

***Lactobacillus* GG Does Not Affect D-Lactic Acidosis in Diarrheic Calves, in a Clinical Setting**

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D-lactate, produced by gastrointestinal fermentation, is a major contributor to metabolic acidosis in diarrheic calves. *Lactobacillus rhamnosus* GG survives gastrointestinal transit in the neonatal calf and does not produce D-lactate. To determine whether this probiotic reduces gastrointestinal D-lactate production or severity of diarrhea or both, 48 calves (mean, 11 days old; range, 2–30 days) admitted to the clinic for treatment of diarrhea were randomly allocated to 2 groups. The experimental group was given *Lactobacillus rhamnosus* GG (1×10^{11} cfu/d) PO, dissolved in milk or oral electrolyte solution, in addition to clinic treatment protocols; the other group served as a control. Serum and fecal samples were obtained at admission and at 24 and 48 hours after initial administration of *Lactobacillus rhamnosus* GG. All samples were analyzed for D- and L-lactate by using high-pressure liquid chromatography. Feces were also analyzed for pathogens, *Lactobacillus rhamnosus* GG recovery, and dry matter. D-lactic acidemia (>3 mmol/L) was present in 37/48 calves at admission. *Lactobacillus rhamnosus* GG was recovered in the feces of 13 experimental calves and 0 control calves 24 hours after administration. No difference in serum or fecal D- or L-lactate between the groups was detected at any time point. After therapy, D-lactic acidosis was absent at 48 hours in all but 1 calf. No relation between fecal pathogen (viral, bacterial, or protozoal) and degree of D-lactic acidosis was observed. The reduction in mortality and greater fecal dry matter in *Lactobacillus rhamnosus* GG-treated calves was not statistically significant.

Key words: D-lactate; Metabolic acidosis; Neonatal diarrhea; Probiotics.

Acute enteric infections are the most important cause of morbidity and mortality in neonatal calves.¹ The major systemic effects of diarrhea are hypovolemia, acidemia, and electrolyte imbalances.² These problems are not unique to calves, but calves may suffer from more severe acidemia than other species.³ Historically, acidosis in diarrhea was assumed to be caused by a loss of bicarbonate in the feces or accumulation of L-lactate from tissue hypoxia resulting from dehydration.⁴ Recently, however, D-lactic acidosis was shown to occur in calves with diarrhea.⁵ D-lactic acidosis is caused by aberrant gastrointestinal fermentation and not to increased production by mammalian cells via the methylglyoxal pathway.^{5–8} D-lactate is present in both the rumen and the colon of diarrheic calves, with the colon contributing more to serum D-lactate concentration than the rumen.⁹ The combination of increased production and compromised metabolism or excretion of D-lactate contribute to the development of acidosis in diarrheic calves. Because the discovery of D-lactic acidosis in diarrheic calves is recent, few therapeutic studies have been aimed at its treatment.¹⁰

Antimicrobials commonly are used to treat calf diarrhea, but growing concern about antibiotic residues in food products of animal origin and the emergence of antimicrobial-resistant pathogens has prompted interest in the development of veterinary probiotics.¹¹ Probiotics are defined as living microorganisms that, upon ingestion in adequate numbers, exert health benefits beyond inherent general nutrition.¹² Few veterinary probiotics have been studied.¹³

Lactobacillus rhamnosus strain GG (LGG) (ATC 53103) is a probiotic that has been isolated from the human gastrointestinal tract and has been extensively studied.^{14–25} LGG is resistant to acid and bile, has strong adhesive properties for intestinal mucosa, suppresses bacterial enzyme activity, and produces antimicrobial substances.^{16,25} Randomized, placebo-controlled studies have confirmed that LGG is successful in the treatment of infectious diarrhea,¹⁸ antimicrobial-associated diarrhea,¹⁷ traveler's diarrhea,²² and relapsing cases of *Clostridium difficile* diarrhea in people.²³ LGG also has been shown to survive transit through the gastrointestinal tract of calves,²⁴ which differs from that of humans because of the presence of the rumenoreticulum. Furthermore, LGG produces only L-lactate, and not D-lactate, and can be safely administered dissolved in milk or in oral rehydration solution (ORS).²⁴

