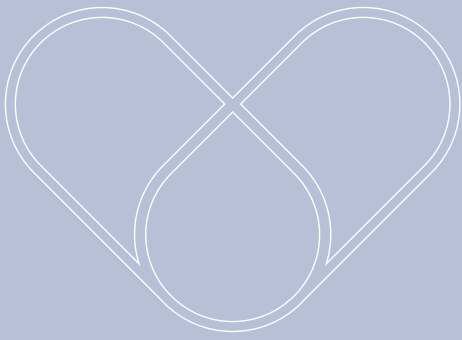
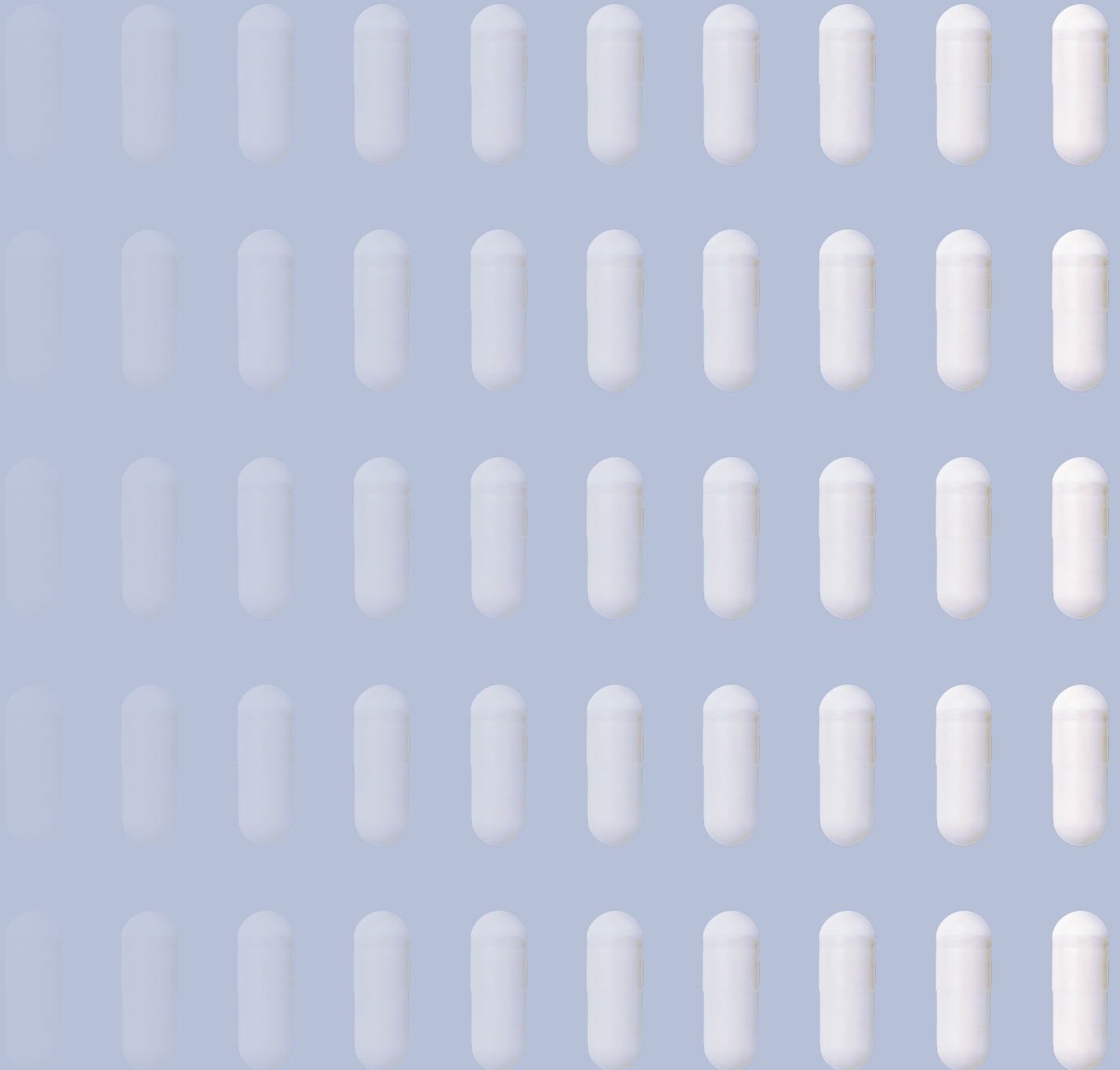




