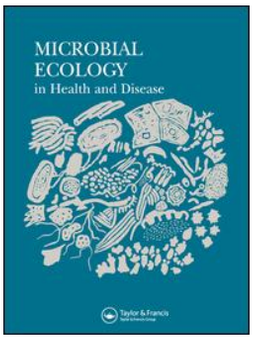

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Safety and Tolerance of *Lactobacillus reuteri* in Healthy Adult Male Subjects

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Thirty healthy, male subjects (age 18 to 75 yrs) were used in a two-group, double-blinded, parallel design to evaluate the safety and tolerance of a potential probiotic organism, *Lactobacillus reuteri*. Subjects (15/treatment) consumed two gelatin capsules daily for 21 d that contained either a freeze dried *L. reuteri* preparation in a cryoprotectant, or a placebo (cryoprotectant). The concentration of *L. reuteri* was such that subjects consumed 1×10^{11} CFU per day. The study was 28 d in length with daily documentation of the presence of gastrointestinal symptoms (nausea, diarrhoea, cramping, distention, flatulence, vomiting, constipation, burping and reflux). In addition, serum chemistries, haematology, urinalysis, urinary indican excretion, and faecal microbiota (*L. reuteri* and total *Lactobacillus* spp. enumeration) were measured weekly (day 0, 7, 14, 21 and 28). A physical exam was given on day 0, 21, and 28. An additional faecal sample was obtained on day 77 for microbial enumeration. Subjects could consume their regular diets; however, alcohol was not allowed. Physical exam and urinalysis parameters were not clinically different between treatments. Supplemental *L. reuteri* reduced ($P<0.05$) urinary indican excretion at day 7, but had no effect ($P>0.05$) on subsequent urine collections. Although significant differences were observed for a few of the serum chemistry and haematology variables, all of the values remained within the expected normal range for healthy adult males. Subjects consuming supplemental *L. reuteri* had increased ($P<0.01$) levels of *L. reuteri* in their faeces on day 7, 14, 21, and 28. However, colonisation was lost within 2 mths of termination of *L. reuteri* consumption (day 77). Levels of total *Lactobacillus* spp. never differed ($P>0.05$) between treatments; however, the ratio of *L. reuteri* : total *Lactobacillus* spp. increased ($P<0.05$) for subjects consuming supplemental *L. reuteri*. Incidence of subjective tolerance factors was infrequent and similar for both treatments. In conclusion, supplemental *L. reuteri* may be fed at 1×10^{11} CFU/day without any clinically significant safety or tolerance problems. Intake of *L. reuteri* (1×10^{11} CFU/day) results in colonisation (as measured by faecal level) within 7 d of consumption and is maintained for at least 7 d post consumption; however, colonisation is lost within 2 mths of washout.

KEY WORDS: *Lactobacillus reuteri*; safety and tolerance; faecal lactobacilli level.

INTRODUCTION

A probiotic has been defined as a mono- or mixed-culture of live microorganisms that beneficially affects the host by improving the properties of the indigenous microbiota when consumed.[1] The belief in the beneficial effects of the probiotic approach is based on the knowledge that intestinal microbiota provide protection against various diseases. Evidence for such a belief comes from numerous sources. First, it has been shown that germ-free animals are more susceptible to disease than are their conventional counterparts with a complete gut microbiota.[3,17] Additional evidence supporting the protective effect of the gut microbiota is the finding that animals, including humans, are more susceptible to infection by organisms such as *Clostridium difficile* after receiving certain antibiotics. A final source of supporting evidence comes from experiments involving faecal suspensions. Faecal enemas derived from healthy human adults have been effective in treating recurrent *C. difficile* infection.[21]

Many probiotic organisms have been investigated with much of the work involving lactic acid bacteria such as the lactobacilli, streptococci, and

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bifidobacteria. It has been hypothesised that re-establishment of the intestinal lactobacilli after discontinuation of antimicrobial therapy will be followed by normal intestinal function. Thus, the consumption of viable intestinal lactobacilli should hasten the return of the intestinal microbiota to a favourable state. One microorganism of particular interest is *Lactobacillus reuteri*, a normal inhabitant of the gastrointestinal tract of healthy humans and many animals.[1,2] Like other lactobacilli, *L. reuteri* produce acidic metabolic end-products (lactic and acetic acids) which have considerable antimicrobial activity, particularly in relatively acidic (pH 5·0) environments. It was recently discovered that metabolism of glycerol by *L. reuteri* can result in excretion of a metabolic intermediate, 3-hydroxypropionaldehyde, which has greater antimicrobial activity against potential pathogens such as *Salmonella*, *Clostridium*, *Listeria*, and *Escherichia* species than against many of the normal constituents of the intestinal microbiota.[1,2] It is thought that this antimicrobial activity contributes to the survival of *Lactobacillus reuteri* cells within their gastrointestinal ecosystem.