An objective of this study was to determine whether LGG is effective in reducing the severity of diarrhea or D-lactic acidosis or both in diarrheic calves. LGG may be useful in treating D-lactic acidosis by competitively inhibiting those lactobacilli that produce D-lactate or by diverting substrate that would be used by D-lactate producers to the more readily metabolized L-isomer. Furthermore, previous studies of D-lactic acidosis in diarrheic calves have measured serum D-lactate only at one time point, usually upon presentation to a veterinary clinic.^{5,9,26} This study also provides information on the time course of D-lactic acidosis during therapy.

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Materials and Methods

Study Design

This study was designed as a randomized, prospective study of the effects of LGG administration on overall in-hospital survival and the severity of diarrhea and acidemia. Based on previous studies, 40 calves were intended to be enrolled in the study. Ultimately, 58 mixed-breed calves (Red Angus, Charolais, Aberdeen-Angus, Simmental, Limousin, Holstein-Friesian, Hereford, and Gelbvieh) <30 days of age were selected from those admitted to the Large Animal Clinic at the Western College of Veterinary Medicine, University of Saskatchewan. Calves were selected for inclusion in the study based on the presence of diarrhea, which was defined as 3 or more profuse or watery stools per day. Calves were randomly placed in 2 groups, 29 in each. All calves received "standard therapy," consisting of IV or IM antimicrobials (except 1 control calf), IV rehydration therapy (except 3 control calves and 1 experimental calf) with sodium bicarbonate in a dose calculated to correct systemic acidosis as measured by blood gas analysis. Calculations of total fluid and bicarbonate administered were performed by the caregiving clinician in accordance with standard practice.² The attending clinician was a hospital clinician and not a member of the research group that was investigating the effect of LGG. The experimental group was fed 1×10^{11} cfu/d of LGG^a mixed with milk or ORS. This dosage was determined in a pilot study.²⁴ If the calf remained in the clinic for a 2nd day after the 1st PO feeding, a 2nd dose of 1×10^{11} cfu/d of LGG was administered. The decision to feed ORS or milk was made by the attending clinician. Oral feeding usually commenced when the calf could stand and had a suck reflex. Time = 0 h was defined as the commencement of oral feeding and usually occurred after the calf had received IV fluid therapy for 12 to 24 hours. The control group received milk or ORS without LGG.

There were 2 secondary objectives. One was to relate the severity of D-lactic acidosis at presentation to the type of pathogen excreted by the calf. For this part of the study, samples collected from all 58 calves at admission were used. The 2nd objective was to measure the rate of clearance of D-lactate during treatment.

This study was approved by the Animal Care Committee of the University of Saskatchewan and was carried out in accordance with the guidelines specified by the Canadian Council of Animal Care.

Sample Collection and Analysis

Immediately after admission of a diarrheic calf to the clinic (time = admission), approximately 10 mL blood was drawn from the jugular vein, allowed to clot at room temperature for 20 minutes, was centrifuged, and serum was separated and frozen at -70°C . A 2nd blood sample was collected into a heparinized syringe for immediate blood gas analysis and determination of plasma sodium, potassium, and chloride concentrations⁵. Approximately 20 g of feces were collected after either perineal massage or digital stimulation of the anus. In this study, time 0 h was the time of the 1st oral feeding, which usually was 12 to 24 hours after admission. Serum and fecal samples were collected again at 24 and, where possible, 48 hours after the initial administration of the milk or ORS with or without LGG. One-gram aliquots of admission fecal samples were separated and frozen at -70°C for pathogen analysis. One-gram aliquots of 24-hour and 48-hour fecal samples were separated and frozen at -70°C for LGG recovery analysis. The remaining feces, designated for D- and L-lactate analysis, were mixed with 15 mL thiomersal (250 $\mu\text{mol/L}$) as a bacteriostatic agent and were frozen at -70°C . After thawing, serum and fecal samples were prepared for high-performance liquid chromatographic (HPLC) analysis for lactate enantiomers, as described previously.^{27,28} Fecal recovery of LGG was assessed by serially diluting 1 g of feces in phosphate-buffered saline solution and