ADVANCED NUTRITION PROGRAMME™

Research Study | **Skin Clear Biome™**





Skin Clear Biome™

Key to skin health is gut health, and key to gut health is a healthy microbiome. All good skin routines start with a biome.

Introduction

The human microbiome is the collection of microbes - bacteria, fungi, viruses and protozoa - that colonise different sites of the body. Both the gut and the skin have well established and specific microbiomes, essential for health.

The gut microbiome is the largest with an estimated 10^{14} microbial cells (1), and has the highest diversity of microbes (1). It is key to gut health, systemic health and skin health. In fact, the impact of the gut microbiome is so significant, that it has been considered an 'essential organ' (2).

Microbes produce numerous compounds which are required for healthy metabolic (1), immune (1), neurologic (3) and endocrine function (3). These include essential nutrients such as vitamin K and B12 (1), hormones and hormone-like substances (4), endocannabinoids (4), as well as neurotransmitters such as gamma-aminobutyric acid (GABA), serotonin, dopamine, tryptophan, noradrenaline, acetylcholine and histamine (5). Microbes also ferment indigestible complex polysaccharides to produce short chain fatty acids (SCFA) such as butyrate, acetate and propionate (1,4,5).

These compounds improve digestion and maintain the health and integrity of the intestinal barrier (5). They are also able to cross the intestinal barrier and enter the blood stream, regulating numerous physiological pathways and impacting systemic health including affecting brain and skin (1,3,4,5).

Gut brain axis

The gut microbiome communicates with, and regulates, the central nervous system (CNS) by neuroimmune and neuroendocrine mechanisms involving the vagus nerve and microbial metabolites (5,6) – known as the 'gut-brain axis'. This 'gut-brain axis' is now thought to contribute to the regulation of a multitude of metabolic, immune, endocrine and nervous system processes (3).

Gut skin axis

The gut microbiome also impacts the health of the skin – the 'gut-skin axis'. Many studies have shown a strong bidirectional link between gastrointestinal and skin health (1). This is largely through modification of the immune system (1). Researchers have gone further to propose that the gut-skin axis is also directly affected by the brain and should be referred to as the gut-brain-skin axis (3).

Effects of dysbiosis

Gut microbial dysbiosis (imbalance in microbial composition) has been associated with a range of medical conditions including irritable bowel syndrome (7), colorectal cancer (7), obesity and related disorders (4,7) and neurological disorders such as anxiety, chronic pain, Parkinson's disease and Alzheimer's Disease (7), amongst numerous other conditions. It also has a negative impact on skin function and integrity (5), and has been linked to numerous skin conditions, in particular conditions related to inflammation (1). This includes inflammatory diseases such as acne, atopic dermatitis, psoriasis and rosacea amongst others (5), as well as skin cancer (1), poor wound healing (1) and accelerating ageing (8).

Intestinal dysbiosis results in the secretion of pro-inflammatory cytokines leading to chronic systemic inflammation and an abnormal immune response that disrupts skin health (4,5,8,9).

Ensuring optimal bacterial balance in the gut is key to regulating the immune system and managing inflammation (5,9).



Probiotics

Probiotics are defined by the World Health Organisation as living microorganisms that confer a health benefit when administered in adequate amounts (10). The use of probiotics leads to improvement and stabilisation of the gut microbiome (11), improvement in gut barrier function (11) as well as regulating the mucosal and systemic immune systems (9) and having an anti-inflammatory effect (9).