Considering the potential of *L. reuteri* to treat and(or) prevent various gastrointestinal infections (maintain microbial homeostasis), *L. reuteri* may serve as an effective probiotic. However, prior to use in a patient population, it is necessary that safety and tolerance data be collected in healthy adult subjects. The objectives of this study were: (1) to assess the effect of *L. reuteri* on gastrointestinal symptoms; (2) to determine the effect of *L. reuteri* on serum chemistries, haematology, and urinary profiles; and (3) to determine the effect of consumption of *L. reuteri* on the faecal level of *L. reuteri* and total *Lactobacillus* spp. in healthy adult male subjects.

METHODS

Subject selection

Thirty healthy males were selected as subjects according to the following criteria: (1) Interest in participating in the study after (a) being fully informed about the experimental treatments and the experimental procedures, (b) reviewing the study procedures, and (c) signing a subject consent form. (2) Subjects' willingness to complete all necessary study questionnaires on a daily basis. (3) Blood chemistries within a normal range or not considered clinically significant if outside the normal range. (4) Subjects' assurance that they are free from known metabolic or gastrointestinal diseases and have no known food allergies. (5) Subjects' assurance that they have not taken antibiotics for a period up to 1 mth prior to the start of the study. (6) Subject is not taking medications that would interfere with nutrient absorption, metabolism, or excretion, or compromise gut microbiota.

Experimental design

This study protocol was approved by the Ohio Valley Institutional Review Board (Evansville, IN). Thirty healthy, male adults (age 18 to 75 yrs) participated in this two-group, double-blind, parallel 28 d study. Subjects were randomly assigned to two treatments. Fifteen subjects consumed a placebo and 15 subjects consumed *L. reuteri*. The *L. reuteri* (strain MM53; ATCC SD2112) used in this study was isolated from human milk. *L. reuteri* was grown in proprietary selective media (Marschall Products a division of Rhône-Poulenc, Madison, WI, USA) and then lyophilised in the presence of a cryoprotectant (nonfat dry milk powder, maltodextrin, and sucrose). The lyophilised organism was weighed into gelatin capsules at a level of 5×10^{10} CFU per capsule (0·42–0·45 g). Similarly, placebo capsules were filled with the cryoprotectant. Subjects consumed two capsules daily (1×10^{11} CFU) for the first 21 d of the study. Subjects were urged to take the capsules 12 h apart. With the exception of alcohol, subjects were allowed to maintain their normal dietary habits. Throughout the 28 d study, the occurrence and severity of gastrointestinal symptoms (nausea, diarrhoea, cramping, distention, flatulence, vomiting, constipation, burping, and reflux) were recorded. Serum chemistries, haematology, urinalysis, indican excretion, faecal fat analysis, and counts of faecal microbiota were measured weekly (day 0 [baseline], day 7, day 14, day 21, and day 28). Physical exams were performed at baseline and on day 21 and 28. Following review of the day 28 *L. reuteri* data, the study protocol was amended to include the collection of an additional faecal sample on day 77 of the study (actually day $79{\cdot}6 \pm 0{\cdot}96$).

Physical exam

Physical exams were conducted on day 0, 21, and 28. The following variables were obtained at each exam: body weight, blood pressure (systolic

and diastolic), pulse rate, respiratory rate, and oral temperature. These parameters along with serum chemistries, haematology and urinalysis documented the subject's 'health' prior to study initiation, after 21 d on the treatment, and after a 7 d washout (day 28). Washout refers to the time in which subjects are not consuming their dietary supplement.

Collection of biological samples

Faeces. Faecal samples were taken from the first defaecation on day 0, 7, 14, 21, 28 and 77. One faecal sample for each day was placed in a preweighed screw cap plastic tube containing 15 ml Cairy-Blair transport media and one sample was placed in a plain screw cap plastic tube. The plain sample (no Cairy-Blair) was analysed for faecal fat. The remaining sample (in Cairy-Blair) was weighed, quickly frozen and stored at − 70°C until shipped to BioGaia Biologics, Inc. (Raleigh, NC) on dry ice for enumeration of bacteria (*L. reuteri* and total *Lactobacillus* spp.).

Urine. Total urine (24 h collection) was obtained on day 0, 7, 14, 21, and 28. Each 24 h collection was preserved with a 100 mg tablet of boric acid. An aliquot of urine was taken for urinalysis and indican analysis.

