

Enteral feeding of premature infants with *Lactobacillus GG*

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Abstract

The objectives of this study were to determine whether or not the probiotic *Lactobacillus GG* can colonise the immature bowel of premature infants and if so, does colonisation result in a reduction of the size of the bowel reservoir of nosocomial pathogens such as enterobacteriaceae, enterococci, yeasts or staphylococci, and does colonisation with *Lactobacillus GG* have any effect on the clinical progress and outcome.

Twenty preterm infants with a gestational age of 33 weeks or less who were resident on a neonatal unit were studied from the initiation of milk feeds until discharge. The infants were randomised to receive either milk feeds or milk feeds supplemented with *Lactobacillus GG* 10⁸ colony forming units twice a day for two weeks. The clinical features of the two groups of infants were similar.

Orally administered *Lactobacillus GG* was well tolerated and did colonise the bowel of premature infants. However, colonisation with *Lactobacillus GG* did not reduce the faecal reservoir of potential pathogens and there was no evidence that colonisation had any positive clinical benefit for this particular group of infants.

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The bacterial flora of the bowel of premature newborn infants in neonatal intensive care units differs from that of normal full term infants.^{1,2} Colonisation with lactobacilli and bifidobacteria may be delayed.³ Preterm infants are predisposed to infection from bacteria encountered in the hospital environment. The bacteria that most frequently cause nosocomial infections of infants in intensive care are coagulase negative staphylococci and enterobacteriaceae. The bowel provides the major reservoir both for enterobacteriaceae,⁴ which may also contribute to the pathogenesis of neonatal necrotising enterocolitis,^{5,6} and in the first few weeks of life coagulase negative staphylococci.

A probiotic can be defined as 'a live microbial feed supplement which beneficially affect the host animals by improving intestinal microbial balance'.⁷ It has been suggested that induced colonisation of preterm infants with a probiotic may produce bacteriological, metabolic, and clinical benefits for these infants.⁸ In human adult and animal studies lactobacilli have been among the commonest organisms used in probiotic studies.^{9,10} In addition to preventing bowel colonisation by other

microbes by competing for binding sites and substrates in the bowel, lactobacilli can produce a wide range of antibacterial substances such as organic acids, bacteriocins, microcins, reuterin, volatile fatty acids, hydrogen peroxide, and hydrogen ions.¹¹⁻¹³ *Lactobacillus GG* is a strain of *Lactobacillus casei* that can colonise the bowel of adults and has been used in the treatment of pseudomembranous colitis and traveller's diarrhoea.^{14,15} Although *Lactobacillus GG* has not been reported to have been given to newborn infants, it has been used to treat relatively young infants suffering from acute gastroenteritis with no reported side effects.¹⁶

The objectives of this study were (i) to determine whether or not the probiotic *Lactobacillus GG* can colonise the immature bowel of premature infants and (ii) if so, does colonisation result in a reduction of the size of the bowel reservoir of nosocomial pathogens such as enterobacteriaceae, enterococci, yeasts or staphylococci, and (iii) does colonisation with *Lactobacillus GG* have any effect on clinical progress and outcome.

Patients and methods

In a preliminary investigation we determined the tolerability of feed supplementation with *Lactobacillus GG*. Three infants with gestational ages 25, 31, and 32 weeks and birth weights 860, 1380, and 2130 g were recruited after informed parental consent had been obtained. This part of the study was not blinded in order to allow the opportunity for evaluation of changes in clinical condition. *Lactobacillus GG* was supplied as a freeze dried powder containing 10¹¹ colony forming units (cfu)/g dry weight and was obtained from Valio Finnish Co-operative Dairies Association, Finland. Each day a stock solution of *Lactobacillus GG* was prepared using a sterile technique by suspension of freeze dried powder in sterile distilled water to give 10⁹ cfu/ml. The suspension was stored at 4°C for up to 12 hours. The suspension was then diluted in milk to give the required concentration of bacteria in feeds. The first dose was usually given with the initiation of milk feeds. All of these infants received parenteral antibiotics. The study was approved by the local ethics committee.

