The Effect of Calcium Supplementation on Gastrointestinal Survival of *Lactobacillus* spp.

Presented by Tyler Culpepper, Ph.D.

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Criteria for selecting organisms as probiotics include the ability to modulate immune response, antagonize pathogens, prevent infection, adhere to epithelial tissue, and to transiently persist in the gastrointestinal tract.

Lacidofil® has been shown to reduce antibiotic-associated diarrhea, *Clostridium difficile* and *Helicobacter pylori* carriage, and *C. difficile* toxins.

Lacidofil® has been shown to reduce symptoms of irritable bowel syndrome and lactose intolerance.

The addition of mineral supplements to probiotics may increase their survival and thus augment their beneficial effects on the host.
Hypotheses

• Consumption of Lacidofil® will increase viable counts of *Lactobacillus* in stools.

• Mineral supplementation with calcium carbonate or calcium triphosphate will enhance survival of Lacidofil® in the gut.

• Mineral supplementation alone will not appreciably alter *Lactobacillus* spp. viable counts or amounts of lactic acid bacteria in stools.
Studies

• Study 1:
  – Randomized, double-blind, placebo-controlled crossover study design (n = 18)
  – Supplementation:
    • Lacidofil® \textit{(Lactobacillus helveticus} R0052 and \textit{Lactobacillus rhamnosus} R0011; $2 \times 10^9$ CFU/capsule) \textbf{AND}
    • 500 mg calcium carbonate or tricalcium phosphate, or no calcium supplement daily for two 2-week periods with a 2-week washout.

• Study 2:
  – Randomized, double-blind, placebo-controlled crossover study design (n = 15)
  – Supplementation:
    • 1000 mg calcium carbonate or tricalcium phosphate daily for two 2-week periods with a 2-week washout.
Study Design – Study 1

Obtain consent
Begin study

Start

(n = 6) Begin supplement
2 weeks
(n = 6) End supplement
Stool collection

(n = 7) Begin supplement
2 weeks
(n = 6) End supplement
Stool collection

(n = 5) Begin supplement
2 weeks
(n = 7) End supplement
Stool collection

Probiotic
Probiotic + calcium carbonate

Probiotic + calcium triphosphate
Experimental methods

- Stool samples obtained and processed within 4 hours of defecation

- Viable counts
  - MRS agar (for Lactobacillus spp.)
  - Vancomycin (5 μg/mL) plates to select for L. rhamnosus R0011 (Study 1)
  - Clindamycin (0.125 μg/mL) plates to select for L. helveticus R0052 (Study 1)
  - Plates were incubated for 48 hours at 37°C in candle extinction jars.

- qPCR (LAB) primers: Genome equivalents (GE) – Study 2
Study 1 – Interpersonal variability (viable counts)

There is a high degree of interpersonal variability between subjects.
Viable counts of *Lactobacillus* spp. in stools were significantly higher after Lacidofil® + calcium triphosphate intervention (*p*<0.05).
Viable counts of vancomycin and clindamycin-resistant *Lactobacillus* spp. in stools were not significantly higher after any intervention.
Study 1 - Conclusions

• Consumption of Lacidofil® did not significantly increase viable counts of *Lactobacillus* spp. detected in stools.

• Mineral supplementation in conjunction with Lacidofil® supplementation did not significantly increase vancomycin- or clindamycin-resistant strains of *Lactobacillus* spp.

• Mineral supplementation with calcium triphosphate but not calcium carbonate significantly enhanced survival of *Lactobacillus* spp. in stools.

• The increase in *Lactobacillus* spp. may be due to Lacidofil® supplementation or increases in resident *Lactobacillus* spp.
Study Design – Study 2

- Obtain consent
- Begin study

Calcium triphosphate (n = 10)

2 weeks

Calcium triphosphate (n = 5)

2 weeks

Calcium carbonate

Stool collection

End supplement

Begin supplement

Stool collection

End supplement

Begin supplement

Stool collection
There is a high degree of interpersonal variability between subjects.
Mineral supplementation alone did not significantly increase *Lactobacillus* spp. counts in stools.
Mineral supplementation alone did not significantly increase lactic acid bacteria GE in stools.
Study 2 - Conclusions

• Neither viable counts nor genome equivalents changed significantly as a result of mineral supplementation alone.

• *Lactobacillus rhamnosus* R0011 and *Lactobacillus helveticus* R0052 may associate with the intestinal mucosa and thus not be detected in high numbers in stools.

• High interpersonal variability may contribute to the difficulty in detecting significant differences.

• The increase in *Lactobacillus* viable counts observed in Study 1 may be dependent upon Lacidofil® supplementation.
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