

PILOT CLINICAL STUDY SHOWED INCREASED
PRODUCTION OF N-BUTYRATE AND TOTAL
SHORT CHAIN FATTY ACIDS IN SUBJECTS
CONSUMING TWO DIETARY SUPPLEMENTS
INDICATING COLON BENEFITS

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ABSTRACT

Background: Dietary supplements containing probiotics and/or prebiotics have gained much notoriety for gastrointestinal support. Often supplements are formulated but not clinically evaluated for possible beneficial effects or side effects. A digestive dietary supplement with probiotic, prebiotics and digestive enzymes was developed. It was of interest to investigate subjects consuming this supplement along with another popular formula that consumers use as a primary supplement from this company. The supplements are called DIGESTIVE⁺⁺⁺ and Laminine[®] (LifePharm, Inc. Lake Forest, CA). A preliminary study was undertaken to evaluate if 4 weeks consumption of the dietary supplements supported short chain fatty acids production as an indicator of benefits to the gastrointestinal tract and to observe preliminarily if subjects experienced discomfort or untoward side effects.

Purpose/Objectives: Supplements are increasingly used for nutritional support. However, often supplements are formulated based on scientifically investigated ingredients, and have safety profiles or are considered Generally Recognized as Safe. When combining supplements or different ingredients, it would be advantageous to have preliminary clinical testing which shows parameters that may be affected as well as checking for adverse side effects, especially in a digestive formula. The DIGESTIVE⁺⁺⁺ product contains prebiotics, probiotics and an array of digestive enzymes. It contained *Bacillus coagulans* (a spore forming lactobacillus) with a prebiotic blend of short chain Fructooligosaccharides, Jerusalem artichoke (90% inulin), Dandelion and Yacon root. Unlike similar formulas on the market, the capsule includes digestive enzymes: amylase, several proteases, alpha-galactosidase, glucoamylase, invertase, lipase, peptidase, lactase and maltase. Laminine, the additional popular supplement, contains a proprietary fertilized chicken egg extract, fish and pea protein powders.

Methods: Four subjects signed informed voluntary consent and consumed one DIGESTIVE⁺⁺⁺ capsule with each of the two heaviest daily meals. Subjects also consumed 2 capsules daily of Laminine. Subjects were asked to refrain from using other supplements and provided a baseline and a four-week stool sample. Samples were evaluated, and data was collected from the Comprehensive Digestive Stool Analysis (Genova Diagnostics, Asheville, North Carolina).

Results: Stool results showed a 28% averaged increase in n-butyrate production and a 28% averaged increase in total short chain fatty acids (n-butyrate, propionate and acetate) compared to baseline. Both total short chain fatty acids and n-butyrate production showed similar increase. Although the data was statistically evaluated by student's t test, no significance was observed with the small sample size, however this initial trend was observed. Because butyrate and total short chain fatty acids both showed almost identical change in increase, it was considered a possible trend in affectivity.

Conclusions: The pilot or proof-of-concept study showed that no subjects experienced any untoward side effects or discomfort from taking the two supplements together daily. Noticing the trend in increased butyrate production is an indicator that colon lining is being supported. Butyrate is the primary energy source for the cells lining the colon. It helps the colonic epithelial cells maintain their functionality and resist mutations by stimulating healthy cellular growth and reducing DNA damage. In certain imbalances of the colon and GI tract, butyrate has been shown to decrease markers of inflammation and lipopolysaccharide-induced Nuclear factor (NF) kappa- activity in intestinal cells. The increased production of these beneficial components is an indicator that the supplements may have supported beneficial changes. These results warrant further clinical investigations with larger sample size, to continue to evaluate n-butyrate production, and other GI parameters.

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OBJECTIVES

Dietary supplements containing prebiotics and probiotics are considered by the World Health Organization to be beneficial towards gastrointestinal and colon health. Some dietary formulations geared to support digestive health are formulated with ingredients that should support digestive health but may not be directly tested in human clinical studies for parameters that will show effectivity towards gastrointestinal (GI) health. It was the purpose of this study to evaluate effectivity of the combination of two dietary supplement formulas and their effects on colonic health when consumed together in subjects.

METHODS

Four subjects signed informed voluntary consent. Subjects were asked to refrain from using other dietary supplements prior to the study and to maintain their same lifestyle patterns during the trial. They were to consume one DIGESTIVE⁺⁺⁺ capsule and one Laminine capsule during each of the two heaviest meals consumed daily. Each subject gave a stool test sample before the beginning of the trial period and once at the end of the 4 week trial period.