The first infant studied received 10⁴ cfu *Lactobacillus GG* in a single feed for five days. This resulted in bowel colonisation with counts ranging from 10⁴ cfu/g dry weight of stool in the first week after administration to 10⁶ cfu/g dry weight of stool five weeks later with a maximum count 10⁸ cfu/g dry weight of

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stool two weeks after receiving the first dose of *Lactobacillus GG*. The next infant received 10^6 as a single dose for five days and in this infant colonisation was not so consistent – indeed *Lactobacillus GG* was isolated on only one occasion, at a count of 10^{10} cfu/g dry weight of stool one week after the start of feed supplementation. The third infant received 10^6 twice daily for 14 days. *Lactobacillus GG* was isolated again on only one occasion at a count of 10^9 cfu/g dry weight of stool two weeks after the start of feed supplementation. *Lactobacillus GG* feed supplementation was well tolerated and there appeared to be no detrimental clinical effects.

Subsequent to these preliminary studies a randomised, double blind study of the effects of *Lactobacillus GG* in preterm infants was undertaken. Twenty preterm infants with a gestational age of 33 weeks or less who were admitted to the neonatal unit of the Princess Anne Hospital, Southampton between 1 September 1991 and 31 January 1992 were studied. After informed parental consent had been obtained the infants were randomised to receive either milk feeds with *Lactobacillus GG* (group A) or unsupplemented milk feeds (group B). The infants received a variety of milks, including expressed breast milk, formula, or preterm formula. In some instances a mixture of milks was given, according to the parent's choice and the infant's clinical requirements. The median gestational age and birth weight in group A were 30.5 weeks (range 26–33) and 1445 g (range 800–2560) and in group B were 30 weeks (range 24–33) and 1500 g (range 830–2150). Six of 10 in group A and two of 10 in group B were born by caesarean section. Two in each group were born after prolonged rupture of membranes (>24 hours).

Those infants randomised to receive *Lactobacillus GG* were given 10^8 cfu twice a day in milk from the initiation of milk feeds for 14 days. All the infants were studied for their full stay in the neonatal unit. Every infant was examined daily by a physician who was actively involved in the care of the infants on the neonatal unit but who was not aware of the study randomisation schedule. The following clinical details were recorded daily: general well being, any signs of abdominal distension, vomiting or regurgitation, feed intolerance, the incidence of perineal rash, the frequency and consistency of stools, the number of suppositories used, and the fluid intake. The exact type and amount of fluid was recorded and the total energy intake was then calculated. The weight of each infant was recorded three times weekly. Other clinical variables evaluated included any clinical or laboratory evidence of sepsis, antibiotic treatment or any other concomitant medication, and oxygen and ventilatory requirements; the duration of hospital stay was also calculated for each group.

Samples of milk supplemented with *Lactobacillus GG* were collected daily for culture on selective and non-selective media to ensure that they were not contaminated with other bacteria or with yeasts. Faecal samples

were collected each day and sent in a GasPak Pouch (BBL Microbiology Systems, PO Box 243, Cockeysville, MD 21030, USA) to the microbiology laboratory for quantitative studies. Samples were not collected from infants after discharge from hospital. Samples were labelled with the names of infants but the randomisation group was not given to the laboratory staff carrying out the quantitative bacteriology. Weighed faecal samples were stored at -70°C in glycerol citrate broths usually within 24 hours of collection. The maximum time that elapsed before samples were frozen was 72 hours. The dry weight for a known wet weight was determined for each sample that was stored. Quantitative bacteriology was performed within four months of sample storage using the methods that have been previously described.⁴ Samples selected for quantitative bacteriology were those collected on the day of oral feeding and seven, 14, 21, 28, and 35 days after the introduction of oral feeds. If samples were not available for the exact day, then the next sample passed was selected, providing it was within the next 72 hours. The day on which enterobacteriaceae were first isolated was determined by inoculation of an aliquot from all of the stored samples from each infant on to MacConkey agar. Identification of isolates was with standard laboratory methods.¹⁷ Members of the family enterobacteriaceae were speciated using the Mast ID system (Mast Laboratories, Bootle). Isolates described as *Lactobacillus GG* were defined as Gram positive bacilli, catalase negative, forming large white colonies on Rogosa's agar in air after 48 hours incubation at 37°C , hydrolysing aesculin and fermenting mannitol, sorbitol, amygdalin, glucose and dulcitol, and which did not ferment lactose, maltose, xylose, rhamnose, sucrose, melibiose, or inositol.