Postbiotics are defined as a “preparation of inanimate microorganisms and/or their components that confers a health benefit on the host” (12). Effective postbiotics must contain inactivated microbial cells or cell components, with or without metabolites, that contribute to observed health benefits (12). This includes heat-killed (tyndallized) bacteria.

Pro- and postbiotics have been shown to lead to improvements in numerous medical conditions including gastrointestinal disease, type 2 diabetes mellitus, obesity and related conditions, infections, anxiety and depression (13,14).

They've also been shown to support skin health, improve the management of acne and atopic dermatitis (11,13,14) and postulated to be beneficial in the treatment of general skin inflammation (3,5).

Skin Clear Biome™

Skin Clear Biome™ was formulated to target acne-prone skin, utilising probiotic strains selected for their anti-inflammatory properties and their ability to modulate key factors contributing to acne pathogenesis.

Lactobacillus paracasei has been shown to have a calming effect on the gut-brain axis, thereby reducing stress (15). Additionally, it can enhance the skin's barrier function by supporting the production of ceramides and other lipids essential for maintaining skin integrity (16), modulate the immune system (17), reduce oxidative stress and attenuate inflammation mediated by substance P (18).

Lactobacillus helveticus, *Lactobacillus paracasei*, *Bifidobacterium bifido* have been shown to improve immune function and exert an anti-inflammatory effect. Levels of *Lactobacillus* and *Bifidobacterium* species have been observed to be reduced in individuals with acne.

Saccharomyces boulardii helps strengthen the intestinal barrier, reducing gut permeability. It also enhances the mucosal immune response and secretory IgG intestinal levels and reduce inflammatory cytokines involved in acne (19).

All strains promote a healthy gut microbiome (19, 20, 21).



Objective

This study had two aims: the first was to evaluate the effect of Skin Clear Biome™, a food supplement containing probiotics and zinc, on problem-prone skin; the second was to assess whether or not taking this probiotic combination internally could alter the composition of the skin microbiome.

Study method

Participants and procedure

This study was conducted over 8 weeks. Thirteen women and 1 man, aged 18 to 40 years (average 26 years), were enrolled. Participants were healthy but displayed signs of skin blemishes such as redness, bumps and inflammatory lesions.

Participants were required to take one capsule of Skin Clear Biome™ daily with a main meal.

They were instructed to avoid excessive UV light exposure, and not to make any changes to their skincare routine, undergo facial treatments or cosmetic alterations, or take food supplements except for medically prescribed supplements such as iron or vitamin B12. No topical antibiotics, alpha hydroxy acids, astringents, alcohol-based preparations, medicated shaving creams, or tea tree oil products could be used during the trial. People who had taken antibiotics in the past 6 months or had soy or dairy allergies were excluded. A 2-week washout period was required before the start of the study to allow for the clearance of any previously taken food supplements. All subjects provided informed consent before participation.

Test products and ingredients

Each capsule of Skin Clear Biome™ contained *Lactobacillus paracasei*, Lafti® L26 AF, *Bifidobacterium bifidum*, Rosell®-71, *Lactobacillus helveticus*, Lafti® L10 ND, *Saccharomyces boulardii* and zinc.

Assessment

Subjects were assessed at baseline, week 4 and week 8. Facial areas measured included the lateral canthus area and mid-cheek, and for sebum levels the forehead, nose and chin.

The Courage & Khazaka™ equipment was used to measure properties of the skin. Courage & Khazaka™ are worldwide leaders in the production of dermatology testing devices.

Assessment included:

○ Direct measurement of skin using Courage & Khazaka™ probes:

- **Hydration** in the stratum corneum is assessed with a Corneometer® which is a capacitance measure of the di-electric properties of the stratum corneum. Hydration is measured in arbitrary units (AU), with very dry being < 30, dry being 30 – 40 and normal > 40.
- **Transepidermal water loss (TEWL)** is assessed with a Tewameter® which uses open chamber evaporimeters to measure TEWL. This together with the electrical method used by the Corneometer are the preferred techniques for measuring the water balance in the stratum corneum. TEWL > 20 is defined as increased.
- **Skin elasticity** is assessed with a Cutometer®, which uses suction methods to deform skin and a non-contact optical measurement system to measure the deformity and recoil, which is used to calculate the elastic and viscoelastic properties of skin.
- **Melanin and erythema (haemoglobin)** are assessed with a Mexameter®, which emits 3 specific wavelengths of light and measures the light reflected (reflectance of skin). Erythema is grouped into: none < 170, minimal 170 – 330, diffuse redness 330 – 450, high 450 – 750 and extreme > 570. Melanin varies per skin type.
- **Sebum** levels are assessed with the Sebumeter®, which uses grease spot photometry. On the forehead and t-zone, a value of < 100 is defined as dry/less sebum, 100 – 180 as normal, and > 180 as oily.



- **Imaging** of the full face using the Canfield® Visia equipment. This uses IntelliFlash®, cross-polarized and UV lighting to record and measure surface and subsurface skin conditions.
- **16S rRNA testing using Diversigen® Core Plus Analysis.** This technique is employed in microbiology to analyse and understand microbial communities present in a sampling area. Samples were collected from the forehead area using Norgren collection tube kits and buffer solution.
- **Self-assessment questionnaire** administered at the end of the study.
- **MYMOP2 (Measure Yourself Medical Outcome Profile) questionnaire**

MYMOP2 is a generic patient-specific outcome tool designed to evaluate a wide range of healthcare interventions. Participants can identify up to two symptoms of greatest concern to them and subjectively assess the severity.

The MYMOP2 consists of 7 questions: 2 related to the symptoms identified, and the rest related to the impact of symptoms on wellbeing, the impact of physical activity on the symptom and the medication used.

It uses a 7-point Likert scale to score the questions with 0 being 'as good as it could be' and 6 being 'as bad as it could be'. The change in symptoms is then assessed over time. A change in score from beginning to end should be between 0.5 and 1.0 to be clinically important, and all changes greater than 1.0 can be considered clinically significant.

Results

Skin measurements

The initial hydration levels in the lateral canthus area were normal, and no changes were observed throughout the study. In the cheek area, one participant exhibited very dry skin with no improvement, while two participants started with dry skin and showed improvement. The rest of the group experienced no changes in hydration levels.

After 8 weeks, improvements in TEWL were observed in the lateral canthus area. Of the 14 participants, 8 showed an increase of more than 9% with improvements ranging from 9% to 39% (average increase 26%). In the cheek area, 7 out of 14 participants had improvements greater than 9%, with a range of 9% to 30% (average increase 20%).

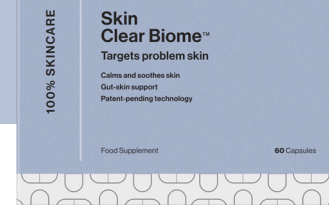
In the forehead area, 2 participants had oily skin and 4 had dry skin; 5 out of 14 showed a sebum reduction of 30% or more, ranging from 30% to 72% (average decrease 49%). In the nose area, 4 participants had oily skin and 6 had dry skin; 6 participants showed a sebum reduction of 19% or more, ranging from 19% to 72% (average decrease 36%). For the chin area, 2 participants had oily skin and 5 had dry skin; 5 participants showed a sebum reduction of 15% or more, ranging from 15% to 54% (average decrease 36%).

Elasticity increased in the lateral canthus area by more than 5% in 8 participants, with improvements ranging from 5% to 33% (average increase 16%). In the cheek area, 2 participants experienced an increase of more than 19%, with improvements ranging from 19% to 26% (average increase of 22%).

Diversigen® Core Plus Analysis

Swabs were taken to sequence the skin microbiome both before and after the intervention. They were taken from the forehead of 10 subjects before and after the trial, totalling 20 samples to sequence the skin microbiome.

To assess the microbiome of the environment, each sample was submitted to Diversigen® for sequencing of the specific V1V3 region of the 16S rRNA gene using amplicon sequencing. A CorePlus analysis aimed to provide detailed insights into the composition, diversity, and dynamics of microbial populations. The analysis evaluated differences in within-sample diversity (Alpha Diversity), between-sample diversity (Beta Diversity), and the abundance of different taxa across different time points.