Blood. Fasting blood samples were drawn by venipuncture on day 0, 7, 14, 21, and 28. Blood was drawn into EDTA tubes for haematology and serum tubes for serum chemistries. All blood was obtained prior to the morning meal following an 8–12 h fast.

Analysis

Gut microbiota. Lactobacillus species and *L. reuteri* were enumerated using standard anaerobic microbiological techniques and techniques developed by BioGaia Biologics, Inc. (Raleigh, NC). Briefly, serially diluted specimens were seeded on LBS agar (BBL 11327, Becton Dickinson, Cockeysville, MD 21030 USA) containing an additional 1·32 ml glacial acetic acid/l and incubated anaerobically utilising GasPak Plus (BBL 71040) at 37°C. Colonies which showed good growth after 48 to 72 h, stained gram-positive, and exhibited bacillus morphology were considered to be lactobacilli. *L. reuteri* (strain MM53) were differentiated from other lactobacilli by their ability to produce reuterin. When necessary, carbohydrate fermentation patterns of selective isolates were determined by the API 50CH system. Strain MM53 was readily distinguished from other strains of *L. reuteri* by its unique colony characteristics (2 to 4 mm in diameter, white, opaque, smooth circular, convex, entire edge and glistening). In this study *L. reuteri* colonisation has been defined as greater than 1×10^4 CFU *L. reuteri*/g wet faeces. This level was chosen as study subjects that were colonised at baseline determination had approximately 10^4 CFU *L. reuteri*/g wet faeces.

Faecal fat. Faecal samples were analysed for total fat (qualitative analysis). Briefly, a small amount of stool was emulsified with 95% ethanol, stained with Sudan IV, viewed microscopically and scored as follows: none seen, within normal limits, or abnormal high.

Serum chemistries. Serum samples were analysed for the following: albumin; albumin/globulin ratio; alkaline phosphatase; alanine amino transaminase (ALT); aspartate amino transaminase (AST); total bilirubin; blood urea nitrogen (BUN); BUN/creatinine ratio; calcium; chloride; cholesterol; high density lipoprotein (HDL) cholesterol; low density lipoprotein (LDL) cholesterol; very low density lipoprotein (VLDL) cholesterol; total cholesterol/HDL ratio; creatinine; gamma glutamyl transpeptidase (GGT); globulin; glucose; iron; lactic acid dehydrogenase (LDH); phosphorus; potassium; total protein; sodium; triglycerides; and uric acid.

Haematology. Blood samples were analysed for the following: differential per cent (neutrophils, lymphocytes, monocytes, eosinophils, and bands); haematocrit; haemoglobin; mean corpuscular volume (MCV); mean corpuscular haemoglobin (MCH); mean corpuscular haemoglobin concentration (MCHC); platelet count; red blood cell (RBC) count; and white blood cell (WBC) count.

Urinalysis. An aliquot of preserved urine was analysed for the following: clarity; colour; specific gravity; pH; protein; glucose; occult blood; ketones; and leukocyte esterase.

Indican. Indican is a putrefactive product produced from tryptophan and may be an indirect indicator of microbial balance (see Discussion). Indican concentration in the urine was performed by an HPLC method. Briefly, a 100 µl sample of

urine was diluted with 900 µl deionised water and filtered through a 0·4 µm teflon membrane. Aliquots (20 µl) of the diluted and filtered specimens were then analysed by gradient reverse phase HPLC using fluorescence detection. The detector was operated at an excitation wavelength of 280 nm and an emission wavelength of 345 nm. In addition, a 305 nm high pass filter was installed on the emission side. A six point calibration was done with standards ranging from 0·00976 to 0·312 mol/dl. Samples that fell outside the calibration window (high) were reanalysed at a higher dilution.

Subjective tolerance factors. Using a daily questionnaire, subjects were asked to report the frequency and severity of the following symptoms: nausea; diarrhoea; cramping; distention; flatulence; vomiting; constipation; burping; reflux; and other adverse reactions. Subjects used the following scale to rate severity of subjective tolerance factors if they were present: 1=mild; 2=moderate; 3=severe.

Statistical methods

For all continuous parameters (except levels of faecal microbiota), weekly change scores from baseline were calculated. Concentrations of faecal microbiota were transformed and reported in $\log_{10}$ scale and groups were compared for the means at each day of data collection. All continuous data were analysed with a Two Sample t Test or Wilcoxon Rank Sum Test where appropriate. Discrete data were analysed with a Fisher's Exact Test on each study day for which data were collected. All results were considered statistically significant if the significance level was less than 5 per cent.