The Mann-Whitney U test was used to compare any differences in the numbers (\log_{10} cfu/g dry weight) of bacteria at each time after feeding in the two groups. With regard to the clinical data, both the Mann-Whitney U test and Student's *t* test were used depending on the distribution of the data.

Results

Lactobacillus GG produced colonies with a distinct appearance after 48 hours at 37°C on Rogosa's agar. Colonies were discrete, domed, opaque, white, and 2–4 mm in diameter. Isolates indistinguishable from *Lactobacillus GG* were isolated from the faeces of all but one of the infants who received feed supplementation with *Lactobacillus GG*. Figure 1 shows the numbers of *Lactobacillus GG* and the proportion of infants with bowel colonisation declined after feed supplementation was discontinued, but samples from four of the seven infants from whom samples were available at five weeks after starting feeds and three weeks after discontinuing feed supplementation still showed bowel colonisation with *Lactobacillus GG* in numbers ranging from 5.8 to $10.0 \log_{10}$ cfu/g dry weight. The mean numbers (mean \log_{10} cfu/g dry weight) of *Lactobacillus GG*

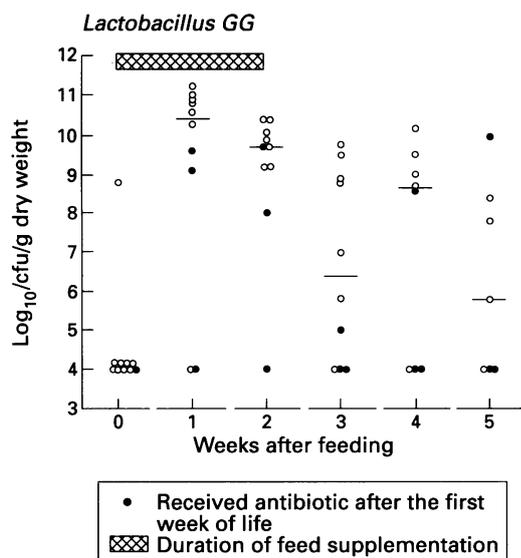


Figure 1 Quantitative changes in *Lactobacillus GG* and age after feeding; bars show median.

were 9.16 at seven days after the start of nasogastric feeding, 9.04 at 14 days, 6.7 at 21 days, 7.23 at 28 days, and 6.3 at 35 days.

Lactobacillus GG was isolated from one infant who did not receive feed supplementation with *Lactobacillus GG*. This infant was one of twins, the other infant was receiving supplementation with *Lactobacillus GG* and was being nursed in an adjacent cot. The micro-organisms isolated from stool samples included enterobacteriaceae (*Escherichia coli*, *Klebsiella* sp, *Enterobacter* sp, *Serratia* sp, *Citrobacter* sp, *Proteus* sp, and *Acinetobacter* sp), staphylococci (coagulase negative and *Staphylococcus aureus*), yeasts, *Enterococcus* sp, *Bacteroides* sp, *Clostridium* sp, *Veillonella* sp, *Bifidobacterium* sp, and other unidentified anaerobic or microaerophilic Gram positive bacilli, *Bacillus* sp, and *Lactobacillus GG*. The proportion of infants colonised by the different bacterial species at each week after feeding was similar in the two groups. Despite clear evidence of bowel colonisation in the majority of those who received feed supplementation with *Lactobacillus GG*, there were no significant differences between the two groups with regard to the numbers of enterobacteriaceae, coagulase-negative staphylococci, *Enterococcus* sp and anaerobes at any week after feeding. The numbers of *Lactobacillus GG* and the proportion of infants with bowel colonisation declined after feed supplementation was discontinued. The mean numbers of distinct colonial types in faecal samples in the two groups at each week after feeding are shown in the table.

Enterobacteriaceae were first isolated at a mean of 7.75 days after feeding in group A compared with 8.0 days in group B.

Mean number of colonial types/infant at each week after feeding (excluding *Lactobacillus GG*)

Group	Week					
	0	1	2	3	4	5
A	1.9	3.7	4.3	4.8	5.6	5.6
B	1.9	3.7	4.8	5.0	5.8	5.4

The number of enterobacteriaceae, coagulase negative staphylococci, *Enterococcus* sp, anaerobes, *Lactobacillus GG* at each week after feeding is shown in figs 2-5. There were no significant differences between the two groups in the quantitative microbiology of the faeces at any week after feeding.