Due to the challenges in collecting microbiome samples from skin, only 5 of the 10 samples contained enough bacterial material for analysis.

Alpha diversity: This shows the within sample diversity. A Shannon index was used for each sample. This index considers both how many different types of microorganisms are present (richness) and how evenly distributed they are (evenness). Higher values of the Shannon index mean there is a more diverse mix of microorganisms in the community. The result of this can be seen in the plot below (Figure 1).

While there was no significant difference in the group before and after, we can see that the change over time appears to be individualized, with 3 of 5 subjects increasing in alpha diversity after treatment and 2 of 5 subjects decreasing.

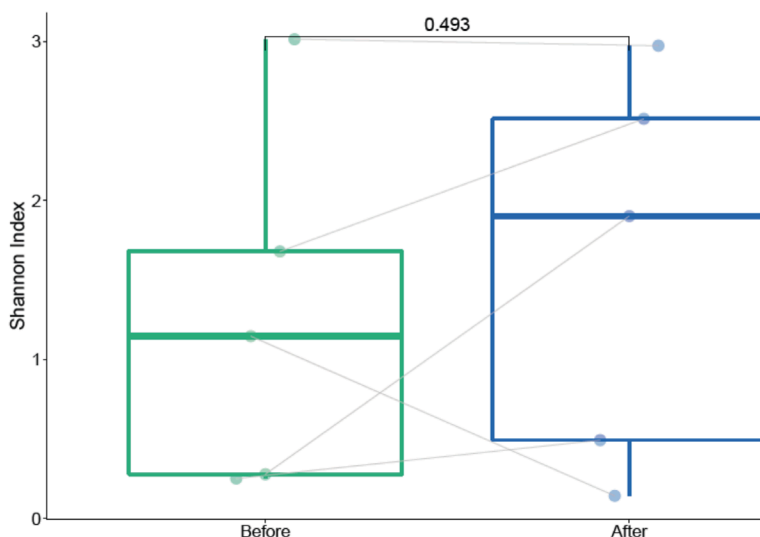


Figure 1. Box plot depicting Shannon index values for paired Before and After samples. Grey lines connect paired samples from the same subject between time points. The p-value from paired t-testing is depicted at the top of the plot with a bracket connecting the two groups. From this plot, we can observe that, overall, there is no significant difference in alpha diversity values between the Before and After samples. However, it's interesting to note that the changes over time seem to vary among individuals. Specifically, out of the five subjects, three showed an increase in alpha diversity after treatment, while two showed a decrease. This suggests that the response to treatment may be individualized rather than uniform across all subjects.

Beta Diversity: This shows the similarity between samples using the Bray-Curtis dissimilarity assessment, which measures how different the overall microbial communities are between pairs of samples. Beta diversity was not significantly different between before and after paired samples. Similar to the alpha diversity analyses, we can see that the shift in beta diversity is individualized, with some subjects changing only slightly, while others change to a greater degree

Individual taxa

No amplicon sequence variants were significantly different between before and after paired samples after multiple hypothesis correction. This is likely due to low sample size, which can make it difficult to see consistent trends in differential abundance for given taxa due to high inter-subject variability.

However, for exploratory purposes, the results of statistical testing prior to multiple hypothesis correction were reviewed. By examining uncorrected p-values, we found that three ASVs decreased significantly over the trial:

- f__Corynebacteriaceae;g__Lawsonella;s__NA;t__ASV_134
- f__Propionibacteriaceae;g__Cutibacterium;s__NA;t__ASV_10
- f__Propionibacteriaceae;g__Cutibacterium;s__NA;t__ASV_9 (figure 2)

