368.
33. **Pusztai, A., and R. M. Palmer.** 1977. Nutritional evaluation of kidney beans (*Phaseolus vulgaris*): the toxic principle. J. Sci. Food Agric. **28:**620–623.
34. **Radberg, K., M. Biernat, A. Linderoth, R. Zabielski, S. G. Pierzynowski, and B. R. Weström.** 2001. Enteral exposure to crude red kidney bean lectin induces maturation of the gut in suckling pigs. J. Anim. Sci. **79:**2669–2678.
35. **Rademaker, J. L. W., M. Peinhopf, L. Rijnen, W. Bockelmann, and W. H. Noordman.** 2005. The surface microflora dynamics of bacterial smear-ripened Tilsit cheese determined by T-RFLP DNA population fingerprint analysis. Int. Dairy J. **15:**785–794.
36. **Reeves, P. G., F. H. Nielsen, and G. C. Fahey, Jr.** 1993. AIN-93 purified diets for laboratory rodents: final report of the American Institute of Nutrition ad hoc writing committee on the reformulation of the AIN-76A rodent diet. J. Nutr. **123:**1939–1951.
37. **Tannock, G. W., K. Munro, H. J. Harmsen, G. W. Welling, J. Smart, and P. K. Gopal.** 2000. Analysis of the fecal microflora of human subjects consuming a probiotic product containing *Lactobacillus rhamnosus* DR20. Appl. Environ. Microbiol. **66:**2578–2588.
38. **Ten Bruggencate, S. J., I. M. Bovee-Oudenhoven, M. L. Lettink-Wissink, M. B. Katan, and R. van der Meer.** 2004. Dietary fructo-oligosaccharides and inulin decrease resistance of rats to salmonella: protective role of calcium. Gut **53:**530–535.
39. **Thomsson, A., D. Rantzer, B. R. Weström, S. G. Pierzynowski, and J. Svendsen.** 2007. Effects of crude red kidney bean lectin (phytohemagglutinin) exposure on performance, health, feeding behavior, and gut maturation of pigs at weaning. J. Anim. Sci. **85:**477–485.
40. **Zoetendal, E. G., A. D. Akkermans, and W. M. De Vos.** 1998. Temperature gradient gel electrophoresis analysis of 16S rRNA from human fecal samples reveals stable and host-specific communities of active bacteria. Appl. Environ. Microbiol. **64:**3854–3859.