inoculating 100- μL aliquots of dilutions 10^{-2} , 10^{-3} , 10^{-4} onto Lactobacillus agar plates (De Mar, Rogosa, and Sharpe).^c Plates were incubated microaerophilically (candle jar) for 72 hours at 37°C . Colonies were identified as LGG based on colonial morphology (i.e., large, white, creamy, opaque) and Gram stain appearance (small uniform rods in chains).²⁴ In a previous study, colony identity was further confirmed as LGG on 20 samples randomly selected from calf feces by using a biochemical identification assay^d with 100% accuracy.²⁴ Fecal dry matter was measured at all 3 time points by using the AOAC method 930.15.²⁹ Briefly, 2 g of feces were dried at 135°C until constant weight, cooled in a desiccator, and weighed.

Fecal pathogens (*Salmonella*, *E coli*, coronavirus, rotavirus, *Cryptosporidia*) were isolated and identified.^e *Salmonella* spp. and *E coli* were identified using culture; *E coli* were subcultured on Minca agar to enhance fimbrial production and tested for the F5 antigen using slide agglutination. Coronavirus and rotavirus were identified by a fecal fluorescent antibody test. *Cryptosporidia* were identified by microscopic evaluation of fecal flotations.

Statistical Analysis

All statistical analyses were carried out using SPSS 11.0.^f Serum and fecal D- and L-lactate concentrations, and fecal water percentages of experimental and control calves were compared by using analysis of variance with repeated measures. The relationship between causative agent and D- and L-lactate concentrations was investigated to determine any relation between the effectiveness of the probiotic and the etiologic agent using analysis of variance with repeated measures.

Using analysis of variance with repeated measures, the data were analyzed for differences between experimental and control groups in serum and fecal D- and L-lactate, selecting those experimental calves that LGG colonized successfully, selecting only calves in which D-lactic acidosis was particularly severe upon admission (>10 mmol/L) or selecting only cases in which a viral pathogenesis was responsible for the diarrhea.

Results

Calves

Fifty-eight calves were admitted into the study. Ten calves were excluded (5 from each group), either because they died (5 calves: 4 from the control group, 1 from the experimental group) or were discharged from the clinic because they had recovered sufficiently that they no longer needed in-hospital care <24 hours after the 1st oral feeding (5 calves, 1 from the control group, 4 from the experimental group). In the early phases of the study, 8 calves (4 calves in each group) received IV lactated Ringer's solution (LRS) per clinicians' orders. Because LRS contains equimolar amounts of L- and D-lactate, these calves were intended to be excluded from the study. However, because no difference in blood D- or L-lactate was detected in these calves compared with those who received no LRS ($P = .9$, independent t -test), they were included, leaving a total of 24 calves in each group. Many calves recovered rapidly enough that they were discharged from the clinic before a 48-hour sample could be taken, with only 12 remaining in the control group and 10 remaining in the treatment group at this time point. No mortalities occurred between the 24- and 48-hour sample times, and the reduction in numbers was because of the discharge

Table 1. Admission parameters (mean \pm standard deviation) from diarrheic calves in control (no *Lactobacillus rhamnosus* GG [LGG]) and experimental (LGG) groups.^a