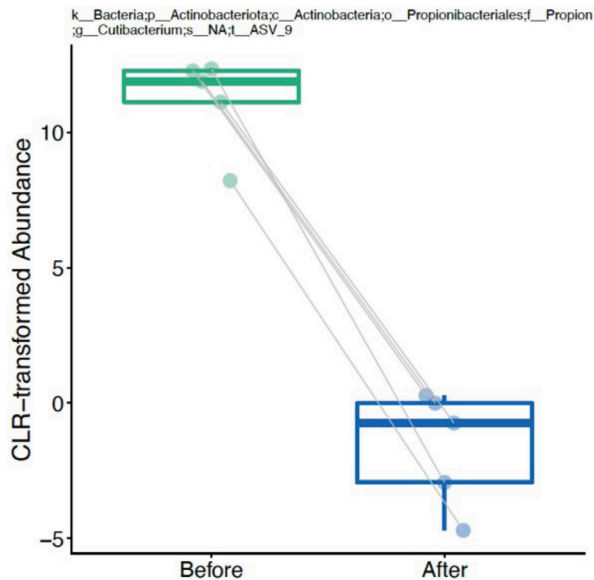
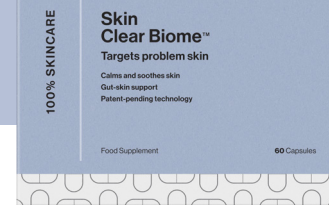


Figure 2. Box plot of CLR-transformed abundance for ASV_9 (*Cutibacterium* genus) for before and after samples. Gray lines connect paired samples from the same subject between time points.

From this plot, we can see an intriguing pattern where this specific ASV consistently decreases in abundance across time for each of the 5 subjects in the data set. While this result does not hold once p-values are corrected for multiple hypothesis testing, it is still an interesting observation that may be worth future study, and the same applies to the other 2 ASVs mentioned above.

MYMOP2 (The Measure Yourself Medical Outcome Profile)

The MYMOP2 uses a 7-point scale, where a minimum clinically important change after intervention ranges between 0.5 and 1.0. A change of 1 or more is considered clinically significant. All participants identified breakouts and redness as their primary symptoms of concern.

Based on the MYMOP2 questionnaire results (Figure 3):

- 8 out of 14 participants (57%) experienced a clinically significant improvement in overall well-being at 8 weeks.
- 10 out of 14 participants (71%) experienced a clinically significant reduction in the number of breakouts at 8 weeks.
- 9 out of 14 participants (64%) experienced a clinically significant reduction in redness at 8 weeks.

These results suggest that daily use of Skin Clear Biome™ led to clinically significant benefits for participants.

