

Maternal consumption of *Lactobacillus plantarum* 299v affects gastrointestinal growth and function in the suckling rat

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After birth, the gastrointestinal (GI) tract undergoes vast structural and functional adaptations to be able to digest mother's milk and later, during the weaning period, solid food. Studies on germ-free animals have shown the role of the gut microbiota for stimulating GI maturation, but which groups are involved is unclear. In the present study, we administered the probiotic bacterium, *Lactobacillus plantarum* 299v (Lp299v), in the drinking water to pregnant and lactating rat dams until their pups had reached an age of 14 d. It was found that Lp299v colonizing the mothers were also able to colonize the pups, which had an impact on their gut growth and function. The small intestine, pancreas and liver weighed more in the 14 d-old pups born from dams exposed to Lp299v than in the control pups from dams given only water. Furthermore, the Lp299v pups showed decreased gut permeability. Despite a heavier spleen in the Lp299v pups, as compared to the control pups, no significant increase in the acute-phase protein, haptoglobin, was found. In conclusion, the results reported here clearly show that manipulating the maternal microflora by exposing expecting mothers to a Gram-positive, probiotic bacterium prior to parturition and during lactation impacts the gut growth and function in the offspring.

Gastrointestinal tract: Microbiota: Suckling rats: Probiotic: *Lactobacillus plantarum* 299v

Rodents are born with an immature gastrointestinal (GI) tract, which rapidly adapts to be able to digest the various nutrients in the mother's milk. For example, intestinal mucosal weight and expression of brush-border lactase increase after birth, while the pancreas and liver are still not fully functional during the suckling period⁽¹⁾. In addition, a permeable intestine is required during the suckling period for transfer of maternal antibodies and growth factors. The neonatal Fc receptor (FcRn) expressed in the proximal small intestine, mediates transport of IgG from the mother's milk by receptor-mediated endocytosis, whereas macromolecules are endocytosed in an unspecific manner, and degraded in large, supranuclear vacuoles situated in the distal part. Gradually, the young rat starts consuming solid food at about 2–3 weeks of age, which leads to a second adaptation period of the GI tract, where there is a need for high expression of the brush-border disaccharidases maltase and sucrase, instead of lactase, together with an increased pancreatic enzyme secretion. In addition, the permeability of the small intestine declines until gut closure occurs at 3 weeks of age^(1,2).

At birth, the GI tract is sterile, but the microbial colonization commences as soon as the neonate is exposed to the outside world. The maternal microflora is important, but the surrounding environment also influences which bacterial groups will colonize the GI tract of the newborn^(3,4). During

the suckling period, lactobacilli and bifidobacteria are favoured by the milk, while at weaning, the diversity of the gut microbiota increases⁽⁵⁾.

Studies on germ-free animals have highlighted the importance of the microflora for GI development, since these animals have thinner intestinal villi and lamina propria, enlarged caecum, decreased intestinal motility and altered mucosal enzyme patterns⁽⁶⁾. Few studies have, however, been designed to determine which genera of the bacterial community are responsible for this stimulation of the GI tract. By destabilizing the flora of rat dams using antibiotics, it has been shown that the microflora in the offspring was affected, but possible effects on the gut were not studied⁽⁷⁾. However, Schumann and colleagues showed that antibiotics administered to neonatal rats increased the expression of several developmental genes in the GI tract⁽⁸⁾.

Probiotics, bacteria that exert a health benefit for the host, have been shown to have a wide range of effects when administered to both man and animals, such as increased secretion of IgA, protection against inflammatory bowel diseases and prevention of allergies, all related to stimulation of the gut immune system^(9,10). However, few studies have been conducted to determine the effect of probiotic bacteria on the GI development, despite the fact that attempts have already been made to prevent the occurrence of necrotizing

Abbreviations: BSA, bovine serum albumin; BIGG, bovine immunoglobulin G; GI, gastrointestinal; Lp299v, *Lactobacillus plantarum* 299v.