There were no significant differences between the two groups for any of the clinical parameters recorded. There were no differences between the two groups in the numbers of infants ventilated, the duration of ventilation, frequency of oxygen treatment, and the incidence of chronic lung disease. The infants in group A spent a median time of 51.1 days (range 23-136) on the neonatal unit compared with 42.8 days in group B (range 19-114). This was not a significant difference using the Mann-Whitney U test. The mean (SD) weight gain was 21.55 (9.22) g/kg/day in group A and 22.40 (7.91) g/kg/day in group B. Fluid intake was 242 ml/kg/day in group A and 243 ml/kg/day in group B, and energy intake 690 and 719 kJ (165 and 172 kcal)/kg/day.

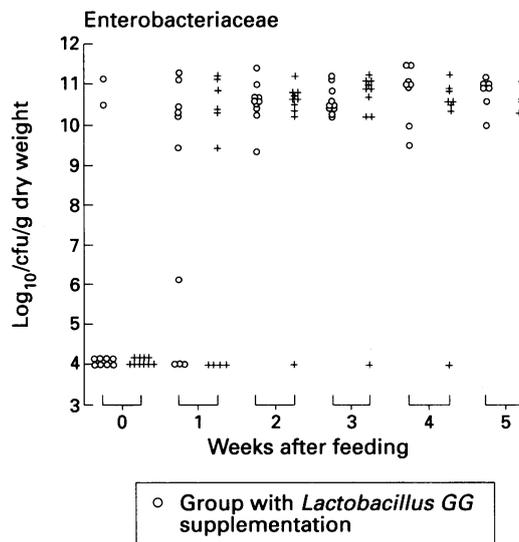


Figure 2 Quantitative changes in enterobacteriaceae and age after feeding.

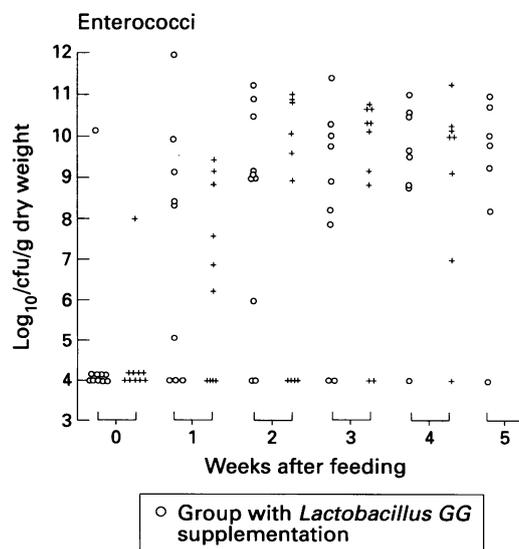


Figure 3 Quantitative changes in enterococci and age after feeding.

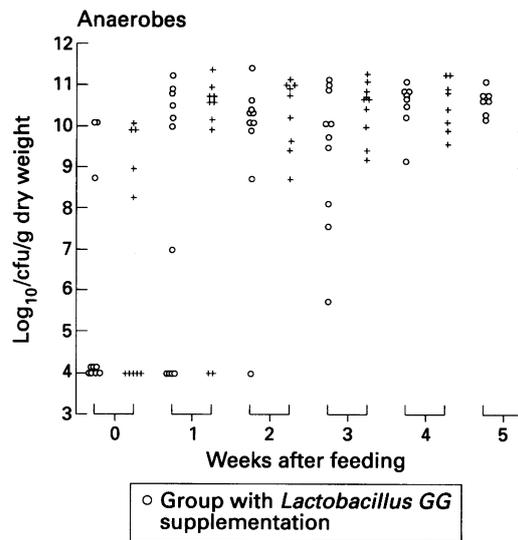


Figure 4 Quantitative changes in anaerobes and age after feeding.