Variable	Control Group n = 24	Treatment Group n = 24
Age, d	12 \pm 7	14 \pm 11
pH	7.10 \pm 0.18	7.08 \pm 0.19
pCO ₂ , mm Hg	45.0 \pm 12.6	46.3 \pm 13.2
pO ₂ , mm Hg	38.2 \pm 0.71	41.0 \pm 14.4
Bicarbonate, mmol/L	15.3 \pm 9	14.6 \pm 8
Base excess, mmol/L	-13.5 \pm 11.2	-15.1 \pm 10.7
Sodium, mmol/L	137.8 \pm 9.5	142.8 \pm 7
Potassium, mmol/L	5.5 \pm 1.7	5.5 \pm 1.7
Chloride, mmol/L	99.9 \pm 9.2	103.2 \pm 8.9
Anion gap, mmol/L	28.0 \pm 9.6	30.5 \pm 9.6
Glucose, mmol/L	5.5 \pm 2.2	5.8 \pm 2.2
Temperature, °C	37.7 \pm 2.2	37.9 \pm 2.1

^a No significant difference existed between control and experimental groups for all variables measured ($P > .05$, independent t -test).

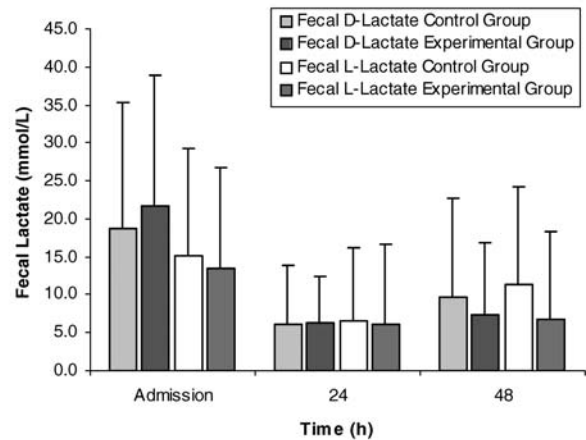
of successfully treated calves. No significant differences were identified between the control and treatment groups in age, sex, or breed.

Blood and Fecal Variables

Upon admission to the veterinary clinic, calves with acute diarrhea had increased plasma anion gap ($\text{Na}^+ + \text{K}^+ - (\text{Cl}^- + \text{HCO}_3^-)$) (mean \pm SD, 29.2 \pm 9.5 mmol/L, $n = 58$), decreased blood pH (7.09 \pm 0.19) and decreased bicarbonate (15.2 \pm 8.6 mmol/L) compared with reference values.^{9,30} Many calves were hyperkalemic; 28 calves had plasma potassium concentrations greater than 5 mmol/L (range, 2.9–9.7 mmol/L) (Table 1). Fecal dry matter contents (%) at 0, 24, and 48 hours were 10.6 \pm 5.8, 12.9 \pm 8.7, and 13.1 \pm 11.6 for control calves, and 14.6 \pm 10.6, 18.2 \pm 16.5, and 14.2 \pm 9.5 for LGG-treated calves, respectively, these differences were not significant. Serum D-lactate concentration was significantly decreased in both treatment and control calves after 24 and 48 hours ($P < .01$), but there was no difference between the groups (Figs 1 and 2).

LGG colonies were distinguished from other *Lactobacillus* spp. by the appearance on MRS agar and Gram staining. LGG was recovered in the feces of 13 of 24 (54%) experimental calves and 0 of 24 control calves (0%). Fecal pathogen analysis of all 58 admitted calves (virology, bacteriology, parasitology) failed to identify the causative agent in 32 calves. No *Salmonella* or F5-positive *E coli* species were isolated, possibly because of administration of antimicrobials before admission to the clinic. Twenty-three cases of viral infection (either coronavirus, rotavirus, or both) were identified, and 6 cases of *Cryptosporidium* spp. were identified. In 3 cases, both *Cryptosporidium* and viral pathogens were isolated. No differences in serum or fecal D- and L-lactate were noted between experimental and control calves selecting the cases where LGG colonized successfully ($n = 13$).

A.



B.

