

Article

Oral Probiotic Supplementation Improves Skin Aging Biomarkers: An 8-Week Double-Blind, Randomized, Placebo-Controlled Trial

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Abstract

Background: Skin aging is influenced by intrinsic and extrinsic factors, and growing evidence suggests that nutritional interventions, particularly probiotics, may modulate key biological pathways involved in skin barrier function, hydration, and oxidative stress through the gut–skin axis. **Methods:** In this randomized, double-blind, placebo-controlled clinical trial, 66 adults received a probiotic formulation containing *Lactocaseibacillus rhamnosus* LRH020, *Lactiplantibacillus plantarum* PBS067, and *Limosilactobacillus reuteri* PBS072 for 56 days, followed by a 28-day follow-up. Skin structure, function, and oxidative status were assessed using 3D profilometry, high-frequency ultrasound, corneometry, transepidermal water loss (TEWL), FRAP assay, and expert clinical scoring. **Results:** Compared with placebo, the probiotic group showed significant improvements in wrinkle depth (−15.0% at T56), and roughness (−6.1% at T56). Moreover, other skin parameters were significantly enhanced by the end of treatment, including greater skin density, hydration, and antioxidant capacity, alongside a reduction in TEWL. Improvements persisted at follow-up, suggesting a durable, lasting benefit of the probiotic formulation beyond the treatment period. **Conclusions:** These findings support the role of targeted probiotic supplementation as a promising strategy to improve structural, functional, and oxidative markers of skin aging.

Keywords: randomized clinical trial; gut–skin axis; wrinkles; TEWL; hydration; antioxidant capacity; mature skin; probiotics; skin aging



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1. Introduction

Skin aging is a multifactorial biological process driven by intrinsic and extrinsic factors that progressively alter skin structure, function, and appearance [1,2]. Intrinsic mechanisms, including hormonal changes and reduced collagen and elastin synthesis, contribute to the progressive decline in elasticity, hydration, and barrier function [1,3]. Extrinsic aging is primarily driven by ultraviolet radiation (UVR), pollution, lifestyle factors, and nutritional habits, collectively described as the “skin aging exposome” [4–6]. These external stressors accelerate oxidative damage, inflammation, and degradation of skin components, thereby exacerbating the visible signs of aging [7].

Recent research has highlighted the essential role of the skin microbiome in maintaining homeostasis, regulating immune responses, and supporting barrier integrity [2,8,9]. In parallel, growing evidence supports a bidirectional communication between the gut

microbiota and the skin, known as the gut–skin axis, which links intestinal dysbiosis to inflammatory and barrier-related skin disorders [10–12]. Although increasing evidence suggests an association between gut microbiota composition and skin health, the causal relationships and the underlying biological mechanisms remain to be fully elucidated. Probiotic supplementation has emerged as a promising strategy to improve skin health by modulating microbial balance [13–15]. Because oxidative stress contributes to collagen degradation and skin aging, biomarkers of antioxidant capacity represent relevant indicators of skin health and treatment efficacy [7,14]. Several clinical studies have shown that specific probiotic strains can reduce oxidative stress, enhance hydration, and attenuate wrinkle depth through systemic and local mechanisms [13–17], supporting skin vitality through strategies that promote well-aging. More broadly, growing evidence indicates that nutritional interventions can modulate cutaneous disease biology by influencing several factors, such as immune response, supporting their potential role as complementary strategies for maintaining skin health and promoting healthy aging [18]. However, strain-specificity, dosage variability, and heterogeneous study designs (including differences in participant age, treatment duration, and methods used to assess skin outcomes) contribute to the inconsistent findings reported across clinical trials, highlighting the need for well-designed studies evaluating defined probiotic combinations [19–22]. While some studies report significant improvements in skin elasticity and moisture retention, others have found minimal or no effects, underscoring the need for further research [19,20].

