

# Preclinical Evaluation of a Ratio-Optimized BALIMONT Multi-Strain Synbiotic Platform for Intestinal Flora Regulation

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## ABSTRACT

We evaluated a BALIMONT multi-strain synbiotic platform built around *Bifidobacterium longum*, *Bifidobacterium adolescentis*, and *Lactiplantibacillus plantarum*, with fructooligosaccharide and stachyose as prebiotic supports. We reorganized the formulation dossier into a journal-style manuscript and interpreted the disclosed mouse outcomes alongside current probiotic and synbiotic literature. Across the disclosed high-dose groups, all six ratio-optimization embodiments increased bifidobacteria and lactobacilli while reducing *Escherichia coli*; total short-chain fatty acids and butyrate rose in parallel with lower cecal pH, higher fecal-pellet output, and greater fecal moisture. Within the *B. longum*-enriched branch, the 3:1:1 ratio produced the strongest reported metabolic profile, including total SCFA 93.27  $\mu\text{mol/g}$  and butyrate 19.51  $\mu\text{mol/g}$ . Within the *B. adolescentis*-enriched branch, the 1:3:1 ratio reached total SCFA 89.48  $\mu\text{mol/g}$ , butyrate 18.81  $\mu\text{mol/g}$ , bifidobacteria 11.38 IgCFU/g, and cecal pH 5.52. Published evidence indicates that FOS increases colonic bifidobacteria in humans, raffinose-family oligosaccharides can be utilized by bifidobacteria and lactobacilli, and synbiotics can raise *Lactobacillus* and propionate in healthy adults [4-8]. Taken together, these results support BALIMONT as a strain-defined, freeze-dried, prebiotic-supported synbiotic platform whose strongest disclosed effects were observed when bifidobacterial weighting was increased.

## KEYWORDS

BALIMONT; *Bifidobacterium longum*; *Bifidobacterium adolescentis*; *Lactiplantibacillus plantarum*; Synbiotic; Short-chain fatty acids; Freeze-drying; Intestinal flora

## 1. INTRODUCTION

We view probiotic performance not as a property of genus labels alone, but as the combined consequence of strain identity, preservation technology, and the metabolic environment encountered after ingestion. This perspective is especially relevant for multi-strain formulations intended to regulate intestinal flora, where viability loss during processing or insufficient fermentable substrate can weaken downstream biological effects.

The BALIMONT platform examined here uses three defined strains—*B. longum* ATCC 15707, *B. adolescentis* ATCC 15703, and *L. plantarum* ATCC 14917—rather than unspecified mixed cultures. ATCC describes the two bifidobacterial strains as adult-intestine type strains, and lists *L. plantarum* ATCC 14917 as a whole-genome-sequenced type strain [1-3]. This strain-defined architecture gives the platform a reproducible microbiological foundation and supports a more rigorous interpretation of ratio effects.

We further note that BALIMONT is not a probiotic-only system. The formulation couples the three strains to fructooligosaccharide and stachyose, two prebiotic substrates that provide a rational post-

ingestive fermentation environment. In a systematic review and meta-analysis of human trials, FOS supplementation increased colonic *Bifidobacterium* spp. abundance [5]. A recent review of raffinose-family oligosaccharides also highlighted that these carbohydrates can be utilized by bifidobacteria and lactobacilli and can contribute to short-chain-fatty-acid production [6].

Against this literature background, our objective was to examine whether the ratio-optimization logic disclosed for BALIMONT was associated with stronger intestinal-microecology outcomes. We therefore preserved the original formulation branches and animal endpoints, and interpreted them alongside current synbiotic and SCFA evidence [7-10].

## 2. MATERIALS AND METHODS

We organized the available technical material into a conventional original-article structure. The active component consisted of *B. longum*, *B. adolescentis*, and *L. plantarum* prepared as single-strain freeze-dried powders following fermentation, centrifugation, protectant addition, pre-freezing, and vacuum freeze-drying. The final sachet matrix also contained fructooligosaccharide, stachyose, maltodextrin, and silicon dioxide.