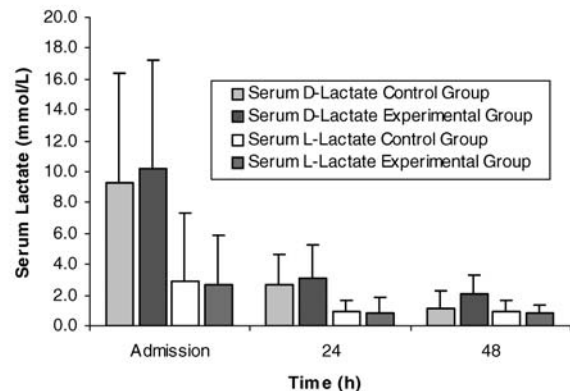


Fig 1. Mean fecal and serum D- and L-lactate concentrations in control and experimental calves. Experimental group fed 1×10^{11} cfu/d of LGG at 1st oral feeding, time = 0 h. Admission, $n = 24$ per group; 24 h, $n = 24$ per group; 48 h, control group $n = 12$; experimental group $n = 10$. Error bars represent standard deviation.

No differences in serum or fecal D- and L-lactate concentrations were noted when only calves in which D-lactic acidosis was particularly severe upon admission ($n = 10$ control calves, $n = 12$ experimental calves; >10 mmol/L) were selected. No differences were detected in serum or fecal D- and L-lactate in the cases where viral pathogenesis was responsible for the diarrhea ($n = 9$ control calves, $n = 8$ experimental calves). Similarly, there was no difference in serum or fecal D- and L-lactate concentrations between those diarrheic calves from which a virus or cryptosporidium could be isolated and those in which no pathogen was found.

Discussion

Although the difference was not statistically significant, 2 more calves in the LGG treatment group were discharged alive from the clinic between 24 and 48 hours after the 1st oral feeding. If calves in the clinic for

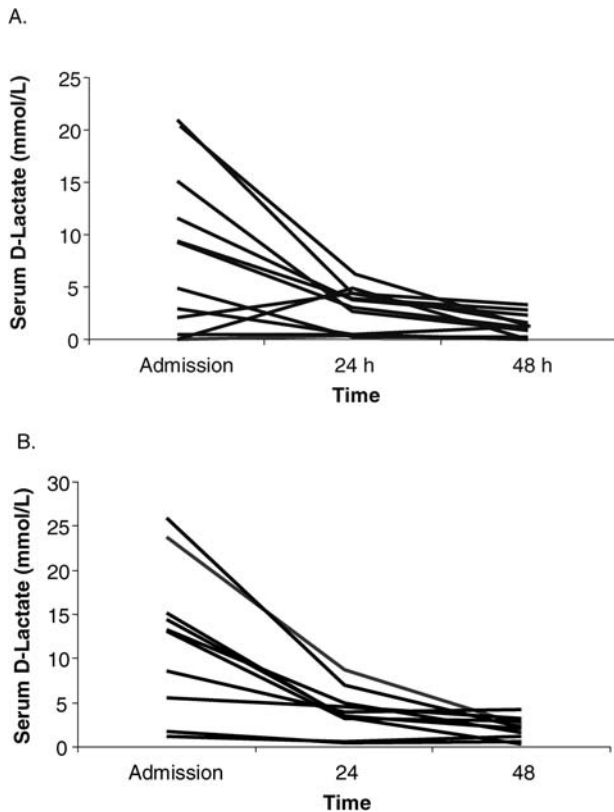


Fig 2. (A) Reduction in serum D-lactate in individual diarrheic calves in control group over time. (B) Reduction in serum D-lactate in individual diarrheic calves in experimental group over time. After admission to the Large Animal Clinic at the Western College of Veterinary Medicine, calves undergo treatment, typically including antimicrobials and intravenous fluid and electrolytes. Current therapy effectively reduces severity of D-lactic acidosis. Times 24 h and 48 h are from the 1st oral feeding in the clinic.

<24 hours are included, 6 more calves in the LGG group were discharged alive within 48 hours of initiation of probiotic treatment. A study with twice the number of calves could potentially determine if these differences are repeatable and make a difference of this magnitude statistically significant.