The digestive dietary supplement was composed of the probiotic *Bacillus coagulans*, and a prebiotic blend containing short chain Fructooligosaccharides, Jerusalem artichoke (90% inulin), Dandelion and Yacon root. The DIGESTIVE⁺⁺⁺ formula also contained a proprietary blend of digestive enzymes: amylase, several proteases (that cleave proteins at various pH ranges), alpha-galactosidase, glucoamylase, invertase, lipase, peptidase, lactase and maltase. It was the purpose of this study to evaluate subjects consuming the DIGESTIVE⁺⁺⁺ supplement to measure possible beneficial gut health parameters. The second dietary supplement to be consumed along with the DIGESTIVE⁺⁺⁺ supplement contained a proprietary natural extract from cage-free, quality grain-fed, highly monitored, incubated fertilized chicken eggs (*Gallus domesticus*). This supplement, Laminine, contained polypeptide growth factors now identified as Fibroblast Growth Factor-2, Platelet Derived Growth Factor β -1 and Transforming Growth Factor. Laminine also contained fish protein and pea protein powder to provide a full range of amino acids.

PREBIOTIC BLEND INGREDIENTS



Short Chain
Fructooligosaccharides



Jerusalem Artichoke



Dandelion



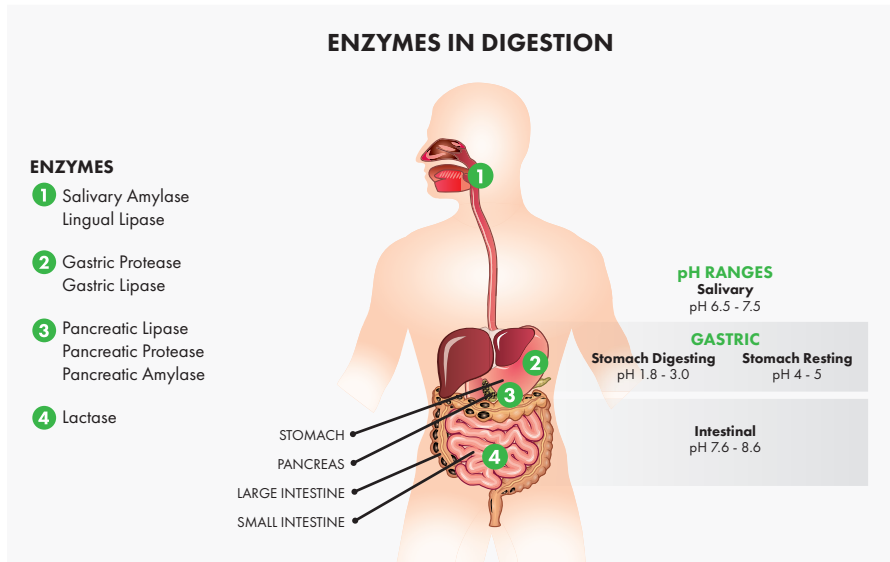
Yacon Root

RESULTS

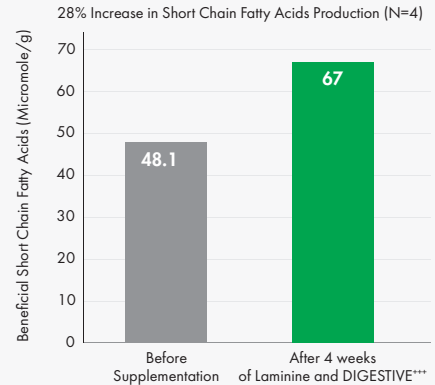
Stool samples data were evaluated from the Comprehensive Digestive Stool Analysis (Genova Diagnostics, Asheville, North Carolina). Quantitative results showed a substantial improvement in total Short Chain Fatty Acids (SCFAs) and butyrate production (Graph 1, Graph 2). Averaged values showed a 28% increase in n-butyrate production and a 28% averaged increase in total short chain fatty acids (n-butyrate, propionate and acetate) compared to baseline. Both total short chain fatty acids and n-butyrate production showed similar increase. Although the data was statistically evaluated by students t test, no significance was observed with the small sample size, however this initial trend was observed. Because butyrate and total short chain fatty acids both showed almost identical change in increase, it was considered a possible trend in effectivity. The increase of these components compared to the subjects' individual baselines before consuming the supplements, is an indicator that the gut is reestablishing a healthier microbiome. Beneficial probiotics are fermenting the prebiotic dietary fiber, which supports many factors relating to gut health.