The ratio-optimization design included two branches. Embodiments E1–E3 progressively increased only the proportion of *B. longum* (1.5:1:1, 2:1:1, and 3:1:1), while embodiments E4–E6 progressively increased only the proportion of *B. adolescentis* (1:1.5:1, 1:2:1, and 1:3:1). This structure allowed us to compare how greater bifidobacterial weighting affected microbiota counts and fermentation-related readouts without changing the three-strain backbone.

For each embodiment, BALB/c mice were reported to receive saline, 0.5 g/kg bw/day, 1.0 g/kg bw/day, or 2.0 g/kg bw/day by gavage for 28 days. We focused primarily on the high-dose results because they were the most complete and provided the clearest cross-embodiment separation. Core endpoints included fecal counts of bifidobacteria, lactobacilli, *Escherichia coli*, and *Clostridium perfringens*, cecal total SCFA and butyrate, cecal pH, fecal-pellet output, and fecal moisture.

We used published literature only to contextualize the disclosed findings rather than to replace them. Our interpretation therefore remained anchored to the reported internal comparator set and did not extrapolate superiority beyond the conditions explicitly tested.

**Table 1.** Strain identity and platform composition

| Component                            | Catalog / designation | Role in formulation              | Documented feature                 |
|--------------------------------------|-----------------------|----------------------------------|------------------------------------|
| <i>Bifidobacterium longum</i>        | ATCC 15707 / E194b    | Core bifidobacterial strain      | Adult-intestine type strain        |
| <i>Bifidobacterium adolescentis</i>  | ATCC 15703 / E194a    | Secondary bifidobacterial strain | Adult-intestine type strain        |
| <i>Lactiplantibacillus plantarum</i> | ATCC 14917 / Lp 39    | Lactic-acid partner strain       | Whole-genome-sequenced type strain |

The finished sachet platform combined single-strain freeze-dried powders with fructooligosaccharide, stachyose, maltodextrin, and silicon dioxide at 1 g per sachet.

## 3. RESULTS

Across all six disclosed embodiments, the high-dose groups showed a consistent directional response: bifidobacteria and lactobacilli increased, whereas *E. coli* decreased. In the *B. longum*-enriched series, bifidobacteria rose from 10.58 lgCFU/g in E1 to 11.52 lgCFU/g in E3, while *E. coli* declined from 5.94 to 5.52 lgCFU/g. In the *B. adolescentis*-enriched series, bifidobacteria rose from 10.47 lgCFU/g in E4 to 11.38 lgCFU/g in E6, with *E. coli* decreasing from 6.00 to 5.59 lgCFU/g.