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enterocolitis in premature infants by exposing them to different probiotic strains of bacteria^(11,12). In fact, it was shown in one report that young, post-weaning mice had better body growth if lactobacilli were administered in their drinking water, but the impact on the GI organs was not investigated⁽¹³⁾. Previously, we have shown that the probiotic *Lactobacillus* strain, *Lactobacillus plantarum* 299v (Lp299v), could improve the barrier properties of the small intestine of young rats when fed for 1 week starting at 3 d of age⁽¹⁴⁾. Moreover, we have demonstrated that manipulating the maternal microflora before parturition by increasing the numbers of Gram-negative *Enterobacteriaceae*, resulted in increased numbers of *Enterobacteriaceae* also in the 14 d-old pups, and an increased growth of their GI tract together with an increase in the acute-phase protein haptoglobin⁽¹⁵⁾.

In the present study, the aim was to expose pregnant rat dams to a probiotic Gram-positive bacterium, Lp299v, which has been shown to have protective effects on the gut barrier and to have anti-inflammatory properties^(16,17), to investigate whether it could be transferred to the offspring and thereby influence gut growth and maturation during the suckling period. The densities of lactobacilli and *Enterobacteriaceae* in both mothers and pups were analyzed, as well as gut and lymphoid organ growth, digestive enzyme activities and intestinal macromolecular permeability. Furthermore, to elucidate whether any inflammation occurred due to the Lp299v exposure, the plasma concentration of the acute-phase protein haptoglobin, was measured in the rat pups.

Materials and methods

Animals

Rats of the Sprague–Dawley strain were purchased (Taconic, Ry, Denmark). Female rats of the same age (10 weeks old) and weight (200 g) were chosen to minimize gut microflora differences at mating. The dams were housed separately from 1 week before parturition in polycarbonate cages on a good laboratory practice chopped aspen wood bedding (Becky bedding, Scanbur BK AB, Sollentuna, Sweden) with free access to a breeding diet (RM1, containing wheat–barley–wheatfeed (88.5%), de-hulled extracted toasted soya–soya protein concentrate (6.0%), soya oil (0.5%), whey powder (2.5%), amino acids–vitamins–minerals (2.5%); SDS, Essex, England) and tap water in our animal facility under standardized conditions (21 ± 1°C, 50 ± 10% relative humidity, 12 h–12 h light–dark cycle). After birth (day of birth was assigned day 0) each litter was restricted to six to eight pups and was held with its respective mother for 2 weeks.

Bacterial preparation

Lp299v (DSM 9843) was grown in *Lactobacillus*-carrying medium supplemented with 1% glucose in still culture overnight at 37°C. The cells were harvested by centrifugation at 3000 g for 5 min, resuspended in freezing medium (3.6 mM-K₂HPO₄, 1.3 mM-KH₂PO₄, 2.0 mM-sodium citrate, 1.0 mM-MgSO₄, 12% glycerol) and kept in portions at –70°C until feeding. At the day of use, the tubes were thawed and the cells washed with saline and centrifuged. The pellet was dissolved in the dams'

drinking water. Samples from the water were analyzed to determine the viable count of Lp299v, which gave a final dose of 7 × 10⁶ colony-forming units/ml water.

Study procedure

One week prior to expected day of parturition, Lp299v was administered in the drinking water of two dams and was continued during the lactation period until the pups had reached an age of 2 weeks. Control dams (*n* 2) were given tap water only. Both bacterial water and control water was changed daily and the mean water consumption throughout the study period was approximately 40 ml/d for both the bacteria treated and control dams. Repeated faecal sampling of the dams took place 1 week before parturition, at the day of parturition and when the pups were 14 d of age, by placing the dam inside an empty cage sterilized with ethanol (*n* 2–6 at each time point and treatment group). This enabled fresh faecal samples to be collected and frozen in freezing medium at –70°C. The local Ethical Review Committee for Animal Experiments had approved the study.