Studies in humans have demonstrated that duration of diarrhea is decreased with LGG treatment.^{18,31} In this study, feces from LGG-treated calves contained more dry matter at 24 hours, but this increase was not statistically significant. The mechanism by which LGG can reduce fecal water is not well established but may be because of an increased rate of intestinal healing.³² LGG may modulate various aspects of host immunity and has been shown to induce production of tumor necrosis factor, interleukin-6 and interleukin-10.³³ The peptidoglycan layer of LGG may activate macrophages; this upregulation may be responsible for the increase in systemic and local immunoglobulin A observed in humans after administration of lactobacilli.³⁴ LGG also decreases the duration of fecal rotaviral shedding.³⁵

No differences in fecal or serum lactate concentrations were observed between the groups. However, because antimicrobials were administered to all calves,

intestinal flora was likely modified, and D-lactate-producing organisms may have been reduced by antimicrobial therapy. This possibly could explain why the condition of calves whose diarrhea is known to be of viral origin can improve with antimicrobial therapy.

The reduction in colonization of LGG in diarrheic calves (13/24) in the present study versus healthy calves (5/5) in a previous study²⁴ may be from increased motility or to administration of antimicrobials, both orally before admission to the clinic, and systemically once admitted to the clinic. LGG is reported to survive intestinal transit even with antimicrobial administration; but this has never been investigated in calves or with veterinary antimicrobials. In a more controlled environment, LGG may show effects that are not detectable in the clinical environment. Failure to detect bacterial pathogens also may have been because of antimicrobial therapy. Future studies in laboratory calves experimentally infected with rotavirus and not given antimicrobials may show that D-lactate production can be reduced by LGG. Given the emergence of antimicrobial-resistant pathogens and the increasing need for alternative therapies to assist clinicians and livestock producers in limiting the use of antimicrobials, further studies of this nature are warranted. Furthermore, studies determining the capacity of LGG to prevent calf diarrhea are warranted.

This is the first study, to our knowledge, to document the presence of D-lactic acidosis in viral diarrhea. This finding strengthens our hypothesis that viral-induced villous atrophy results in malabsorption and over-fermentation, similar to that observed in short bowel syndrome.^{36,37} D-Lactate production and accumulation in short-bowel syndrome is caused by malabsorption after an extensive bowel resection. Excessive fermentation of carbohydrate by *Lactobacillus* spp. occurs, and the subsequent inability of the body to effectively metabolize D-lactate results in acidosis. Overgrowth of *Lactobacillus* spp. initially results from increased organic acid production, which reduces pH in the colonic lumen. This acidic environment permits acid-resistant lactobacilli to grow preferentially, yielding both D- and L-lactate. When the rate of D-lactate production exceeds the body's capacity to metabolize it, D-lactic acidemia and metabolic acidosis occur.⁶

This study also investigates the time course of D-lactic acidemia in calves receiving IV fluids and antimicrobial therapy. Over half of the calves (58%) no longer had D-lactic acidosis at 24 hours after admission (Fig 2), many more were only very marginally above the 3 mmol/L cutoff. No calf had D-lactic acidosis after 48 hours in the clinic. This dramatic reduction in D-lactic acidosis may occur as a result of antimicrobial administration and consequent reduction in D-lactate producing organisms combined with enhanced renal excretion, or possibly metabolism, of D-lactate. Others also have reported decreased D-lactate concentrations after 24 hours of IV therapy with sodium bicarbonate and saline solution.¹⁰ Because most calves showed rapid decreases in fecal and serum D- and L-lactate concentrations after 24 hours in

the clinic, even if LGG did reduce D-lactate concentrations, the effects may not have been detectable.

This study does not provide clear evidence for a benefit of LGG treatment. To some extent, concurrent antibiotic administration may have also had beneficial effects on intestinal bacterial overgrowth that masked benefits to LGG. Also, the dose of LGG did not induce colonization in all diarrheic calves. A larger study with a higher dose of LGG or a bovine-adapted probiotic in nonantibiotic-treated calves is needed. Treating calves with probiotics is an area that warrants further investigation, as consumers and producers become aware of detrimental effects of widespread administration of antimicrobials in livestock. Further development of probiotics of bovine origin may increase the success of future therapeutic interventions.