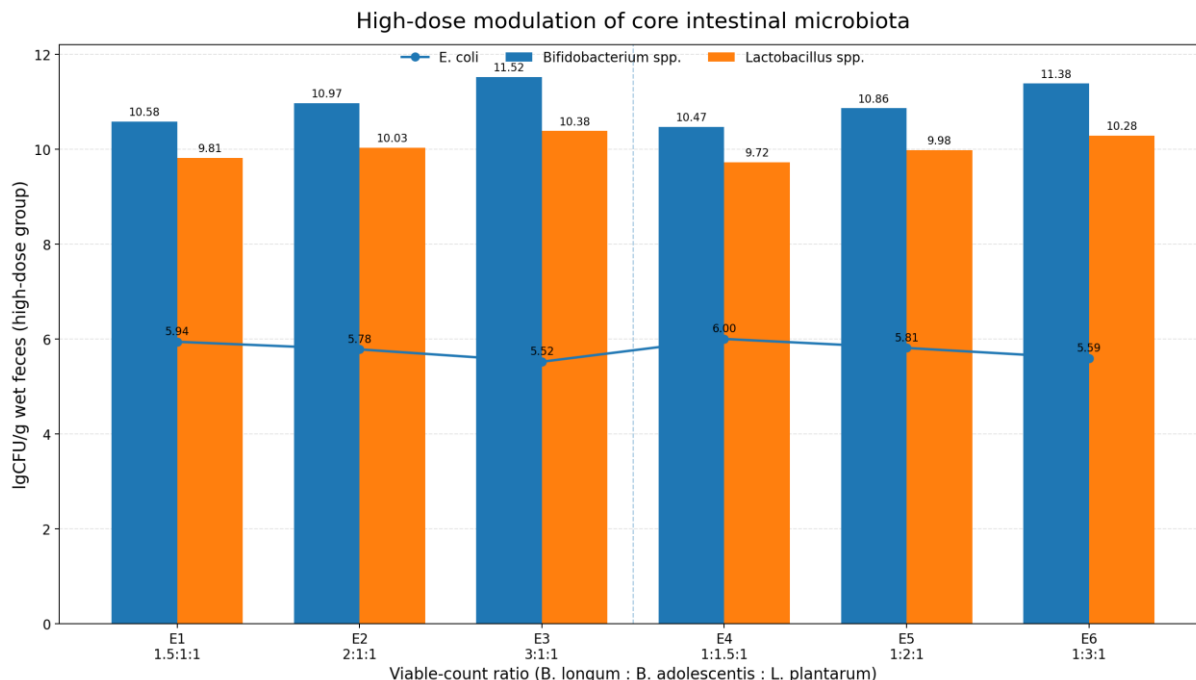
The metabolic branch of the dataset was equally coherent. Total SCFA increased from 78.39 to 93.27  $\mu\text{mol/g}$  and butyrate from 15.89 to 19.51  $\mu\text{mol/g}$  as *B. longum* weighting increased from 1.5 to 3.0. A parallel pattern was observed in the *B. adolescentis*-enriched branch, where total SCFA increased from 75.82 to 89.48  $\mu\text{mol/g}$  and butyrate from 15.35 to 18.81  $\mu\text{mol/g}$ . At the same time, cecal pH moved from near-neutral baseline toward a more acidic fermentation profile, reaching 5.48 in E3 and 5.52 in E6.

The strongest disclosed outcomes therefore clustered in the upper-ratio embodiments. E3 produced the highest values for bifidobacteria, lactobacilli, total SCFA, and butyrate, together with the lowest cecal pH in the *B. longum*-enriched branch. E6 showed the strongest corresponding pattern in the *B. adolescentis*-enriched branch. No deaths, abnormal behavior, diarrhea, or organ-coefficient abnormalities were reported during the 28-day dosing period.

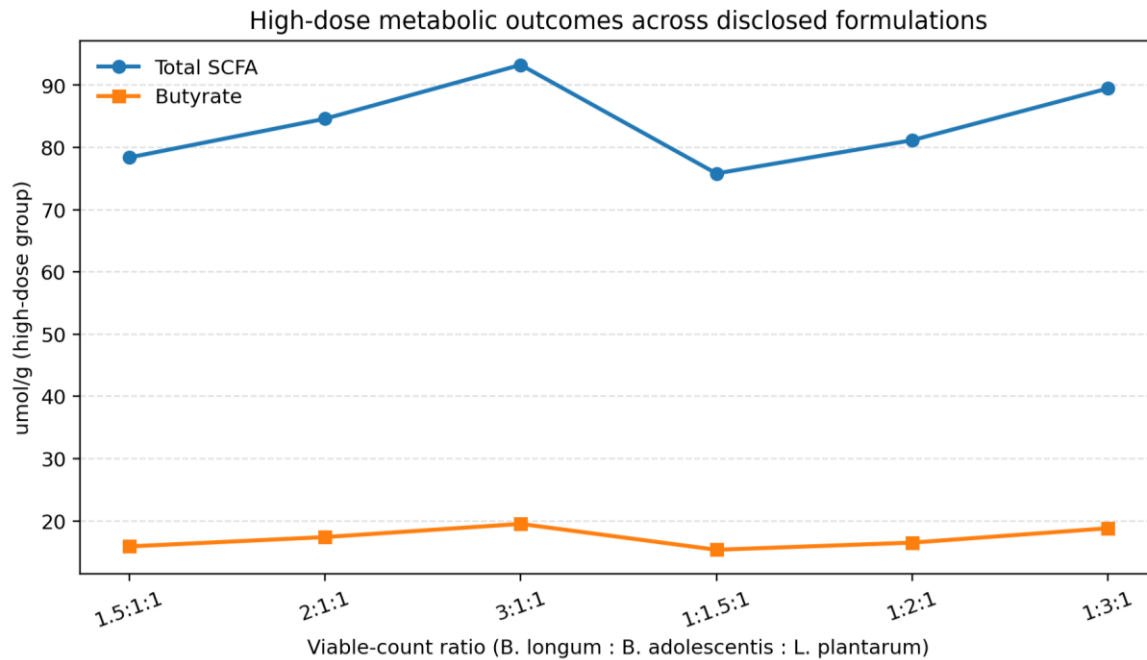
**Table 2.** Key outcomes in the high-dose groups after 28 days