Procedure at sacrifice of pups

At 2 weeks of age, the pups were separated from their mother for 1 h prior to gavage using a teflon stomach tube (Agn Tho's AB, Lidingö, Sweden) with a marker solution containing bovine serum albumin (1.25 mg BSA/g body weight) and bovine immunoglobulin (0.25 mg BIgG/g body weight) (Sigma-Aldrich Co, St Louis, USA). Three hours later, the pups were anaesthetized with a mixture of Ketamin (0.5 mg/g body weight; Ketalar, Parke-Davis, Solna, Sweden) and Azaperon (0.4 mg/g body weight; Stresnil, Janssen-Cilag Pharma, Wien, Austria) in 0.15 M-NaCl. After opening the bowel and chest, approximately 0.5 ml blood was taken by heart puncture into tubes containing 1.5 mg EDTA and 20 000 IU aprotinin (Trasylol, Bayer, Leverkusen, Germany) and ice-chilled. The pancreas was carefully dissected from the small intestine, rinsed in ice-cold saline, weighed and frozen. Thereafter, the stomach was removed, its content was collected and ice-chilled and the stomach tissue was rinsed in saline and weighed. The small intestine was dissected and divided into two halves of equal length (proximal and distal part), flushed with 1 ml saline before being weighed and frozen. The caecum was removed using sterile instruments, weighed and then frozen in freezing medium. After this, the weights of the liver, spleen, thymus and adrenals were recorded.

After completion of dissection, blood samples were centrifuged and plasma was removed and stored at –70°C until further analysis. The stomach content was mixed in 0.5 ml 0.9% NaCl and centrifuged (3000 g for 15 min at 4°C) after which the supernatant pH was measured.

Analyses

The caecum and faecal samples. The caecum with its content or faecal samples in freezing medium was thawed and disintegrated using a sterile pipette. After vortexing the samples, serial dilutions were made in dilution medium (9 mg NaCl/ml, 1 mg peptone/ml, 0.2 mg cysteine/ml, 1 ml

Tween/litre distilled water) and spread on violet red bile glucose and Rogosa agar plates, respectively (Oxoid Ltd, Basingstoke, Hants., UK). After incubating violet red bile glucose plates for 24 h aerobically and Rogosa plates for 48 h anaerobically (AnaeroGen, Oxoid Ltd) at 37°C, the number of colonies was estimated and calculated as colony forming units/g caecum with content or colony forming units/g faeces. Colonies found growing on violet red bile glucose agar plates were considered to be bacteria belonging to the family *Enterobacteriaceae*, while colonies found on Rogosa agar plates were considered to be lactobacilli (Lactobacillus-like bacteria). The specific Lp299v colonies, normally not found in the caecum of rats⁽¹⁸⁾, of both mothers and pups were tentatively identified by colony shape and colour on the Rogosa agar plates.

The pancreas. The pancreas protein content was determined according to the Lowry method modified for ninety-six-well microplates⁽¹⁹⁾. Briefly, the pancreata were homogenized in ice-cold 0.2 mol TRIS-HCl buffer/litre + 0.05 mol CaCl₂/litre, using a glass-glass homogenizer, followed by centrifugation at 15 000 g for 20 min at 4°C. The protein concentration was determined in the supernatant by reading the absorbance of the samples at 690 nm using a plate reader and BSA as the standard. To estimate the trypsin activity, the pancreatic supernatants were activated with enterokinase and thereafter incubated with the substrate Bz-Arg-pNA (Sigma-Aldrich Co, St Louis, USA), and the absorbance change was then measured at 405 nm⁽²⁰⁾. The amount of enzyme causing transformation of 1.0 µmol substrate/min at 25°C was defined as one unit.