Given the increasing interest in microbiota-based strategies for skin health, this study aimed to evaluate whether a targeted probiotic formulation composed of *Lactiplantibacillus plantarum* PBS067, *Limosilactobacillus reuteri* PBS072, and *Lacticaseibacillus rhamnosus* LRH020 could improve structural, functional, and oxidative markers of skin aging through modulation of the gut–skin axis. These strains were originally screened for their anti-inflammatory and antioxidant properties in a gut in vitro model [23], while a UVB-photoaging mouse model confirmed improved hydration and reduced wrinkling via modulation of the intestinal microbiome [24]. In a previous clinical study, all species included in the formulation were detected in the human gut even after the end of supplementation, suggesting their ability to persist and potentially colonize the intestinal environment [25].

Moreover, this formulation has previously demonstrated clinical benefits on wrinkle depth, hydration, and elasticity when combined with topical actives [13], and has shown barrier-protective, anti-inflammatory effects in atopic dermatitis and acne [26–28]. Unlike these earlier trials, which either paired the blend with topical actives or targeted inflammatory skin conditions, the present study is the first to test this three-strain formulation as a standalone oral intervention with validated anti-aging endpoints.

By performing a randomized, double-blind, placebo-controlled study, the objective is to determine whether probiotic supplementation can provide measurable benefits in terms of skin hydration, elasticity, and overall barrier function, thereby contributing to the expanding field of probiotic dermatology.

2. Materials and Methods

2.1. Study Design and Ethical Approval

This study was designed as a randomized, double-blind, placebo-controlled clinical trial conducted at the Complife Italia S.r.l. facility (San Martino Siccomario, PV, Italy) between October 2024 and January 2025. The total study duration was 84 days, consisting of a 56-day supplementation phase (T0–T56) followed by a 28-day post intervention follow-up (T56–T84).

The protocol was approved by the Comitato Etico Indipendente per le Indagini Cliniche Non Farmacologiche (ref. 2024/02, 5 April 2024) and conducted in accordance

with the Declaration of Helsinki and Good Clinical Practice (GCP). The study was prospectively registered at ISRCTN (ISRCTN93811508). All participants provided written informed consent prior to enrolment.

2.2. Participants

A total of 66 healthy Caucasian adults (38–67 years) were enrolled. A homogeneous population was intentionally selected to minimize ethnic-related variability in skin structure and aging patterns.

The planned sample size was defined according to previous clinical experience and published randomized controlled trials investigating oral interventions for skin aging with comparable clinical endpoints [13,20,29]. Inclusion criteria required visible crow's feet wrinkles (score ≥ 2 according to the Skin Aging Atlas—Caucasian Type) [30], normal to dry skin, and stable pharmacological therapy for at least one month. Key exclusion criteria included dermatological diseases, allergies to cosmetic or probiotic ingredients, pregnancy or breastfeeding, recent probiotic supplementation (within 4 weeks), and participation in other clinical studies (Table S1).

Participants were recruited from the Complife volunteer database and screened at baseline (T0). All subjects agreed to maintain their usual lifestyle and to refrain from using any facial cosmetic products other than the standardized SPF base cream provided by the investigators.

2.3. Randomization, Blinding, and Intervention

Participants were randomly assigned (1:1) to receive either the active probiotic supplement (PRO) or placebo (PLA). Randomization was performed using Wey's urn method to ensure balanced allocation. An independent technician dispensed either the active product or the placebo according to the randomization list. Access to the randomization list was restricted to the in-site study director. Investigators, collaborators involved in outcome assessment, and participants remained unaware of treatment allocation throughout the study. Active and placebo capsules were identical in appearance, size, color, smell, and taste, thereby maintaining blinding. The active product consisted of capsules containing three probiotic strains:

- *Lacticaseibacillus rhamnosus* LRH020 (DSM 25568),
- *Lactiplantibacillus plantarum* PBS067 (DSM 24937),
- *Limosilactobacillus reuteri* PBS072 (DSM 25175),

Each providing 1×10^9 CFU/dose, with viability guaranteed until end of shelf life. The placebo capsules contained identical excipients (corn starch, maltodextrin, magnesium stearate) without probiotics. Both the active probiotic product and the placebo were provided by Synbalance S.r.l. (Origgio, Italy).

Participants consumed one capsule daily, preferably away from meals. Compliance was monitored through capsule count and daily diaries, with $\geq 80\%$ considered acceptable.