| Emb. | Ratio   | Bifidobacteria | Lactobacilli | <i>E. coli</i> | Total SCFA | Butyrate |
|------|---------|----------------|--------------|----------------|------------|----------|
| E1   | 1.5:1:1 | 10.58          | 9.81         | 5.94           | 78.39      | 15.89    |
| E2   | 2:1:1   | 10.97          | 10.03        | 5.78           | 84.61      | 17.39    |
| E3   | 3:1:1   | 11.52          | 10.38        | 5.52           | 93.27      | 19.51    |
| E4   | 1:1.5:1 | 10.47          | 9.72         | 6.00           | 75.82      | 15.35    |
| E5   | 1:2:1   | 10.86          | 9.98         | 5.81           | 81.16      | 16.50    |
| E6   | 1:3:1   | 11.38          | 10.28        | 5.59           | 89.48      | 18.81    |

Note: blank-control baseline values were 7.21 lgCFU/g for bifidobacteria, 6.84 lgCFU/g for lactobacilli, 8.32 lgCFU/g for *E. coli*, 32.15  $\mu\text{mol/g}$  for total SCFA, 5.99  $\mu\text{mol/g}$  for butyrate, and cecal pH 7.12.



**Figure 1.** High-dose modulation of core intestinal microbiota across the six ratio-optimization embodiments



**Figure 2.** High-dose metabolic outcomes across the disclosed formulations

## 4. DISCUSSION

We interpret the BALIMONT platform as a three-layer design. The first layer is strain definition: using catalogued strains rather than unspecified genera improves reproducibility and gives ratio changes a clear biological meaning [1-4]. The second layer is freeze-dried delivery: the formulation stores each organism as an independent freeze-dried powder before final blending, a strategy that is consistent with the broader probiotic literature on preserving viability during processing and storage. The third layer is synbiotic support: FOS and stachyose provide fermentable substrate that can strengthen bifidobacterial and lactobacillar metabolism after ingestion [5-7].

The SCFA findings are important because they connect the platform to a plausible host-relevant mechanism. Short-chain fatty acids, especially butyrate, are widely recognized as mediators of gut and metabolic health [9,10]. The 2025 synbiotic meta-analysis in healthy adults found that synbiotics increased *Lactobacillus* and propionate and may increase *Bifidobacterium* [7], while the 2022 meta-analysis of probiotics and synbiotics in chronic constipation supports continued interest in bowel-output endpoints such as stool output and transit-related outcomes [8]. In this context, the disclosed increases in SCFA, lower cecal pH, and higher fecal-pellet output form a biologically coherent pattern rather than isolated numerical changes.

At the same time, our interpretation remains bounded. The dataset is preclinical, uses one species and one dosing period, and should not be read as final clinical proof. What the current manuscript supports is narrower but still meaningful: within the disclosed internal comparator framework, higher bifidobacterial weighting was associated with stronger microbiota and fermentation outcomes, and that effect was embedded in a strain-defined, prebiotic-supported, freeze-dried platform.

## 5. CONCLUSION

We conclude that BALIMONT is best described as a ratio-optimized synbiotic platform rather than a generic probiotic blend. Its scientific distinctiveness rests on three aligned features: defined probiotic strains, freeze-dried preservation, and a prebiotic background capable of supporting post-ingestive fermentation. Within the disclosed comparator set, increasing bifidobacterial weighting

produced the strongest gains in microbiota modulation and SCFA output. These results justify further strain-level validation, storage studies, and controlled human trials.

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