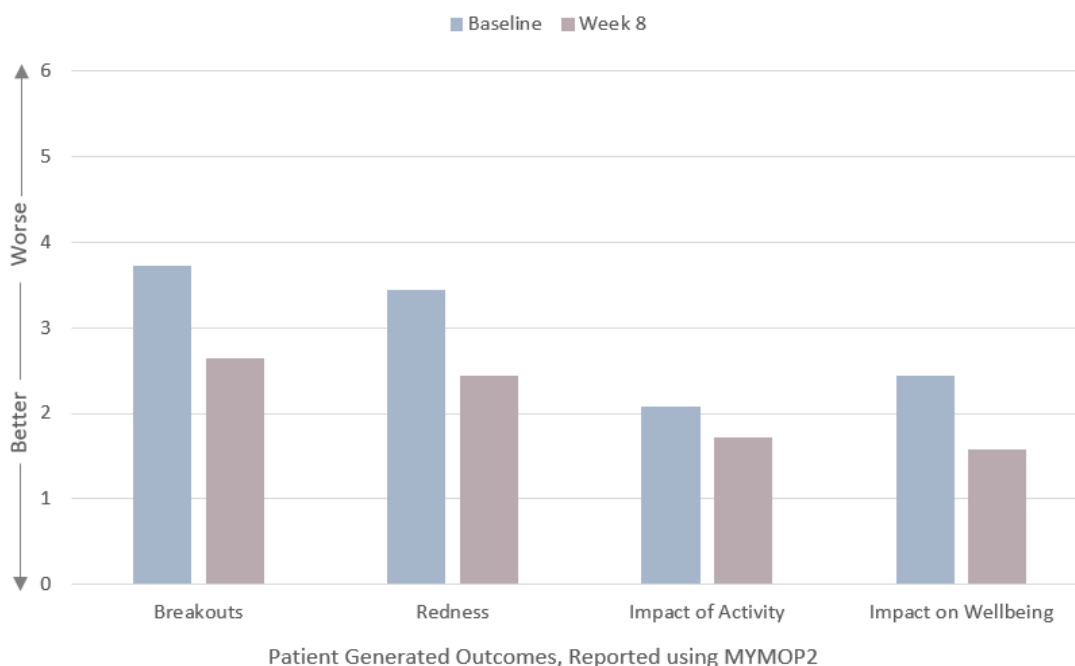
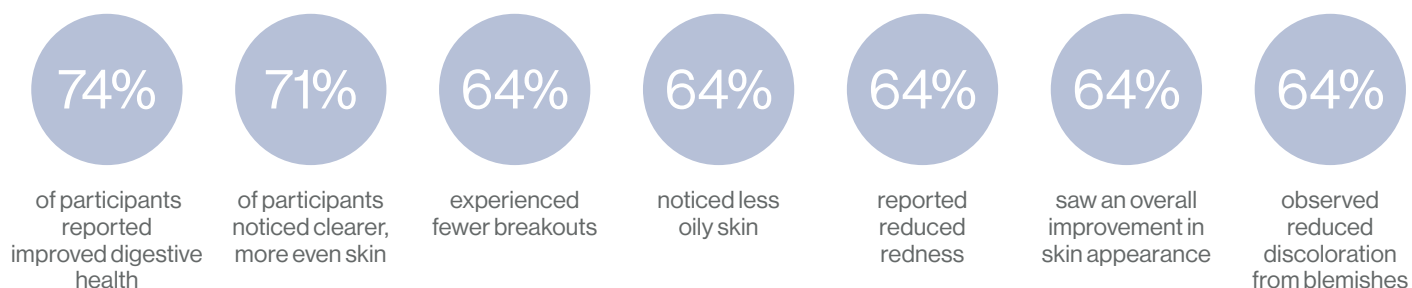


Figure 3. MYMOP2 scores of the severity of breakouts, redness of skin, impact of physical activity on symptoms and impact of symptoms on wellbeing, with 0 being as good as it gets and 6 being as bad as it can be.



Self-administered questionnaire results

The questionnaire consisted of 7 questions with 6 referring to skin health and 1 to digestive health. It was administered at the end of the study only.



Before and afters

Dose: 1 Skin Clear Biome™ daily | Duration 8 weeks

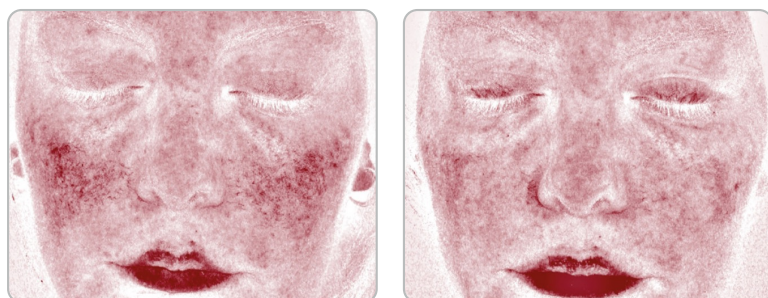


Figure 4. Photographs taken with Canfield® Visia equipment of the full face, focusing on the cheek area, at baseline (before) and after 8 weeks. A noticeable reduction in redness was observed.