2.4. Additional Treatments and Lifestyle Controls

To minimize external influences on the study outcomes, participants were instructed to avoid any additional cosmetic products on the face during the study, except for the provided SPF base cream, which contained no active anti-aging ingredients. They were also asked to record any significant changes in their diet or lifestyle in a daily journal, which would help identify potential confounding factors. This was particularly important in controlling for variables such as changes in diet, skincare habits, or stress levels, which could impact skin health. Treatment adherence was assessed by counting the unused capsules at the end of the 56-day treatment period, with a compliance rate of $\geq 80\%$ considered acceptable.

2.5. Study Procedures and Clinical Visits

Participants attended four visits:

- T0 (baseline): eligibility assessment, randomization, product dispensing, baseline measurements.
- T28: compliance check and intermediate measurements.
- T56: end of treatment measurements and self-assessment questionnaire.
- T84: follow-up measurements to evaluate persistence of effects.

All assessments were performed under controlled environmental conditions (22 ± 2 °C; 40–60% RH). Participants acclimatized for 15–20 min before measurements and refrained from applying any facial products for at least 8 h prior to each visit.

2.6. Outcome Measures and Instrumentation

The study evaluated a comprehensive panel of structural, functional, and biochemical skin parameters to capture the multidimensional nature of skin aging. Measurements were selected to reflect clinically relevant aspects of cutaneous aging, including wrinkle morphology, hydration status, barrier integrity, dermal architecture, and oxidative balance. All assessments were performed under controlled environmental conditions (22 ± 2 °C; 40–60% RH) after a 15–20 min acclimatization period.

2.7. Primary Outcome: Wrinkle Morphology

Wrinkle Depth and Surface Roughness (Ra)

The primary endpoint was the change in wrinkle depth in the crow's feet area, a region highly sensitive to chronological and photo-induced aging. Wrinkle depth and surface roughness (Ra) were quantified using the PRIMOS CR small field 3D optical profilometry system (Canfield Scientific Europe, Utrecht, The Netherlands).

This device projects structured light to generate high-resolution topographic maps of the skin surface, enabling precise quantification of micro-relief features. The system ensures high reproducibility (intra session variability < 5%) through anatomical repositioning masks and fixed support systems. The measurement side (left/right) was randomized to avoid systematic bias.

2.8. Secondary Outcomes

2.8.1. Skin Hydration

Superficial Hydration

Superficial stratum corneum hydration was assessed using the Corneometer® CM 825 (Courage + Khazaka electronic GmbH, Cologne, Germany), which measures changes in skin capacitance. This parameter reflects the water-binding capacity of the outer epidermal layers, a key determinant of smoothness, elasticity, and wrinkle formation. Measurements were performed in quintuplicate, and mean values were used for analysis.

Deep Epidermal Hydration

Deep hydration was evaluated using the MoistureMeterEpiD (Delfin Technologies Ltd., Kuopio, Finland), which quantifies dielectric permittivity to estimate water content in deeper epidermal layers. This measurement provides insight into the skin's ability to retain moisture beyond the superficial barrier.

2.8.2. Skin Barrier Function

Transepidermal Water Loss (TEWL)

TEWL was measured using the Tewameter® TM 300 (Courage + Khazaka electronic GmbH, Cologne, Germany), a widely validated instrument for assessing barrier integrity.

TEWL reflects the passive diffusion of water through the epidermis; reductions in TEWL indicate improved barrier function, tighter lipid organization, and reduced susceptibility to environmental stressors.

2.8.3. Dermal Structure

Skin Thickness and Density

High-frequency ultrasound imaging (22 MHz) was performed using the DUB[®] Skin-Scanner (tpm taberna pro medicum GmbH, Lüneburg, Germany) to assess combined epidermal and dermal thickness and dermal density. These parameters reflect the structural integrity of the extracellular matrix, including collagen and elastin networks, which progressively decline with age. Increased thickness and density are indicative of improved dermal support and reduced wrinkle formation.