Footnotes

- ^a CAG Functional Foods, Omaha, NE
^b Ciba-Corning 288 blood gas system, Ciba-Corning Canada Inc, Markham, ON, Canada
^c Difco, Sparks, MD
^d API 50 CHL, BioMerieux, St. Laurent, Quebec
^e Prairie Diagnostic Services, Saskatoon, SK
^f SPSS Inc, Chicago, IL
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References

- Dutil L, Fecteau G, Bouchard E, et al. A questionnaire on the health, management and performance of cow-calf herds in Quebec. *Can Vet J* 1999;40:649–656.
- Booth AJ, Naylor JM. Correction of metabolic acidosis in diarrheic calves by oral administration of electrolyte solutions with or without bicarbonate. *J Am Vet Med Assoc* 1987;191:62–68.
- Groutides C, Michell AR. Evaluation of acid-base disturbances in calf diarrhoea. *Vet Rec* 1990;126:29–31.
- Lewis LD, Phillips RW, Elliott CD. Changes in plasma glucose and lactate concentrations and enzyme activities in the neonatal calf with diarrhea. *Am J Vet Res* 1975;36:413–416.
- Omole OO, Nappert G, Naylor JM, et al. Both L- and D-lactate contribute to metabolic acidosis in diarrheic calves. *J Nutr* 2001;131:2128–2131.
- Oh M, Alveranga D, Lazar I, et al. Metabolic utilization and renal handling of D- lactate in men. *Metabolism* 1985;34:621–625.
- Phillips S, Mirrlees D, Thornalley P. Modification of the glyoxalase system in streptozotocin-induced diabetic rats. *Biochem Pharmacol* 1993;46:805–811.
- Thornalley P. Modification of the glyoxalase system in human red blood cells by glucose in vitro. *Biochem J* 1988;254:751–755.
- Ewaschuk JB, Naylor JM, Zello GA. D-lactate production and excretion in diarrheic calves. *J Vet Int Med* 2004;18:744–747.
- Solveig V. Investigations on the Influence of D-lactate Blood Levels on Acidosis, Posture and Behaviour in Calves with Neonatal Diarrhea. Munich, Germany: University of Munich; 2004. Inaugural Dissertation.
- Paige JC, Tollefson L, Miller MA. Health implications of residues of veterinary drugs and chemicals in animal tissues. *Vet Clin N Am Food Anim Pract* 1999;15:31–43.
- Guarner F, Schaafsma GJ. Probiotics. *Int J Food Microbiol* 1998;39:237–238.
- Weese SJ. Microbiologic evaluation of commercial probiotics. *J Am Vet Med Assoc* 2002;220:794–797.
- Saxelin M, Elo S, Salminen S, et al. Dose response colonisation of faeces after oral administration of *Lactobacillus casei* strain GG. *Microb Ecol Health Dis* 1991;4:209–214.
- Siitonen S, Vapaatalo H, Salminen S, et al. Effect of *Lactobacillus* GG yoghurt in the prevention of antibiotic associated diarrhoea in children. *Ann Med* 1990;22:57–59.
- Silva M, Jacobus NV, Deneke C, et al. Antimicrobial substance from a human lactobacillus strain. *Antimicrob Agents Chemother* 1987;31:1231–1233.
- Vanderhoof JA, Whitney DB, Antonson DL, et al. *Lactobacillus* GG in the prevention of antibiotic-associated diarrhea in children. *J Pediatr* 1999;135:564–568.
- Guandalini S, Pensabene L, Zikri MA, et al. *Lactobacillus* GG administered in oral rehydration solution to children with acute diarrhea: a multicenter European Trial. *J Pediatr Gastroenterol* 2000;30:54–60.
- Guandalini S. Use of *Lactobacillus* GG in paediatric Crohn's disease. *Dig Liver Dis* 2002;34:S63–S65.
- Kaila M, Isolauri E, Saxelin M, et al. Viable versus inactivated lactobacillus strain GG in acute rotavirus diarrhea. *Arch Dis Childhood* 1995;72:51–53.
- Ling WH, Hanninen O, Mykkanen H, et al. Colonization and fecal enzyme activities after oral *Lactobacillus* GG administration in elderly nursing home residents. *Ann Nutr Metab* 1992;36:162–166.
- Oksanen PJ, Salminen S, Saxelin M, et al. Prevention of traveller's diarrhoea by *Lactobacillus* GG. *Ann Med* 1990;22:53–56.
- Gorbach SL, Chang TW, Goldin B. Successful treatment of relapsing *Clostridium difficile* colitis with *Lactobacillus* GG. *Lancet* 1987;2(8574):1519.
- Ewaschuk JB, Naylor JM, Zello GA. *Lactobacillus rhamnosus* strain GG is a potential probiotic for calves. *Can J Vet Res* 2004;68:49–53.
- Goldin BR, Gorbach SL, Saxelin M, et al. Survival of *Lactobacillus* species (strain GG) in human gastrointestinal tract. *Dig Dis Sci* 1992;37:121–128.
- Schelcher F, Marcillaud S, Braun J. Metabolic acidosis without dehydration and no or minimal diarrhea in suckler calves is caused by hyper D-lactatemia. *Proc XX World Buiatris Congress, Sydney, Australia, 6–10 July, 1998.* 1998;371–374.
- Omole OO, Brocks DR, Nappert G, et al. High-performance liquid chromatographic assay of (±)-lactic acid and its enantiomers in calf serum. *J Chromatogr B* 1999;727:23–29.
- Ewaschuk JB, Naylor JM, Zello GA. High-performance liquid chromatographic assay of lactic, pyruvic and acetic acids and lactic acid stereoisomers in calf feces, rumen fluid and urine. *J Chromatogr B* 2004;805:347–351.
- AOAC, Official Methods of Analysis of AOAC International, 16 ed. Washington, DC: AOAC International; 1997.
- Ewaschuk JB, Naylor JM, Zello GA. Anion gap correlates with serum D- and L lactate concentrations in diarrheic neonatal calves. *J Vet Int Med* 2003;17:940–942.
- Raza S, Graham SM, Allen SJ, et al. *Lactobacillus* GG promotes recovery from acute non-bloody diarrhea in Pakistan. *Pediatr Infect Dis J* 1995;14:107–111.