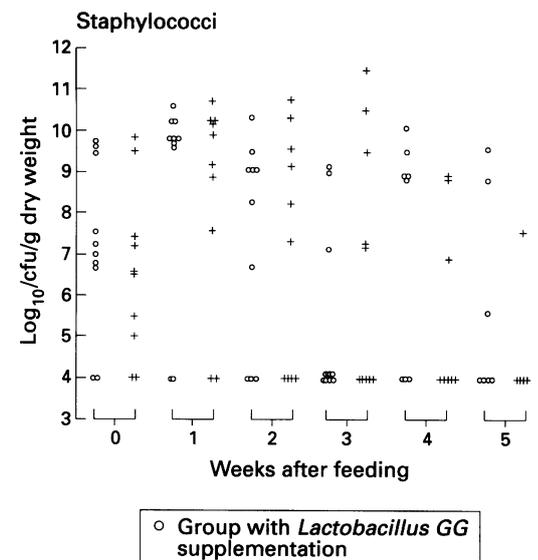


Figure 5 Quantitative changes in staphylococci and age after feeding.

Intravenous antibiotic treatment was administered to eight of the 10 infants who were given feed supplementation with *Lactobacillus GG* and to seven of the infants in group B. Antibiotic treatment in the first week of life did not seem to affect colonisation with *Lactobacillus GG*. However, three of the infants in group A did receive intravenous flucloxacillin and netilmicin after the first week of life and in each case there was a more than four times \log_{10} fall in the numbers of *Lactobacillus GG* in the seven days after the start of antibiotic treatment. The only infant who showed no evidence of colonisation with *Lactobacillus GG* had received intravenous cefotaxime from soon after birth and was then treated continuously with a variety of parenteral antibiotics until the 14th day of life. There were no cases of proved sepsis in either of the two groups at any time, although one of the infants in each group was found to have perineal candida infection that required treatment with topical and oral nystatin.

Discussion

Lactobacillus and *Bifidobacterium* sp in fermented dairy products can survive the gastric acid barrier.^{18 19} However attempts to establish bowel colonisation of adult volunteers with *Lactobacillus bulgaricus*,²⁰ *Bifidobacterium* sp,¹⁸ or with *E coli* strains isolated from humans²¹ or animals have failed to show persistent long term colonisation. The opportunity for bacteria to colonise the bowel of neonates in intensive care units is limited by control of infection procedures and use of broad spectrum antibiotics, so that the bowel flora of these infants is much less complex than that of adults and establishment of colonisation is less likely to be prevented by competitive inhibition. Reuman *et al* reported a study using *Lactobacillus acidophilus*,²² and found that the proportion of infants from whom lactobacilli could be cultured from stools was increased compared with a placebo group. In our study *Lactobacillus GG* colonised the bowel of some infants for

more than three weeks after supplementation was discontinued. They found no evidence that oral feeding of *L acidophilus* reduced bowel colonisation with facultative Gram negative bacteria. Similarly, in our study we found no qualitative or quantitative differences in faecal bacteriology in infants with or without feed supplementation with *Lactobacillus GG* despite evidence of colonisation. As in our study Reuman *et al* demonstrated no beneficial effect on weight gain, formula intake, morbidity, or mortality.

The possible side effects of oral feeding with *Lactobacillus GG* include infection and adverse metabolic effects. There were no episodes of infection attributable to *Lactobacillus GG* in the small number of infants included in this study. *L casei* does not ferment lactose to D-lactate²³ so that D-lactate acidosis is an unlikely complication. The metabolic consequences of oral feeding with *Lactobacillus GG* are addressed in the companion paper in this issue.²⁴

The absence of evidence of colonisation with *Lactobacillus GG* in one infant, the eradication of colonisation in another, and a considerable fall in the level of colonisation of a third infant were associated with the use of flucloxacillin and netilmicin after the first week of life, suggesting that intravenous flucloxacillin and netilmicin may have a profound influence on bowel colonisation with *Lactobacillus GG* in preterm infants. The transient colonisation of one infant not given feed supplementation with *Lactobacillus GG* may have arisen as a result of cross colonisation from a twin given feed supplementation with *Lactobacillus GG* or as a result of accidental feeding with supplemented feed.

The results of this study do not preclude the possibility that *Lactobacillus GG* colonisation modified intestinal substrate metabolism⁹ or the pattern of small bowel colonisation. There is evidence from animal and chemostat models that colonisation with lactobacilli reduces *E coli* colonisation of the small intestine more effectively than the large intestine.²⁵ This study has

clearly shown that *Lactobacillus GG* can colonise the bowel of premature infants. However bowel colonisation with *Lactobacillus GG* did not reduce the large bowel reservoir of nosocomial pathogens. The findings of this study suggest that feed supplementation with *Lactobacillus GG* has little or no clinical or bacteriological benefit for premature infants.

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