2.8.4. Oxidative Stress and Antioxidant Capacity

FRAP Assay on Corneofix[®] Strips

Oxidative stress was evaluated through the Ferric Reducing Antioxidant Power (FRAP) assay performed on stratum corneum samples collected via Corneofix[®] tape stripping (Courage + Khazaka electronic GmbH, Cologne, Germany). The FRAP assay quantifies the skin's antioxidant capacity by measuring its ability to reduce Fe^{3+} to Fe^{2+} , providing a biochemical indicator of protection against ROS-mediated aging processes. The method followed Benzie & Strain [31] and the adaptation by Nobile et al. [32].

2.8.5. Clinical Evaluation

Expert Scoring of Wrinkle Severity and Skin Radiance

Standardized VISIA[®] CR photographs (Canfield Scientific Europe, Utrecht, The Netherlands) were evaluated by trained experts blinded to treatment allocation. Wrinkle severity was scored on a validated 0–7 scale, while skin radiance was assessed on a 1–5 scale. These clinical evaluations complement instrumental data by capturing visible, real-world changes in skin appearance.

2.8.6. Self-Assessment Questionnaire

At T56, participants completed a structured questionnaire evaluating perceived improvements in hydration, smoothness, radiance, elasticity, and overall skin appearance. Responses were collected using a four-point Likert scale (“completely agree” to “completely disagree”).

2.9. Statistical Analysis

Statistical analyses were conducted on the Per-Protocol (PP) population. No imputation of missing data was applied. Normality was assessed using the Shapiro–Wilk test.

For normally distributed variables, repeated measures ANOVA with Tukey–Kramer post hoc test was used. For non-normal data, the Friedman test with Tukey–Kramer post hoc was applied. Intergroup comparisons were performed using Student's *t*-test or Mann–Whitney U test as appropriate.

Baseline values and FRAP pre- vs. post-treatment comparisons were analyzed using paired *t*-tests. Statistical significance was set at $p < 0.05$. Analyses were performed using NCSS 10 Professional (version 10.0.7; NCSS, LLC, Kaysville, UT, USA).

3. Results

3.1. Population, Treatment Compliance, and Tolerability

A total of 66 participants were enrolled and randomized, of whom 65 completed the study and were included in the Per-Protocol (PP) analysis (Figure 1). Baseline demographic and skin characteristics were comparable between groups (Table 1), confirming adequate randomization.

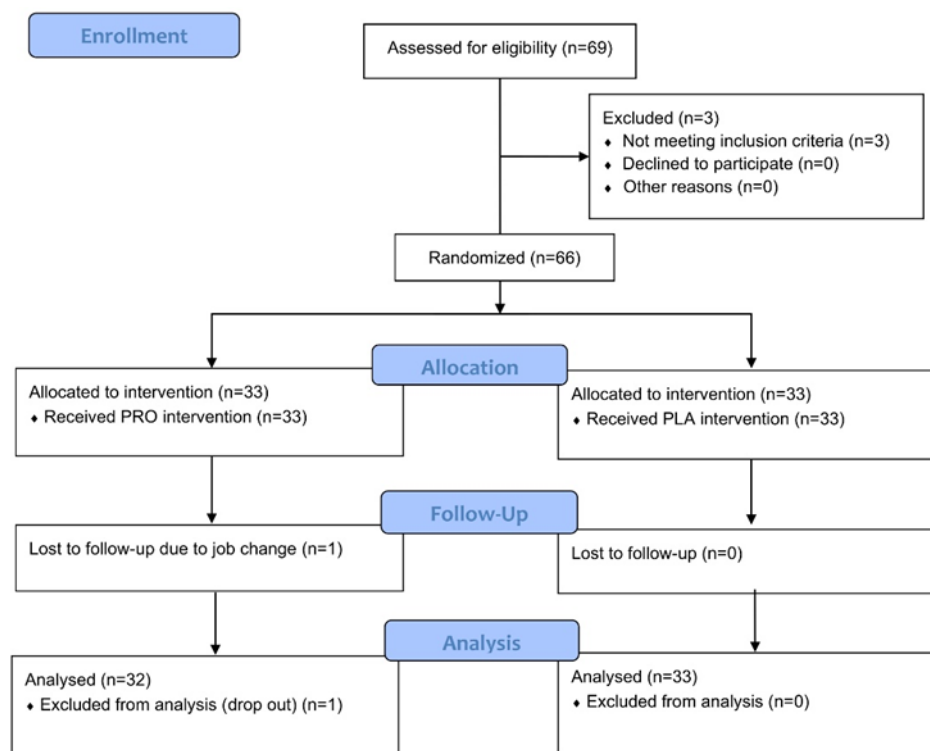


Figure 1. CONSORT flow-chart diagram of the participant.