32. Schultz M, Lind H, Lehn N, et al. Immunomodulatory consequences of oral administration of *Lactobacillus rhamnosus* strain GG in healthy volunteers. *J Dairy Res* 2003;70:165–173.
33. Miettinen M, Vuopio-Varkila J, Varkila K. Production of human tumour necrosis factor alpha, interleukin-6, and interleukin-10 is induced by lactic acid bacteria. *Infect Immun* 1996; 64:5403–5405.
34. Kaila M, Isolauri E, Soppi E, et al. Enhancement of the circulating antibody secreting cell response in human diarrhea by a human *Lactobacillus* strain. *Pediatr Res* 1992;32: 141–144.
35. Guarino A, Cananin BC, Spagnuolo MI, et al. Oral bacterial therapy reduces the duration of symptoms and of viral excretion in children with mild diarrhea. *J Pediatr Gastroenterol Nutr* 1997;25:516–519.
36. Zhang DL, Jiang ZW, Jiang J, et al. D-lactic acidosis secondary to short bowel syndrome. *Postgrad Med J* 2003;79: 110–112.
37. Caldarini M, Pnos S, D'Agostino D, et al. Abnormal fecal flora in a patient with short bowel syndrome: an in vitro study on effect of pH on d-lactic acid production. *Dig Dis Sci* 1996; 41:1649–1652.