RESULTS

Thirty subjects were enrolled. Edits made to the data base are as follows: (1) two subjects were deemed noncompliant as they consumed less than 75 per cent of the study capsules; (2) the study day 0 and 7 serum chemistries obtained for one subject were not fasting blood samples; and (3) the baseline faecal samples for three subjects were actually obtained 1–2 d post 'baseline;' subsequently these data were not tabulated or used for analysis. There were many instances where 'below the detection threshold' was noted for faecal levels of *L. reuteri*. For analyses, the value representing the halfway point between zero and the threshold was used.

No differences ($P>0·05$) were found throughout the study for changes in: body weight; pulse rate; systolic and diastolic blood pressure; and body temperature. There was a significant ($P=0·017$) difference for change from baseline at day 28 for respiratory rate between the two treatments. The data show that the placebo produced a greater reduction in respiratory rate.

Data for serum chemistries associated with heart, liver, and kidney function; protein balance; and bone maintenance are presented in Table 1. Haematology and the other remaining serum chemistry data are not presented. Of the serum chemistries measured, albumin/globulin ratio (*L. reuteri*>placebo), globulin (placebo>*L. reuteri*), and iron (placebo>*L. reuteri*) were significantly ($P<0·05$) different between treatments at baseline. Using Analysis of Covariance, iron was different ($P=0·016$) between treatments (*L. reuteri*> placebo) at day 28. Change from baseline in calcium (day 14, $P=0·002$; placebo>*L. reuteri*), creatinine (day 28, $P=0·015$; placebo>*L. reuteri*), potassium (day 7, $P=0·025$; placebo>*L. reuteri*), and GGT (day 7, $P=0·047$; *L. reuteri*>placebo) were significantly different between treatments. For several haematology parameters the change from baseline was found to be statistically different between treatments. Percentage of neutrophils at day 28 (placebo>*L. reuteri*) and percentage of lymphocytes at day 7 and 28 (*L. reuteri*>placebo, all calculated as change from baseline) were significantly ($P=0·041$, 0·024, and 0·013, respectively) different between treatments. In addition, MCHC was significantly ($P=0·035$) different at day 28 showing a greater change from baseline for subjects consuming the placebo versus supplemental *L. reuteri*.

Neither urinary pH nor specific gravity were affected ($P>0·05$) by supplemental *L. reuteri* (data not shown). Supplemental *L. reuteri* decreased ($P=0·028$) urinary indican excretion at day 7, but had no effect at subsequent urine collections (Table 2). No differences ($P>0·05$) were noted in any of the qualitative urinary parameters measured (data not shown). In addition, the faecal fat qualitative analysis showed either no or normal limits of faecal fat in both treatments (data not shown).

Because *L. reuteri* is a ubiquitous organism of the small intestine, a few subjects had a detectable level of *L. reuteri* at the baseline measurement. In fact, four subjects consuming the placebo, during at least one sampling time, had a detectable level of

Table 1. Effect of supplemental *L. reuteri* on serum chemistries (mean ± SEM) in healthy adult male subjects[a]

Parameter	Treatment	Baseline	Day 7	Day 14	Day 21	Day 28
Calcium[bc], mg/dl	Placebo	9·2 ± 0·11	9·0 ± 0·15	9·4 ± 0·19**	9·0 ± 0·13	9·8 ± 0·14
(8·5–10·4)[b]	*L. reuteri*	9·3 ± 0·10	9·0 ± 0·07	9·1 ± 0·07	9·1 ± 0·10	9·7 ± 0·10
Phosphorus[d], mg/dl	Placebo	3·9 ± 0·11	4·1 ± 0·16	4·3 ± 0·15	4·0 ± 0·14	4·2 ± 0·18
(2·5–4·8)[b]	*L. reuteri*	4·1 ± 0·13	4·3 ± 0·12	4·5 ± 0·19	4·2 ± 0·16	4·3 ± 0·14
Total bilirubin[c], mg/dl	Placebo	0·7 ± 0·11	0·6 ± 0·07	0·6 ± 0·05	0·5 ± 0·07	0·5 ± 0·04
(0·1–1·2)[b]	*L. reuteri*	0·6 ± 0·09	0·5 ± 0·04	0·5 ± 0·06	0·6 ± 0·06	0·6 ± 0·07
Alkaline phosphatase[c], U/l	Placebo	67 ± 4·26	72 ± 3·86	73 ± 4·37	76 ± 4·37	73 ± 4·28
(34–114)[b]	*L. reuteri*	66 ± 4·01	75 ± 3·87	77 ± 4·16	75 ± 4·37	78 ± 4·48
Aspartate amino transaminase[c],	Placebo	23 ± 1·19	25 ± 2·51	26 ± 2·24	22 ± 1·31	20 ± 0·99
U/l (0–33)[b]	*L. reuteri*	22 ± 2·01	22 ± 1·28	25 ± 2·09	21 ± 1·44	25 ± 4·83
Alanine amino transaminase[c],	Placebo	20 ± 1·53	19 ± 1·74	20 ± 3·86	14 ± 2·10	13 ± 2·07
U/l (0–43)[b]	*L. reuteri*	17 ± 2·55	16 ± 2·79	19 ± 3·62	15 ± 3·41	18 ± 6·94
Albumin[d], g/dl	Placebo	4·9 ± 0·08	4·8 ± 0·08	4·8 ± 0·09	4·9 ± 0·09	5·0 ± 0·07
(3·9–5·1)[b]	*L. reuteri*	5·0 ± 0·06	4·8 ± 0·06	4·8 ± 0·06	5·0 ± 0·05	5·1 ± 0·06
GGT[c], U/l	Placebo	21 ± 2·46	20 ± 2·08*	20 ± 2·19	21 ± 1·78	21 ± 1·79
(0–57)[b]	*L. reuteri*	20 ± 2·56	21 ± 2·35	21 ± 2·34	22 ● 2·84	23 ± 3·25