Table 1. Demographic and baseline characteristics of the study population. Data are reported as mean value $\pm$ SEM. *p* values refer to intergroup baseline comparisons (PRO vs. PLA).

Variable	PRO (n = 32)	PLA (n = 33)	Units	<i>p</i> Value
Sex				
Male	16 (48%)	18 (52%)	n.a.	n.a.
Female	17 (52%)	15 (48%)	n.a.	n.a.
Age (years)	53.6 $\pm$ 1.5	52.9 $\pm$ 1.5	Years	n.a.
Wrinkle Depth	320.0 $\pm$ 24.6	296.4 $\pm$ 19.5	μ m	0.455
Ra	32.8 $\pm$ 1.5	34.0 $\pm$ 1.3	μ m	0.534
Skin Thickness	1578.1 $\pm$ 43.7	1614.3 $\pm$ 38.9	μ m	0.537
Skin Density	17.9 $\pm$ 0.4	17.5 $\pm$ 0.4	%	0.588
TEWL	12.4 $\pm$ 0.4	11.1 $\pm$ 0.3	g/h/m ²	0.046
Superficial skin moisturization	35.2 $\pm$ 1.4	37.4 $\pm$ 1.1	c.u.	0.231
Deep skin moisturization	35.0 $\pm$ 1.5	36.9 $\pm$ 1.3	%	0.239
FRAP assay	134.0 $\pm$ 7.0	134.7 $\pm$ 9.4	Fe ²⁺ μ m	0.954

Subjects were randomly assigned to receive either the test product (PRO) or the placebo (PLA). Compliance was high in both groups, exceeding the predefined threshold of 80% (96.7% in PRO; 95.2% in PLA). No serious adverse events or unexpected reactions were reported, and both products were well tolerated throughout the study.

3.2. Skin Profilometry

3.2.1. Wrinkle Depth

Participants receiving the probiotic supplement showed a progressive and significant reduction in wrinkle depth compared with baseline (−10.4% at T28, −15.0% at T56, −11.6% at T84; all $p < 0.01$). No significant changes were observed in the placebo group. Intergroup comparisons confirmed significantly greater improvements in the PRO group at all time points ($p < 0.05$) (Table 2).

Table 2. Skin profilometry. Data are reported as mean $\pm$ SEM. Intragroup statistical analysis (comparisons vs. baseline) is indicated on the raw data with an asterisk (*). Intergroup (PRO vs. PLA groups) statistical analysis is indicated on the % variation with a diamond ($\diamond$). Significance levels are defined as follows: * or $\diamond$ $p < 0.05$, ** or $\diamond\diamond$ $p < 0.01$, *** $p < 0.001$.

	T0	T28	T56	T84
Wrinkle Depth (μm)				
PRO	320.0 $\pm$ 24.6	286.8 $\pm$ 23.0 ** (−10.4%) $\diamond\diamond$	272.1 $\pm$ 19.9 *** (−15.0%) $\diamond\diamond$	282.8 $\pm$ 21.1 *** (−11.6%) $\diamond$
PLA	296.4 $\pm$ 19.5	291.0 $\pm$ 18.6 (−1.8%)	291.6 $\pm$ 21.3 (−1.6%)	290.4 $\pm$ 19.3 (−2.0%)
Ra (μm)				
PRO	32.8 $\pm$ 1.5	31.4 $\pm$ 1.0 * (−4.3%) $\diamond$	30.8 $\pm$ 1.0 ** (−6.1%) $\diamond\diamond$	31.1 $\pm$ 0.9 * (−5.2%) $\diamond$
PLA	34.0 $\pm$ 1.3	34.4 $\pm$ 1.2 (1.2%)	34.5 $\pm$ 1.3 (1.5%)	34.7 $\pm$ 1.5 (2.1%)

3.2.2. Surface Roughness (Ra)

Skin roughness decreased significantly in the PRO group (−4.3% at T28, −6.1% at T56, −5.2% at T84; all $p < 0.05$), while remaining unchanged in PLA. These reductions reflect smoother micro-relief and improved skin texture. Intergroup differences were significant at all time points ($p < 0.05$) (Table 2).