The small intestine. The protein content of the proximal small intestine was determined as described earlier. In addition, the Dahlqvist method⁽²¹⁾ was used to measure the intestinal disaccharidase activities. In short, a knife-homogenizer was used to homogenize the intestine in nine volumes of ice-cold NaCl (w/v). Then the substrates, lactose, sucrose and maltose, were incubated with the intestinal homogenates for 1 h, after which the reaction was stopped with a glucose oxidase reagent and the amount of generated glucose was determined. Glucose (0.05–1.0 mg/ml) was used as standard. The disaccharidase activities were estimated by reading the absorbance at 450 nm.

The intestinal permeability was determined by measuring the concentrations of the marker molecules BSA and B1gG in blood samples taken 3 h after gavage⁽²⁾. BSA and B1gG concentrations in blood plasma were measured by electroimmunoassay (rocket electrophoresis)⁽²²⁾ using specific antisera for BSA (rabbit anti-cow albumin, Dako A/S, Denmark) and B1gG (rabbit anti-B1gG, Dako A/S, Denmark). Purified BSA and B1gG were used as standards (Sigma-Aldrich Co, St Louis, USA).

Haptoglobin in plasma. The concentration of plasma haptoglobin was analyzed using a commercially available kit (Phase™ Range Haptoglobin Assay; Tridel Development Ltd, Ireland) according to the manufacturer's instructions. In short, plasma was incubated with Hb which bound to any haptoglobin present in the samples leading to preservation of peroxidase activity of the Hb. The reaction was performed at low pH, which inhibited the peroxidase activity of free, unbound Hb. A colorimetric reaction showing the peroxidase activity in the samples was then compared

with a haptoglobin standard (0–2 mg/ml). Absorbance was measured at 630 nm. The assay sensitivity was reported to be 0.05 mg/ml haptoglobin.

Calculations

Student's *t* test was performed (unpaired, two-tailed) on all of the results using Microsoft Excel, where values of $P < 0.05$ were considered significant. The Lp299v group of pups ($n = 14$) was compared to the control pups ($n = 12$). The effect of Lp299v exposure on the dams' faecal flora was estimated by comparing bacterial numbers between the Lp299v exposed dams with the control dams at three different time points (one day before exposure, at parturition and at the final day of the experiment, $n = 2–4$ at each time point and group).

To compensate for body weight differences all parameters are given per g body weight, giving a relative organ weight that could be compared between groups.

Results

Effects of Lp299v exposure

Effect on dams' microflora. The Lp299v strain was exclusively found in faecal samples from dams given Lp299v in the drinking water, but no significant increases in the total amount of lactobacilli were observed in the Lp299v dams as compared to samples from control dams given only water (Fig. 1 (a)). The amount of *Enterobacteriaceae* tended to increase due to the Lp299v exposure, but the difference was not significant (Fig. 1 (b)).

Effect of maternal exposure to Lp299v on the pups' microflora. At 2 weeks of age, the caecum with its content weighed less in the Lp299v group than in the control group (Table 1). Lp299v was recovered in the caecum of all but one pup from mothers exposed to the bacterium, while no Lp299v were found in the pups from control mothers. No significant differences in the total amount of lactobacilli were found between the two groups (Table 2). The number of *Enterobacteriaceae* was not significantly altered by the colonization of Lp299v (Table 2).

Effect on body growth, gastrointestinal growth and function. Administering Lp299v in the dams' drinking water during pregnancy and lactation did not affect the body weight of their pups as compared to pups born from control dams and all rats survived the experimental period (Table 1).

No significant differences were found regarding the weight of the stomach, while the pH of the stomach contents was higher in the Lp299v pups in comparison with the control pups (Table 1). The small intestine, both the proximal and the distal part, was heavier in the Lp299v group than in the control group (Fig. 2). No significant differences were found in the total protein content or the lactase and maltase activities in the proximal small intestine between groups (Table 3). The sucrase activity was generally low in all rats, but significantly lower in the Lp299v pups (Table 3).