[a]Difference from baseline between treatments is significant (*$P<0·05$; **$P<0·01$).
[b]These values reflect the normal range. Values were provided by GFI Pharmaceutical Services, Inc. (Evansville, IN).
[c,d]Parameter tested using the Wilcoxon Rank Sum Test or Two Sample *t* Test, respectively.

L. reuteri. Subjects consuming supplemental *L. reuteri* had increased ($P<0·01$) faecal levels of *L. reuteri* at day 7, 14, 21, and 28 compared to the placebo group (Table 3). Only two subjects (both on supplemental *L. reuteri*) had detectable levels of *L. reuteri* on day 77. Supplemental *L. reuteri* had no effect ($P>0·05$) on total *Lactobacillus* spp. enumerated from faeces (Table 3). Subjects consuming *L. reuteri* had an increased ($P<0·01$) ratio of *L. reuteri*: total *Lactobacillus* spp. on day 7, 14, 21, and 28, however, at day-77 ratios were similar for both treatments (Figure 1). More subjects consuming *L. reuteri* ($P<0·01$) were colonised at day 7, 14, and 21 and more tended ($P=0·054$) to be colonised at day 28 (Figure 2).

Table 2. Effect of supplemental *L. reuteri* on urinary indican excretion (mol/day ± SEM) in healthy adult male subjects

Day	Treatment		P[a]
	Placebo	*L. reuteri*	
0 (baseline)	268 ± 34·89	329 ± 42·19	0·278
7	296 ± 45·88	253 ± 35·36	0·028
14	284 ± 40·77	279 ± 50·00	0·146
21	237 ± 27·79	289 ± 39·98	0·842
28	247 ± 34·07	262 ± 45·68	0·327

[a]Other than baseline, *P*-values respresent the level of significance in change from baseline between treatments with Two Sample *t* Test.

There were a few instances where an individual subject reported the same gastrointestinal tolerance symptom on more than one occasion on the same study day. The average severity for that symptom on that day for the subject was calculated. Each symptom was then summarised with frequencies and reported as both overall percentage of patients and percent of patient days with that particular symptom. Of the subjective tolerance factors measured flatulence was the only factor noted in more than 1 per cent of the study days (data not shown). Mild flatulence was noted on 2·3 per cent of the study days for subjects consuming the placebo. Subjects consuming *L. reuteri* reported flatulence as mild on 5·61 per cent, moderate on 0·51 per cent, and severe on 0·51 per cent of the study days. Diarrhea was noted as severe in 0·51 per cent (total of 2 days) of study days for subjects consuming *L. reuteri* whereas no diarrhoea was noted for subjects on placebo. However, one incidence of diarrhoea occurred during the washout period. On the other hand, cramping was noted in 0·77 per cent of study days for

Table 3. Effect of supplemental *L. reuteri* on faecal levels of *L. reuteri* and total *Lactobacillus* spp. (log_{10} CFU/g ± SEM) in healthy adult male subjects[a]