3.2.3. Clinical Evaluation

Instrumental findings were consistent with expert clinical scoring: at T56, 56.3% of PRO participants showed visible wrinkle improvement, with effects persisting in 53.1% at T84 (Figure 2).

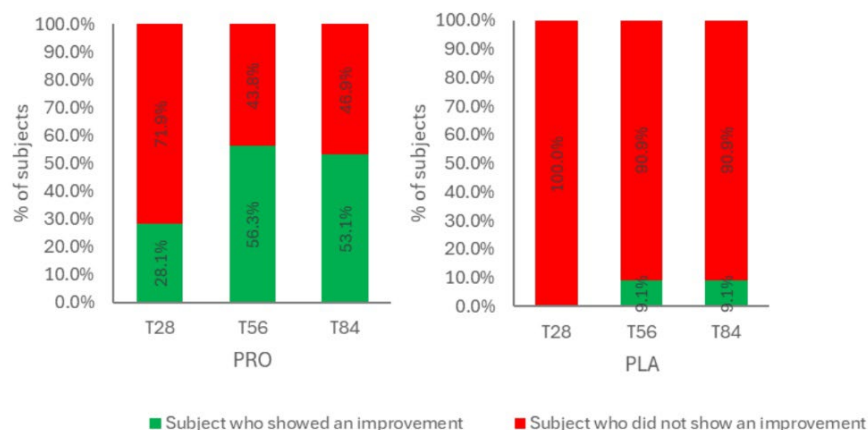


Figure 2. Clinical scoring for wrinkle severity performed by trained experts.

3.3. Skin Thickness and Density

High-frequency ultrasound revealed significant structural improvements in the PRO group. Skin thickness increased by +2.5% at T56 and +2.7% at T84 (both $p < 0.01$), whereas PLA showed no meaningful changes. Intergroup differences reached significance at T84 ($p < 0.05$).

Skin density followed a similar trend, increasing by 3.31 at T56 and 3.36 at T84 percentage points (both $p < 0.001$). No changes were observed in PLA. Intergroup comparisons confirmed significant differences at all time points ($p < 0.001$), suggesting enhanced dermal echogenicity and extracellular matrix support (Table 3).

Table 3. Skin thickness (epidermis + dermis) and skin density. Data are reported as mean $\pm$ SEM. Intragroup (comparisons vs. baseline) statistical analysis is indicated on the raw data with an asterisk (*). Intergroup (PRO vs. PLA groups) statistical analysis is indicated on the % variation with a diamond ($\diamond$). Significance levels are defined as follows: $\diamond$ $p < 0.05$, ** $p < 0.01$, and *** or $\diamond\diamond\diamond$ $p < 0.001$.

	T0	T56	T84
Skin Thickness (μm)			
PRO	1578.1 $\pm$ 43.7	1617.4 $\pm$ 42.4 ** (+2.5%)	1620.8 $\pm$ 44.3 ** (+2.7%) $\diamond$
PLA	1614.3 $\pm$ 38.9	1616.7 $\pm$ 37.9 (+0.1%)	1617.8 $\pm$ 38.8 (+0.2%)
Skin Density (%)			
PRO	17.9 $\pm$ 0.4	21.2 $\pm$ 0.7 *** (+3.31%) $\diamond\diamond\diamond$	21.2 $\pm$ 0.7 *** (+3.36%) $\diamond\diamond\diamond$
PLA	17.5 $\pm$ 0.4	17.8 $\pm$ 0.4 (+0.27%)	17.7 $\pm$ 0.5 (+0.17%)

3.4. Skin Barrier Function and Hydration

3.4.1. Transepidermal Water Loss (TEWL)

TEWL decreased significantly in the PRO group (−9.7% at T28, −14.5% at T56, −15.3% at T84; all $p < 0.001$), indicating strengthened barrier integrity. The PLA showed only minor reductions (−4.5% at T56; −5.4% at T84). Intergroup differences were significant at all time points ($p < 0.001$) (Figure 3a).