Three hours after marker feeding, lower plasma concentrations of B1gG were found in the Lp299v group as compared to the control group, while no significant differences were observed for BSA (Fig. 3).

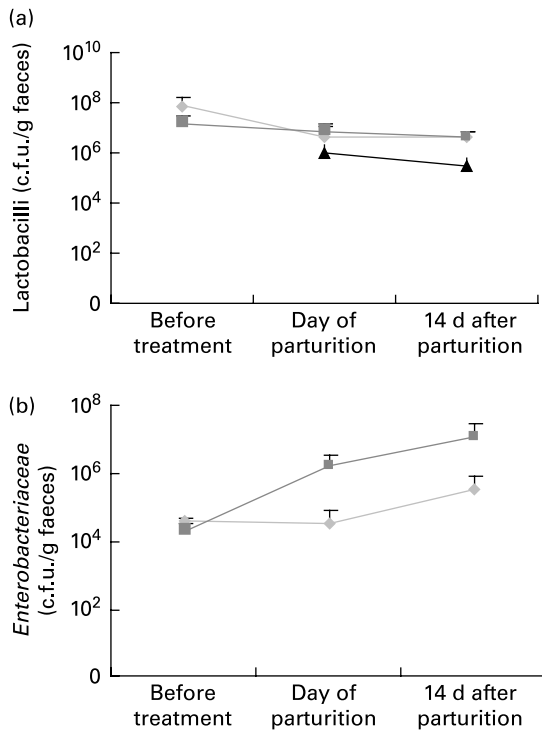


Fig. 1. Amount of lactobacilli, *Lactobacillus plantarum* 299v (Lp299v) (a) and *Enterobacteriaceae* (b) in faeces from dams 1 week before parturition, at day of parturition and at the end of the experiments, treated with Lp299v in the drinking water during late pregnancy and lactation and from control dams given only water (c.f.u., colony forming units). ((a): \circ , control dams, lactobacilli; \blacksquare , Lp299v dams, lactobacilli; \blacktriangle , Lp299v dams, Lp299v. (b): \circ , control dams; \blacksquare , Lp299v dams.) The number of faecal samples was between two and four at each sample time. No Lp299v were found in the control dams. Values are means with standard deviations indicated by vertical bars.

Effect on the gut-associated glands. The Lp299v group had heavier pancreata than the control group, but neither the protein nor the trypsin content differed significantly between groups (Table 4), although there was a trend towards a higher trypsin content in the Lp299v group ($P=0.09$). Liver weight was also found to be higher in the Lp299v group as compared to the control group (Table 1). The weight of the adrenals did not differ between the Lp299v and control pups (Table 1).

Effect on lymphoid organ growth. The Lp299v group had heavier spleens as compared to the control group, while the thymus weight did not differ significantly between the two groups (Table 1).

Effect on plasma haptoglobin levels. The plasma concentration of the acute-phase protein, haptoglobin, did not differ

significantly between the Lp299v group (mean 0.16 (SD 0.13) mg/ml) and the control group (mean 0.11 (SD 0.09) mg/ml).

Discussion

Our results clearly show that manipulating the maternal microflora by administering live Lp299v in the drinking water to pregnant and lactating rat dams led to colonization of their gut, and also of their offspring, having an impact on their growth and function of the gut and its associated organs.

Effect of Lp299v exposure on the gut microbiota

The bacteria consumed by the dams during pregnancy and lactation were recovered in faecal samples taken both after parturition and 2 weeks later. Presumably, Lp299v were able to colonize their gut and appeared to take the place of other strains of lactobacilli, since no general increase in lactobacilli was observed. There was, however, a tendency towards an increase in the total count of *Enterobacteriaceae* which could possibly be a reflection of an increase in the total number of bacteria in the gut.