Parameter	Treatment	Baseline	Day 7	Day 14	Day 21	Day 28	Day 77[b]
L. reuteri	Placebo	1·98 ± 0·28	2·33 ± 0·35**	2·34 ± 0·44**	1·97 ± 0·27**	2·45 ± 0·41**	2·70 ± 0·00
	L. reuteri	2·05 ± 0·25	5·01 ± 0·35	4·73 ± 0·33	5·41 ± 0·32	4·45 ± 0·48	3·09 ± 0·28
Lactobacillus spp	Placebo	7·93 ± 0·27	7·52 ± 0·30	6·86 ± 0·32	6·49 ± 0·32	6·00 ± 0·31	8·31 ± 0·25
	L. reuteri	7·84 ± 0·34	7·16 ± 0·32	6·16 ± 0·33	5·94 ± 0·28	5·81 ± 0·37	7·77 ± 0·35

[a]Difference between treatments is significant (**$P<0·01$) with Wilcoxon Rank Sum Test.
[b]Day 77 *L. reuteri* data was not statistically analysed due to a different detection threshold in microbial enumeration (i.e., the detection threshold for day 0–28 was 10^2 versus 10^3 for data collected on day 77).

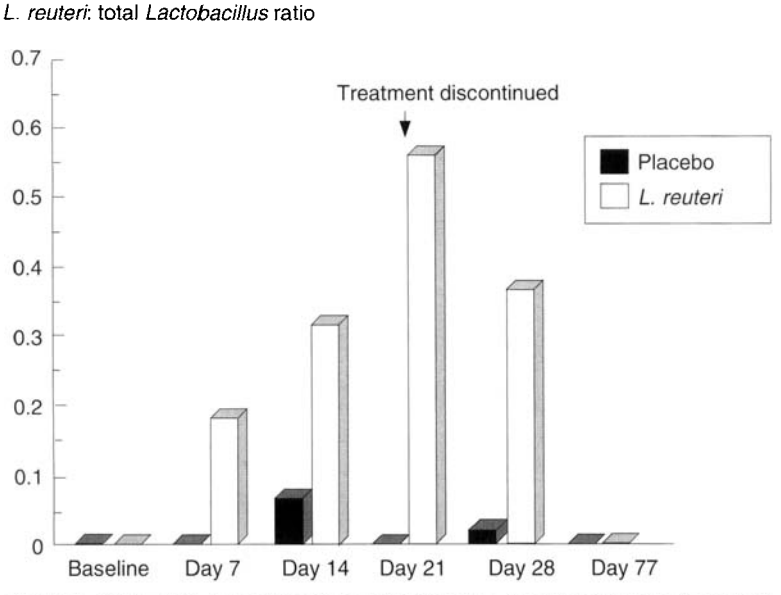

Figure 1. Ratio of *L. reuteri*: total *Lactobacillus* spp. in subjects consuming supplemental *L. reuteri* or a placebo

subjects on placebo and was absent for the *L. reuteri* group.

DISCUSSION

Studies with probiotics (e.g., lactobacilli and bifidobacteria) have shown several putative health benefits to include: aid digestion of lactose, stimulation of the immune system, anti-tumourigenic, anti-carcinogenic, serum cholesterol reduction, prevention/treatment for some diarrhoeas, prevention of vaginal yeast infections, and alleviation of constipation.[20] For a culture to be considered a viable candidate for use as a dietary adjunct, it must be a normal inhabitant of the intestinal tract, survive passage through the upper digestive tract, be capable of surviving and growing in the intestine, produce beneficial effects when in the intestine, and maintain viability and activity in the carrier food before consumption.[9] As mentioned previously, *L. reuteri* is a natural inhabitant of the human gastrointestinal tract (specifically the ileum). *Lactobacillus reuteri* (strain MM53) was isolated from human milk and is currently sold in commercial milk in Sweden (BRA milk).

Most research to date involves the use of *L. reuteri* in the poultry industry. BioGaia Biologics, Inc. currently markets *L. reuteri* (GaiaFeed™) to turkey producers in the US. Commercial data show improved feed efficiency, increased body weight and yields of breast and thigh, along with fewer mortalities with the GaiaFeed™ supple-