3.4.2. Superficial Hydration

Corneometry revealed significant increases in superficial hydration in the PRO group (+11.9% at T28, +16.5% at T56, +16.8% at T84; all $p < 0.001$). PLA showed only modest, non-significant increases. Intergroup differences were significant at all time points ($p < 0.001$) (Figure 3b).

3.4.3. Deep Hydration

Deep epidermal hydration also improved in the PRO group (+4.5% at T56; +4.9% at T84; $p < 0.001$), while PLA exhibited only transient changes. Intergroup comparisons confirmed significantly greater hydration in the PRO group ($p < 0.001$) (Figure 3c).

3.5. Antioxidant Capacity and Skin Radiance

The FRAP assay demonstrated a marked increase in antioxidant capacity in the PRO group (+50.3%; $p < 0.001$), compared with a smaller increase in PLA (+15.2%; $p < 0.001$). Intergroup differences were significant ($p < 0.001$), indicating enhanced protection against oxidative stress (Figure 3d). The modest increase in FRAP observed in the placebo group may partly reflect a placebo effect and normal biological variability commonly observed

in clinical trials. However, the significantly greater improvement in the probiotic group supports a specific effect of the intervention on skin antioxidant capacity.

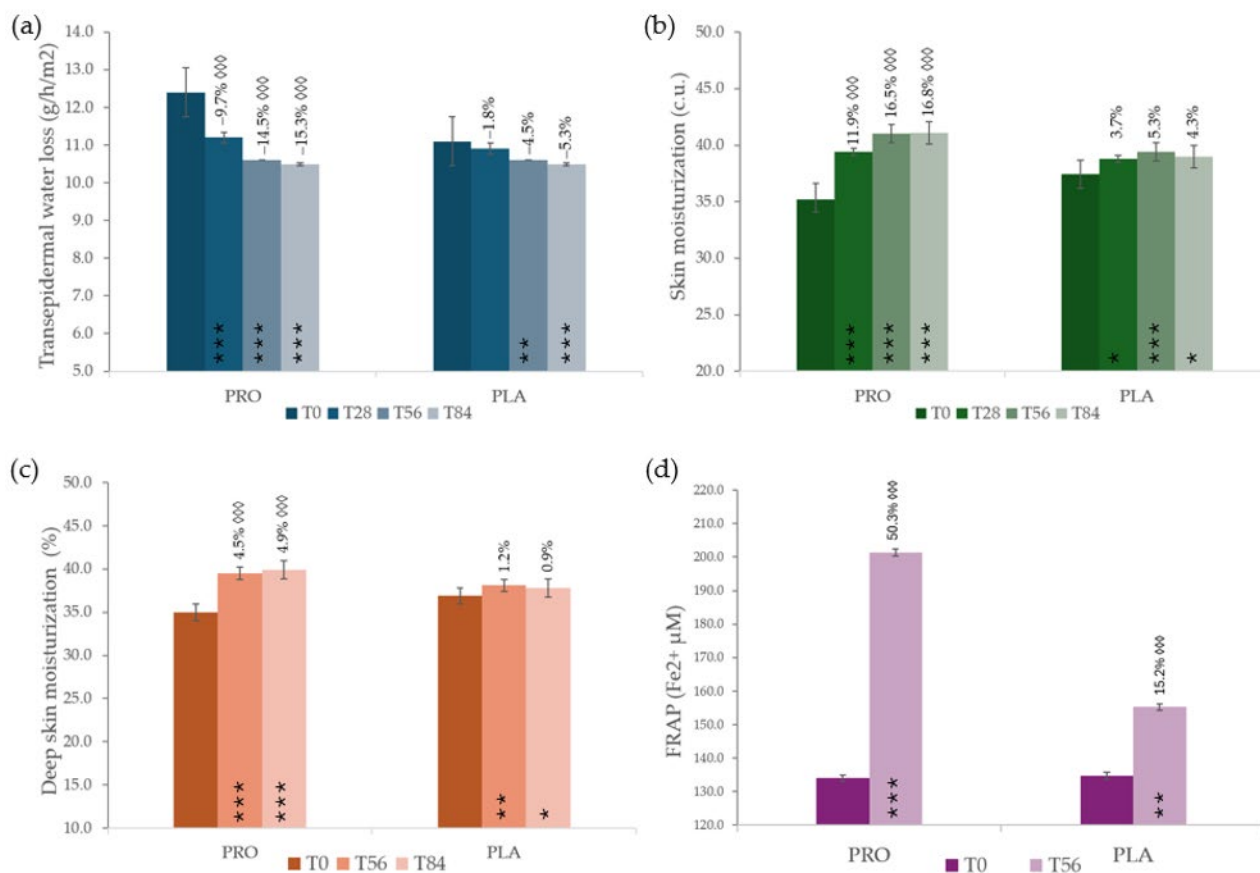


Figure 3. (a) TEWL (g/h/m²). (b) Skin moisturization (Corneometric units). (c) Deep skin moisturization (%). (d) FRAP Antioxidant Capacity. Intragroup statistical analysis (comparisons vs. baseline) is indicated within the bars with an asterisk (*). Intergroup (PRO vs. PLA groups) statistical analysis is indicated above the bars with a diamond (◇). Significance levels are defined as follows: * $p < 0.05$, ** $p < 0.01$, and *** or ◇◇◇ $p < 0.001$.

Expert scoring revealed visible improvements in skin radiance in the PRO group, with 75.8% of participants showing enhancement at T28 and 84.8% at both T56 and T84.

3.6. Self-Assessment Questionnaire

Self-reported outcomes aligned with instrumental findings. A higher proportion of PRO participants reported improvements in hydration, smoothness, radiance, elasticity, and overall appearance compared with PLA (Tables S3 and S4). The treatment was perceived as effective and well tolerated.

4. Discussion