In order to see any effects of a changed bacterial exposure during the suckling period, the sacrifice of the pups took place at 2 weeks of age, before the start of ingestion of solid food and weaning, a process that certainly affects bacterial homeostasis and gut maturation^(1,5,23). Thus, it was found that Lp299v fed to the dams were able to transfer to the pups, most likely by the time of birth. This is consistent with Ley and colleagues who showed that, in mice, the maternal flora is inherited to a large degree by the offspring⁽³⁾. Furthermore, Schultz and co-workers were able to show that *L. rhamnosus* strain GG administration to pregnant mothers led to colonization of their babies as late as 6 months after birth, despite the fact that the bacterial consumption ceased at time of birth⁽²⁴⁾. A cross-fostering study in rabbits emphasized the importance of colonization during the suckling period, as opposed to the colonization at birth, since the microbial flora of the pups was influenced most by the nursing doe, instead of the biological mother⁽⁴⁾.

The pups of the Lp299v exposed dams showed no increase in the total count of *Enterobacteriaceae*, suggesting that the tendency of an increase in this family of bacteria, as was seen in the dams, was not apparent in the pups. In a previous study of ours, we showed that an increase in the number of *Enterobacteriaceae* could indeed affect gut development, but the effects appeared to be dependent on which species of *Enterobacteriaceae* had increased⁽¹⁵⁾.

Table 1. Body weight (g), organ weights (mg/g body weight) and stomach pH of 14-d-old suckling rat pups (n 14) born from dams treated with *Lactobacillus plantarum* 299v (Lp299v) in the drinking water during late pregnancy and lactation or pups (n 12) born from control dams only given water (Values are means with standard deviations)

	Body weight		Liver		Stomach		Stomach pH		Caecum		Adrenals		Spleen		Thymus	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Lp299v	31.1	1.6	31.3**	3.3	7.0	0.4	5.0*	0.4	3.0**	0.5	0.20	0.1	4.8*	0.5	4.4	0.4
Controls	30.4	1.3	28.3	1.6	6.9	0.6	4.4	0.8	3.6	0.5	0.15	0.1	4.4	0.3	4.4	0.4

Significant differences were found between the Lp299v group and the control group: * $P<0.05$; ** $P<0.01$.

Table 2. Numbers of caecal *Lactobacillus plantarum* 299v (Lp299v), lactobacilli and *Enterobacteriaceae* of 14 d-old suckling rats (*n* 14) born from dams treated with Lp299v in the drinking water during late pregnancy and lactation and rats (*n* 12) born from control dams only given water

(Values are means with standard deviations)

	Lp299v		Lactobacilli		<i>Enterobacteriaceae</i>	
	Mean	SD	Mean	SD	Mean	SD
Lp299v†	$3.7 \times 10^{6**}$	2.0×10^6	1.1×10^8	9.5×10^7	7.9×10^6	1.0×10^7
Controls	0	0.0	6.3×10^7	9.2×10^7	4.7×10^6	5.5×10^6

Significant differences were found between the Lp299v group and the control group: ***P* < 0.01.

† In one animal of the Lp299v group no bacteria (Lp299v, lactobacilli or *Enterobacteriaceae*) were found.

The reduced weight of the caecum with contents found in the Lp299v group suggests that these animals have a changed microflora in the gut. Possibly, this altered microflora is more efficient in degrading the digesta, since germ-free animals have been found to have enlarged caeca due to accumulation of water and undigested fibre⁽²⁵⁾. In addition, mice pups, suckling mothers treated with penicillin in the drinking water, developed an enlarged caecum, and it was proposed that the microflora induced by the antibiotic treatment was responsible for this enlargement of the caecum⁽²⁶⁾. Lactobacilli, on the other hand, might have an opposite effect.