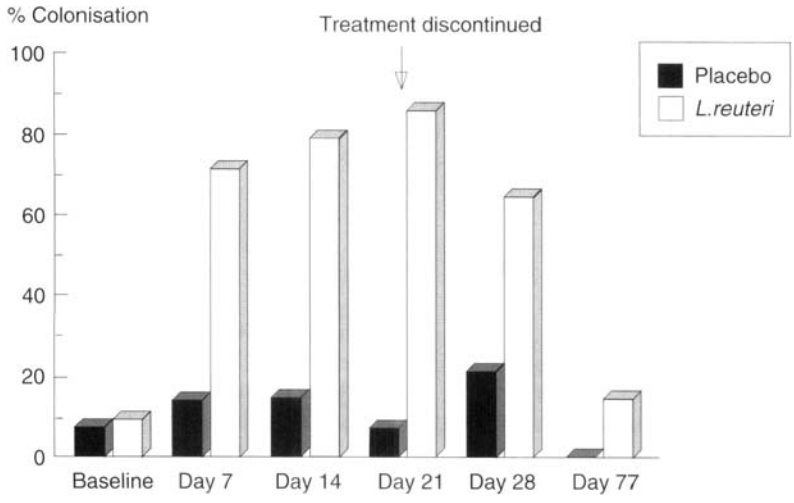

Figure 2. Effect of supplemental *L. reuteri* or placebo on percentage of subjects colonised by *L. reuteri* (colonisation defined as $>1 \times 10^4$ CFU/g wet faeces)

ment.[7] Commercially hatched and reared chicks and poults are poorly colonised by *L. reuteri* because eggs are hatched in an incubator rather than by the mother (natural source of *L. reuteri* for the young). Therefore, BioGaia suggests early colonisation of *L. reuteri* to reduce mortality and morbidity losses associated with environmental and pathogenic (e.g., Salmonella) stressors. Possible modes of action include competitive exclusion, antimicrobial activity of reuterin *in vivo*, elevating levels of blood constituents,[6] and(or) modulating the neonate's immune response.[5]

It has been shown that reuterin production increases in the presence of 'target' cells (e.g., *E. coli*).[2] Interestingly, other species of *Lactobacillus* and *L. reuteri* itself are more resistant to the antimicrobial activity of reuterin than potential pathogens such as *Salmonella*, *Clostridium*, *Listeria*, and *Escherichia* species. Reuterin is produced by *L. reuteri* grown anaerobically in the presence of glycerol.[23] Its spectrum of activity is exceptionally broad and includes gram-positive and gram-negative bacteria, yeasts, moulds and protozoa. This compound can occur in solution in three forms, one of which is a cyclised dimer. It has not been determined if all three forms are active.[24] It is thought that reuterin exerts its activity on ribonucleotide reductase.[24] In the living world, this enzyme universally catalyses the first step in DNA synthesis, which could explain reuterin's broad spectrum of activity. It is suspected that the antimicrobial activity of reuterin contributes to the survival of *L. reuteri* cells within the gastrointestinal ecosystem.

Molin *et al.*,[19] fed rats oatmeal soup fermented by six different *Lactobacillus* strains including two *L. reuteri* strains (one human and one rat isolate). The objective of this experiment was to determine the effect of fermented oatmeal soup on the cholesterol level and *Lactobacillus* colonisation of rat intestinal mucosa. No differences in cholesterol levels were found; however, *L. reuteri* (rat isolate) did colonise the intestinal mucosa. It represented about 30 per cent of the *Lactobacillus* population 24 days after termination of the administration. The human isolate of *L. reuteri* was unable to colonise the rat mucosa, supporting the hypothesis that the mucosal colonisation ability of lactobacilli is host specific.[4,14,22,25]

Similarly, Johansson *et al.*,[12] fed healthy human volunteers oatmeal soup fermented by 19 strains of *Lactobacillus* including two *L. reuteri* strains (one human and one rat isolate). The aim of this study was to compare the *in vivo* capacity of different *Lactobacillus* strains to colonise the human mucosa. Subjects consumed 5×10^8 CFU/day of each isolate in the fermented oatmeal soup. As in the previous study, the host specific nature of the *L. reuteri* was documented. Only the human strain of *L. reuteri* was able to colonise the subject's intestine.

Fabia and coworkers[8] evaluated the potential benefit of feeding *L. reuteri* to rats with acetic acid-induced colitis. Rats fed $3{\cdot}5 \times 10^8$ CFU *L.*

reuteri immediately after acetic acid administration did not develop colitis.

Several putative health benefits may be achieved by incorporating *L. reuteri* into the diet. For example, *L. reuteri* may promote the restoration of normal microbiota following antibiotic therapy or maintain a normal microbiota in patients. This may enhance colonisation resistance to pathogens or improve the microbial balance and prevent small bowel bacterial overgrowth and translocation.

Considering the potential benefits of *L. reuteri* in humans, the use of this ingredient may be warranted. As with all new functional ingredients, it is important that studies be conducted to identify (document) the positive effects of consuming the ingredient. Equally important, however, is the research that relates to the identification of any negative 'side effects' associated with the consumption of the functional food. The purpose of the present study was to access the safety and tolerance of *L. reuteri* in healthy male adults. In addition, documentation of *L. reuteri* survivability through the gastrointestinal tract was obtained.

