DEVELOPMENT AND VALIDATION OF 4LIFE PRE/O BIOTICS[™] THROUGH UNIVERSITY RESEARCH PARTNERSHIPS

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ABSTRACT

OBJECTIVE To develop and validate, through university research partnerships, a digestive health product that combines prebiotics, probiotics, and immune benefitting ingredient, Transfer Factor, in a unique delivery format.

METHODS Prebiotic formula was optimized by developing a rapid method to determine ratios that yielded peak probiotic growth under conditions that mimic the digestive system. Briefly, five probiotics (*B. infantis, B. longum, B. lactis, L. rhamnosus* and *L. acidophilus*) were added to 48-well plates containing varying ratios of prebiotics (xylo-oligosaccharides, galacto-oligosaccharides, fructo-oligosaccharides) and 4Life Transfer Factor Tri-Factor, an ultra-filtrate of colostrum and chicken egg yolk. Plates were incubated at 37°C for 12 hours at selected pHs and analyzed by a spectrophotometer.

EXPERIMENTAL METHODS

IN VITRO **EXPERIMENTS** Individual *Lactobacillus* strains were grown in MRS broth while individual *Bifidobacterium* strains were grown in MRS broth supplemented with L-cysteine. Broth tubes were inoculated from frozen stock cultures that were stored at -70°C.

Both organisms were incubated at 37 °C in gas-paks. Each culture was backdiluted to an OD600 of 0.1 with the appropriate MRS broth then inoculated into sterile 12- or 24-well spectrophotometer plates containing the various concentration ratios for the three prebiotics GOS, XOS, and FOS. All experiments were done at a physiological pH of 5.

Inoculated plates were placed in a programmable spectrophotometer (Tecan Infinite M200) and incubated at 37°C with growth readings taken regularly for up to 12 hours. All trials were run in triplicate with appropriate controls.

IN VIVO RESULTS

MICROBIOME EFFECTS The combination of probiotics, prebiotics, and 4IIFE Tri-Factor Formula showed an increase in alpha diversity compared to that from the Western diet alone. Beta diversity (not shown) was also observed to have a differentiating effect.



Western Diet OnlyWestern Diet + PRE/O



Individual ingredients and product were evaluated in a pre-clinical model that simulates a perturbed microbiome via a Western diet. Briefly, separate groups of C57BL6/J mice were administered test articles through their chow. After four weeks, serum and cecal/fecal samples were taken. Determination of alpha and beta diversity by sequencing a region of 16S gene, short chain fatty acid (SCFA) synthesis by GC/MS, as well as calprotectin and zonulin by ELISA were performed.

RESULTS Prebiotics demonstrated a stimulatory effect on probiotic growth. A 2:2:1 ratio of GOS:XOS:FOS was found to provide optimal benefit. 4Life Tri-Factor Formula demonstrated enhancement ranging from 111% to 868%, depending on the probiotic.

The product demonstrated positive microbiome benefits in pre-clinical model. Alpha and beta diversity significantly increased with a shift towards more beneficial bacterial populations. SFCA trended upwards, primarily driven by the prebiotics. Zonulin was observed to decrease, primarily through the addition of 4Life Tri-Factor Formula.

CONCLUSION University research partnerships yielded an optimally formulated and effective digestive health product.

IN VIVO **EXPERIMENTS** C57BL6J mice were individually caged, and randomized into one of five groups: control, prebiotic, probiotic, 4Life Tri-Factor Formula and PRE/O Biotic. They were fed each ingredient through a Western diet chow for 4 weeks to simulate a perturbed microbiome. Food intake and body weights were measured twice weekly. Fecal samples were collected weekly and blood at the end of the study period.

Composition of the gut microbiome was determined using DNA isolated from feces. The taxonomic abundance and species diversity (alpha- and beta-diversity) of gut bacteria present was determined using 16S sequencing. Amplified fragments were determined on the Ion PGM System at the USU Center for Integrative Biosystems, and analyzed with Ion Reporter[™] workflow.