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LAMININE® AND DIGESTIVE+++ SUPPLEMENTATION (2 CAPSULES OF EACH/DAY FOR 4 WEEKS) IMPROVED GUT HEALTH AS SHORT CHAIN FATTY ACIDS PRODUCTION INCREASED



Graph 1

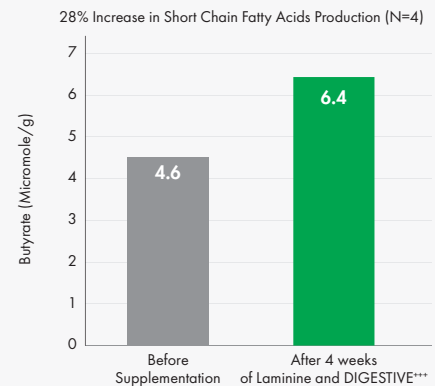
CONCLUSIONS

The volunteer subjects showed an improvement in several indicators of gut health following four weeks of supplementation with two DIGESTIVE+++ capsules and two Laminine capsules taken daily. There are several benefits of increasing short chain fatty acids (SCFA) in the bowel.

Short Chain Fatty Acids serve several important functions:

- Provide energy for cells lining the digestive tract.
- Act as an anti-diarrheal agent by removing sodium and water from the colon.
- Improve colonic blood flow.
- Deter the colonization of pathogens in the bowel.
- Provide 5-30 percent of systemic daily energy requirements.
- Reduce ammonia uptake from the intestine.

LAMININE® AND DIGESTIVE+++ SUPPLEMENTATION (2 CAPSULES OF EACH/DAY FOR 4 WEEKS) IMPROVED GUT HEALTH AS BUTYRATE PRODUCTION INCREASED



Graph 2

DIGESTIVE+++ and Laminine Consumption Enhanced Butyrate Production - the Optimal Substrate for Integrity of Cells Lining the Gastrointestinal Tract.

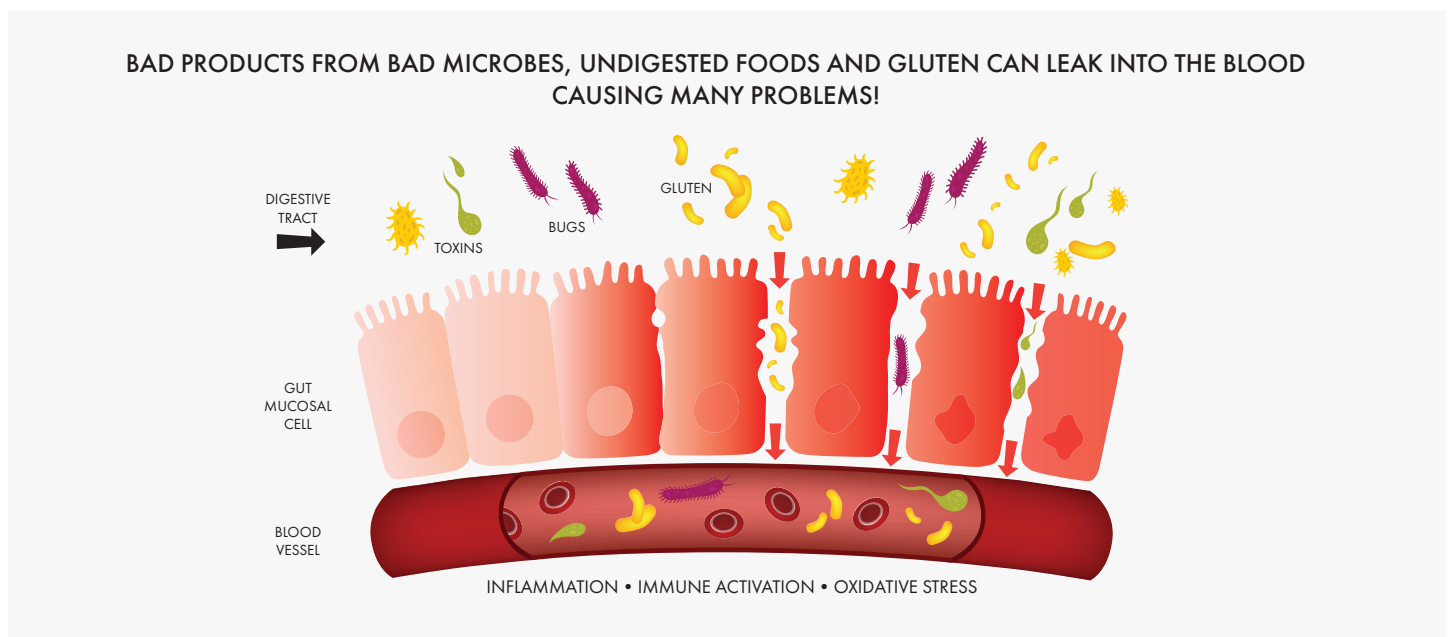
Butyrate is the preferred substrate for colon cells, assisting in the maintenance of colon cell integrity. Butyrate helps prevent mutations in colon cells by stimulating healthy cellular growth and reducing DNA damage. Although it has been known for decades that the commensal metabolite butyrate suppresses inflammation and carcinogenesis in colon, the exact identity of the molecular target(s) of butyrate in this process remained elusive. Current studies have identified Gpr109a as an important mediator of butyrate effects in colon and, also as a critical molecular link between colonic bacteria, dietary fiber and the host. These findings have important implications for prevention as well as treatment of inflammatory bowel disease and carcinogenesis.¹²

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During the four-week study, observing the increase in butyrate would suggest the dietary supplements had a supporting role in reducing inflammation of the bowel by acting as a good fuel for the cells lining the colon and keeping them properly nourished. This may protect the bowel lining against inflammation. When butyrate can act as the preferred substrate for the cells lining the digestive tract, inflammation can be reduced and tight junctions between cells lining the GI tract can be maintained. This prevents the gaps that can lead to the leaky gut syndrome.