Several serum chemistry and haematology variables were statistically different between treatments, however, these differences are not considered clinically significant. All values were within the normal range for healthy male adults throughout the study. These results suggest that supplemental *L. reuteri* does not compromise these specific serum chemistry or haematology variables.

Indican is a putrefactive product produced from tryptophan. Putrefactive products absorbed from the gut are conjugated with sulphuric or glucuronic acid in the liver and excreted in the urine. It has been hypothesised that by improving the microbial balance (i.e., reducing putrefactive organisms) less putrefactive substances would be produced, thus reducing the amount of potential carcinogens in the colon.[16,18] These data may suggest that *L. reuteri* is improving intestinal microbial balance thus reducing the amount of putrefactive substances produced in the intestine and then excreted in the urine. It should be noted that those subjects consuming *L. reuteri* with initially high levels of indican excretion were more responsive to the treatment (data not shown). However, the significant reduction in indican excretion seen at day seven did not continue to day 14 and 21 as would be expected (Table 2).

A faecal fat analysis was conducted to determine if feeding supplemental *L. reuteri* had a negative impact on fat absorption (i.e., inducing bacterial overgrowth). The qualitative analysis showed either no or normal limits of faecal fat in both treatments. Thus, it appears that supplemental *L. reuteri* had no negative impact on fat absorption.

Because *L. reuteri* is a ubiquitous organism of the small intestine a few subjects had a detectable level of *L. reuteri* at the baseline measurement. In fact, four subjects on placebo, during at least one sampling time, had a detectable level of *L. reuteri*. Subjects consuming supplemental *L. reuteri* had increased ($P<0{\cdot}01$) faecal levels of *L. reuteri* at day seven, 14, 21, and 28 compared to placebo. It should be mentioned that in this double-blind study, the reuterin producing strain MM53 type colony morphology was found only in samples of individuals fed *L. reuteri*. This evidence supports the validity of this method. Because *L. reuteri* was still present in the faeces after seven days of washout, a faecal sample was taken on approximately day 77 (2 mths washout). Only two subjects (both on *L. reuteri*) had detectable levels of *L. reuteri* on day 77; one of the two subjects which was colonised at day 77 was also colonised on day 0. This finding would be expected, as it is very difficult to displace the existing microbial population of a healthy individual. Similarly, Lidbeck *et al.*,[13] saw a reduction of lactobacilli back to normal within nine days after discontinuing *L. acidophilus* administration. Similar results have been reported.[10,15] From our data we can conclude that supplemental *L. reuteri* are able to survive passage through the stomach and maintain viability through the gastrointestinal tract, however, colonisation is lost sometime after one week. Thus, this finding suggests that supplemental *L. reuteri* should be taken continuously to maintain high levels of the probiotic, thus obtaining the expected effect on the intestinal microbiota. Colonisation is difficult to define using only faecal microbial levels. A true colonisation study would require sampling of gut tissue and enumeration of attached microbiota. It is uncertain how viability is affected during transit from the small bowel to the rectum. More subjects consuming supplemental *L. reuteri* ($P<0{\cdot}01$) were colonised at day 7, 14, and 21 and more tended ($P=0{\cdot}054$) to be colonised at day 28.

Supplemental *L. reuteri* had no effect ($P>0{\cdot}05$) on total *Lactobacillus* spp. enumerated from faeces, similar to the results reported by Goldin and co-authors.[10] Results suggest that feeding healthy humans supplemental *L. reuteri* does not increase the total level of *Lactobacillus* spp. but

increases the proportion of *L. reuteri* in that genus. This finding is contrary to that of Lidbeck and co-authors[13] who found that it was possible to increase the concentrations of lactobacilli by ingesting cultured low-fat acidophilus milk.

The incidence of flatulence appeared to be higher in the *L. reuteri* supplemented group, however, in over 93 per cent of the study days flatulence was reported as absent. Other adverse reactions noted in the study were headache and cold symptoms and are not considered to be treatment related. Few subjective tolerance problems were noted for either treatment throughout the study. We conclude that differences in subjective tolerance between treatments are not clinically significant.

The level of *L. reuteri* fed in this study (1×10^{11} CFU/day) is probably near the maximum that would be fed to a patient. With this in mind, we are confident in the tolerance and safety of this organism. In conclusion, supplemental *L. reuteri* may be fed at 1×10^{11} CFU/day with no clinically significant safety or tolerance problems. Intake of *L. reuteri* (1×10^{11} CFU/day) results in colonisation (as measured by faecal level) within seven days of consumption that is maintained for at least seven days post consumption, however, colonisation is lost within two months of washout.

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