Effect of Lp299v exposure on gut development in the offspring

Colonization with Lp299v in the pups led to significant effects on gut growth and function. The small intestine and its associated glands, the pancreas and liver, of the Lp299v pups were all heavier than in the control pups. In addition, the Lp299v had an impact on the gut function. The mechanism behind this stimulation of the gut and its associated organs remains to be elucidated. It is likely that Lp299v affected the gut-associated lymphoid tissue leading to a cytokine surge, as occurs at weaning in rats⁽²⁷⁾, which could possibly affect GI development. Additionally, it has been shown that activated $\gamma\delta$ -T cells in the

gut secrete keratinocyte growth factor, which is a potent growth factor to enterocytes⁽²⁸⁾. Moreover, the spleen in the Lp299v group was heavier than in the control group, indicating an activation of the immune system. These effects were not, however, concomitant with any change of the acute phase protein, haptoglobin, a sensitive marker for inflammation⁽²⁹⁾. This is supported by results from our study on the effects of an increased number of *Enterobacteriaceae* during the suckling period, where it was shown that increased concentrations of haptoglobin were not correlated with increased growth of the GI tract⁽¹⁵⁾. It appears as if Lp299v can balance pro- and anti-inflammatory cytokines in a way that stimulates gut growth without causing any major inflammation.

The decreased intestinal transport of BIGG to the blood circulation is not surprising, since Lp299v and other lactobacilli have been found to have a similar effect also in adult animals^(16,17), as well as in suckling rats^(14,30). An increase in the gut-barrier function during the neonatal period might protect the infant against developing diseases such as diabetes, allergies or Crohn's disease later in life⁽³¹⁾.

The higher pH of the stomach contents in the Lp299v group as compared to the controls reflects a lower production of hydrochloric acid by the stomach mucosa. The reason for this is unclear and the significance of this finding can be discussed, as the stomach weight did not differ between groups.

Previously, we conducted a study where we directly fed suckling rats of various ages Lp299v for 1 week, where it was found that early exposure, i.e. oral feeding from 3 d of age, as opposed to later exposure during the suckling period, influenced the gut function the most⁽¹⁴⁾. In the present study, we observed similar effects on gut permeability, and also growth increases of several gut organs. The colonization levels of Lp299v and lactobacilli appear to be similar in the

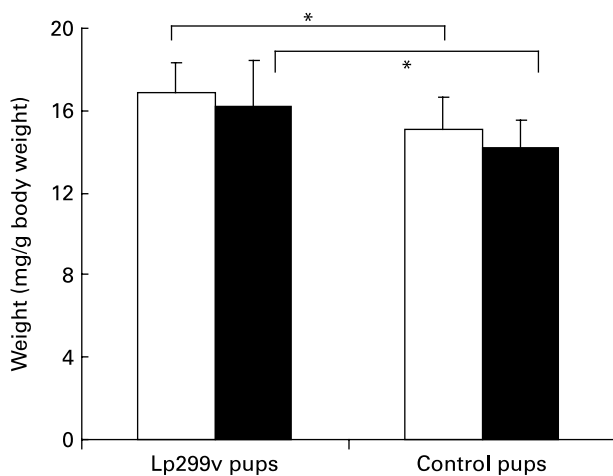


Fig. 2. Weight of the proximal (□) and distal (■) halves of the small intestine in 14 d-old suckling rats born from dams treated with *Lactobacillus plantarum* 299v (Lp299v) in the drinking water during late pregnancy and lactation and rats born from control dams given only water. Values are means with standard deviations indicated by vertical bars. Mean values were significantly different between the pups from Lp299v-treated dams and the controls: **P* < 0.05.

Table 3. Small intestinal protein content (mg/g body weight) and disaccharidase activities (U/g body weight) from the proximal part of 14 d-old suckling rat pups (*n* 14) born from dams treated with *Lactobacillus plantarum* 299v (Lp299v) in the drinking water during late pregnancy and lactation or pups (*n* 12) born from control dams given only water

(Values are means with standard deviations)

	Protein		Lactase		Maltase		Sucrase	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Lp299v	202	41	89	21	86	16	0.08**	0.3
Controls	182	49	92	13	76	12	2.3	2.3

Significant differences were found between the Lp299v group and the control group: ***P* < 0.01.