Short chain fatty acid synthesis was measured in fecal and the cecal samples using gas chromatography with flame ionization detection. Calprotectin and haptoglobin (zonulin) were determined from fecal samples by ELISA using commercially available kits from Hycult Biotech and MyBio, respectively.

Treatment effects and interactions were determined by ANOVA.

The combination of probiotics, prebiotics, and 4Life Tri-Factor Formula yielded a shift towards a more beneficial population of gut bacteria.



SHORT CHAIN FATTY ACID SYNTHESIS The combination of probiotics, prebiotics, and 4Life Tri-Factor Formula showed a trend toward increased SCFA synthesis.



BACKGROUND

Over the past 30 years, digestive health has been an increasingly prevalent area of study as it pertains to improving overall human health. Much of the research that has emerged has centered around utilizing probiotics as a functional food or dietary supplement to modulate the intestinal flora for digestive benefit. With an increasing understanding of the gut microbiome in the last five years, prebiotics have also come into focus as a major contributor in digestive health.

IN VITRO RESULTS

FORMULA OPTIMIZATION Prebiotics demonstrated a well-characterized stimulatory effect on probiotics which could be measured and differentiated. A 2:2:1 ratio of GOS:XOS:FOS provided the optimal enhancement across all 5 probiotic strains.



4Life Research has had a dedicated interest in immune health from the beginnings of the company over 20 years ago. Recently, we have focused on the interplay between immune health and digestive health which has led to the development of a product that contains probiotics, prebiotics as well as 4Life Tri-Factor Formula, a proprietary ingredient derived from bovine colostrum and chicken egg yolk, all contained in a unique delivery format. This product development process was supported by collaborative research partnerships with two universities, Weber State and Utah State.

PRODUCT INGREDIENTS



Supplement Facts	
Serving Size: One (1) stick pack (3.1 g) Servings Per Container: 15	
mount Per Serving	% DV*
robiotic Beadlet Blend	l billion CFU∮ †
Bifidobacterium longum (BB536)	
Bifidobacterium lactis (BI-04)	
Bifidobacterium infantis (M-63)	
Lactobacillus rhamnosus (Lr-32)	
Lactobacillus acidophilus (NCFM)	
rebiotic Blend (2:2:1)	2.5 g †
Galactooligosaccharides (GOS)	
Xylooligosaccharides (XOS)	
Fructooligosaccharides (FOS)	
Life® Tri-Factor® Formula	100 mg 🛛 🕇
UltraFactor XF®	
A proprietary concentrate of ultra-filtered 4Life Transfer Factor® proteins and other peptides from cow colostrum	
OvoFactor [®]	
A patented concentrate of 4Life Transfer Factor® proteins	
and other peptides from chicken egg yolk	
NanoFactor [®]	
A proprietary concentrate of nano-filtered cow colostrum	
Daily Value Daily Value not established At expiration	
THER INCREDIENTS: Microheadlats /hwimmenated coconut and	
alm kernel oil, fish delatin, divcerin, sov lecithin, and nectin), cane	
ugar, maltodextrin, natural flavor, salt, and stevia.	
ONTAINS INGREDIENTS FROM MILK, EGG, SOY, FISH	

AND TREE NUTS



4LIFE TRI-FACTOR FORMULA IMPACT 4Life Tri-Factor Formula demonstrated a varying degree of enhancement for each probiotic strain



GUT INTEGRITY The combination of probiotics, prebiotics, and 4Life Tri-Factor Formula as well as Tri-Factor alone demonstrated a significant improvement in zonulin, a serum biomarker related to intestinal permeability.



CONCLUSIONS

Through university research partnerships, a unique product was developed and validated. *In vitro* experiments were used to optimize the prebiotic ratios and demonstrate the value of adding 4Life Tri-Factor Formula to the product. *In vivo* experiments were utilized to establish, in the presence of a Western diet, the efficacy of the product as a digestive health supplement.