The leaky gut can lead to various products of the digestive tract such as gluten, microorganisms, by-products and toxins leaking from the digestive tract into the blood stream without being metabolized and broken down first by the actual cells lining the GI tract. Leaky gut can affect various organ systems of the body such as the joints, the adrenal glands, the mouth and sinuses, the brain, the skin, the adrenal glands and thyroid gland and the health and function of the colon. In certain imbalances of the colon and GI tract, butyrate has been shown to decrease markers of inflammation and lipopolysaccharide-induced Nuclear factor (NF) kappa- β activity in intestinal cells. People that experience bowel irritation conditions (especially those that may include mild diarrhea) tend to have lower total SCFAs.

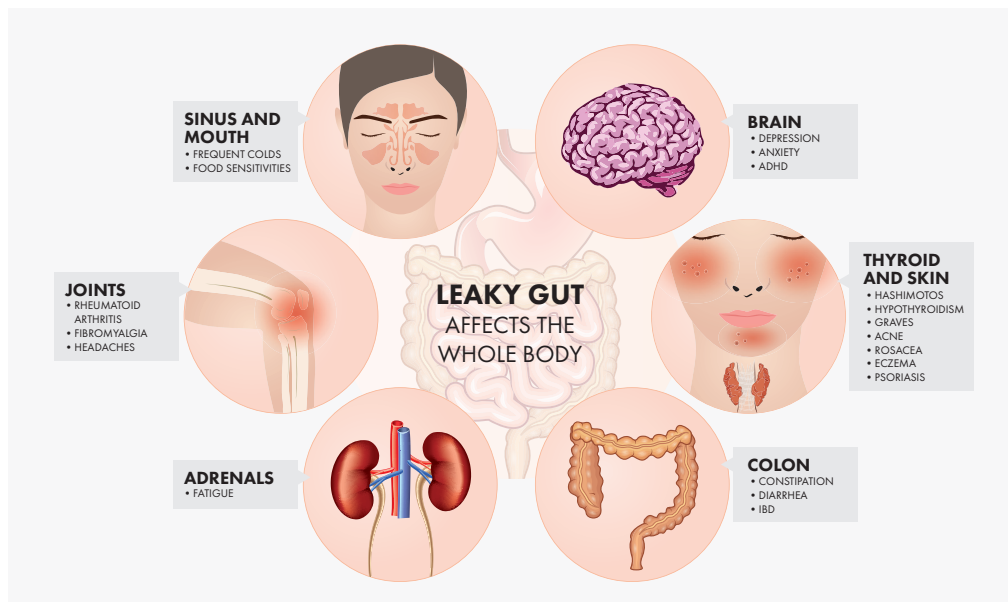


In general, the more slowly fermented (insoluble) forms of fiber as used in DIGESTIVE⁺⁺⁺, maintain low pH and raise SCFAs (especially butyrate) along the entire length of the bowel, while soluble fibers (such as oat bran and guar gum) have less effect on pH and SCFA production. While both soluble and insoluble fiber foods are important for health, digestion and preventing diseases, insoluble fiber passes through our intestines largely intact until reaching the colon where anaerobic bacteria finally break these down into SCFA.

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In conclusion, supplementing with Laminine and DIGESTIVE⁺⁺⁺ dietary supplements showed indication that short-chain fatty acids especially butyrate are increased over a relatively short time. This is a promising result indicating the supplements are supportive to the health of the gastrointestinal tract. Consumers that take the Laminine supplement in their regime for its benefits also may want to consume DIGESTIVE⁺⁺⁺, therefore it was important to observe possible side effects or a synchronistic benefit of taking both supplements together. The formula created by LifePharm for a probiotic, prebiotic and digestive enzyme product was formulated using science-backed ingredients. Testing its affects in human subjects confirms that the product ingredients are working at some levels to improve the bowel function. Observing the increase in butyrate and SCFA after four weeks of supplementation with 2 capsules each of Laminine and DIGESTIVE⁺⁺⁺ as directed is a very positive intervention to support optimal digestive tract and colon health. No untoward side effects were observed in the subjects. Further studies are warranted.



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