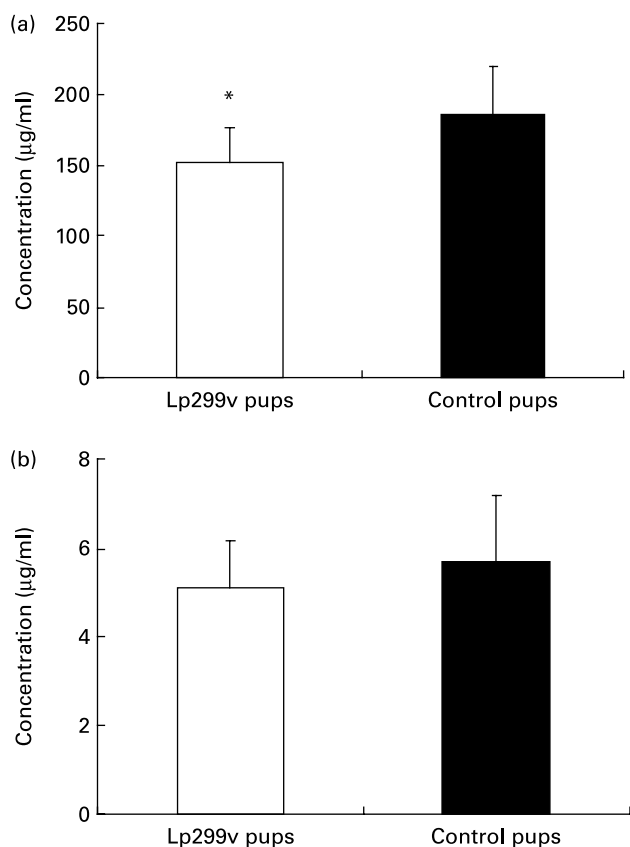


Fig. 3. Plasma concentrations of bovine IgG (a) and bovine serum albumin (b) in 14-d-old suckling rats born from dams treated with *Lactobacillus plantarum* 299v (Lp299v, □) in the drinking water during late pregnancy and lactation and rats born from control dams (■) given only water. Values are means with standard deviations indicated by vertical bars. Mean values were significantly different between the pups from Lp299v-treated dams and the controls: * $P < 0.05$.

two studies, despite the different administration procedures of Lp299v. Possibly, the longer exposure period in the present study (1 week prenatally + 2 weeks postnatally v. 1 week postnatally) causes a greater impact on the GI tract. Furthermore, in the present study, there was no handling stress since the pups were not fed orally, which might have had an impact on the results in the previous study. It cannot be discounted that the prenatal exposure affected the growth of the fetuses, or that the dam's milk production was somehow influenced by the Lp299v consumption.

Table 4. Pancreas weight (mg/g body weight), pancreas protein (mg/g body weight) and trypsin content (U/g body weight) of 14-d-old suckling rats ($n = 14$) born from dams treated with *Lactobacillus plantarum* 299v (Lp299v) in the drinking water during late pregnancy and lactation and rats ($n = 12$) born from control dams only given water

(Values are means with standard deviations)

	Weight		Protein		Trypsin	
	Mean	SD	Mean	SD	Mean	SD
Lp299v	3.1*	0.6	258	91	16	4.1
Controls	2.6	0.5	221	133	13.1	4.1

Significant differences were found between the Lp299v group and the control group: * $P < 0.05$.

Conclusions

The results reported here clearly show that manipulating the maternal microflora by exposing expecting mothers to a single bacterial strain prior to parturition and during lactation impacts the gut growth and function in the offspring. Whereas Gram-negative *Escherichia coli* appear to have similar effects on the gut⁽¹⁵⁾, this study shows for the first time that Gram-positive, probiotic Lp299v can induce gastrointestinal growth effects without involvement of a systemic inflammation.

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