Dose: 1 Skin Clear Biome™ daily | Duration 8 weeks

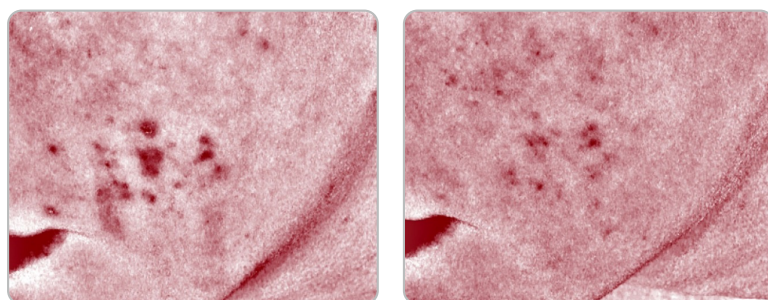


Figure 5. Photographs taken with Canfield® Visia equipment of the left cheek at baseline (before) and after 8 weeks, displaying changes to red, problematic areas.

Conclusion

The findings of this study provide some insights into the relationship between gut health and skin health. While the analysis of the skin microbiome before and after the intervention remained largely unchanged, there were noteworthy trends uncovered when examining the amplicon sequence variants (ASVs). Specifically, three ASVs from two bacterial genera exhibited significantly lower abundance post-intervention, suggesting that supplementation with Skin Clear Biome™ may influence specific microbial populations. The MYMOP2 assessment revealed clinically significant improvements in symptoms like breakouts and redness, supporting the efficacy of Skin Clear Biome™. These findings highlight the potential of targeting gut health with probiotic combinations to manage skin conditions and underscore the importance of further research to optimise such therapeutic strategies.



ADVANCED
NUTRITION
PROGRAMME™



8 of 10

100% SKINCARE

**Skin
Clear Biome™**

Targets problem skin
Calms and soothes skin
Gut-skin support
Patent-pending technology

Food Supplement

60 Capsules

Awards



Nutritionist Choice 2023

Winner - Best Supplement for Acne-Prone Skin

Advanced Nutrition Programme™
Skin Clear Biome™



The Beauty Shortlist Wellbeing Awards 2022

UK - Editor's Choice Award Winners

Advanced Nutrition Programme
Skin Clear Biome™



Marie Claire Skin Awards 2021

Winner - Best Skin Innovation - Supplement Category

Advanced Nutrition Programme
Skin Clear Biome™



Pure Beauty Global Awards 2021

Winner - Best New Skin and Body Supplement

Advanced Nutrition Programme
Skin Clear Biome™



Woman & Home Clever Skincare 2021

Winner - Best Supplement For Clear Skin

Advanced Nutrition Programme
Skin Clear Biome™



Testimonials

*I noticed some improvements in the way that my skin felt and looked during the first 4 weeks of using the product. I had fewer spots on my face and the red bumps/spots on my chest and neck area seemed to fade a bit. In the last 4 weeks of using the product the skin on my face started to feel much smoother. I had considerably fewer spots on my face and my chest and neck are almost entirely spot-free. **Laura S** (29)*

*I have had such a good experience with the product. My skin has cleared up a lot, from the oiliness of my skin to the number of breakouts I have had, has massively decreased. Also, the healing time, if I did get a breakout, they would dry out and clear up a lot quicker than normal. **Kate M** (19)*

*When first using the product, I experienced breakouts, and I was unsure if it was working. After the second assessment session, it kicked in and slowly started to clear up my skin and the spots were less sore. My face also became less oily. **Leah P** (21)*

*During the 8 weeks, I noticed a difference in redness and inflammation around my spots and they weren't as sore as normal. I also experienced less bloating and cramps while I was taking this product. **Sharon I** (40)*

*I noticed clearer skin, fewer monthly breakouts, and faster healing of blemishes. My digestion has also been a lot better, and I noticed a significant reduction in craving for sugar. My complexion is also a lot brighter. **Candice F** (29)*

*I am overwhelmed with the results, it has exceeded my expectations. I will be lost without the product, it has really boosted my confidence, lots of people have noticed, and over the last 8 weeks I have made more progress on my skin than I have in 7 years. It's been completely life-changing.. **Charlotte L** (22)*

*I found the product overall good, I felt that my problem such as oily skin and blemishes hugely improved. My skin felt more radiant, and the overall quality was improved by the products. **Jassynta N** (23)*



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