Skin aging results from the combined influence of intrinsic biological processes and extrinsic environmental stressors, leading to progressive alterations in skin structure, barrier integrity, hydration, and oxidative balance [2,33]. These mechanisms manifest clinically as wrinkles, reduced elasticity, impaired barrier function, and diminished radiance—features directly assessed in this randomized, double-blind, placebo-controlled trial.

Increasing evidence suggests that modulation of the gut microbiota may contribute to improved skin health [10–12]. Accordingly, probiotic supplementation has emerged as a promising approach to support skin function and counteract skin aging [13–15]. We

investigated a multi-strain probiotic formulation, composed of *Lactiplantibacillus plantarum* PBS067, *Limosilactobacillus reuteri* PBS072, and *Lacticaseibacillus rhamnosus* LRH020, that has previously shown persistence in the human gut and beneficial effects on photoaging, acne, and atopic dermatitis [24–28].

The present findings demonstrate that 56 days of supplementation with a specific probiotic formulation produced significant improvements across multiple validated markers of skin aging, with several benefits persisting 28 days after discontinuation.

The reductions in wrinkle depth and surface roughness observed in the probiotic group align with emerging evidence probiotics may influence skin aging markers via the modulation of the gut–skin axis. Previous studies have shown that specific strains can influence collagen metabolism, reduce inflammation, and enhance antioxidant defenses, ultimately contributing to improved skin texture and reduced wrinkle formation [14,17].

Similarly, the persistence of effects at T84 suggests a sustained systemic modulation, consistent with reports indicating that probiotic-induced changes in microbial metabolites and immune signaling may extend beyond the supplementation period. However, in the absence of direct biological measurements in this study, the involvement of the gut–skin axis in the observed effects remains speculative and cannot be directly confirmed by our data.

Structural improvements observed through high-frequency ultrasound suggest enhanced dermal organization. Future studies should combine high-frequency ultrasound with complementary approaches, including direct assessment of extracellular matrix components (e.g., collagen and elastin), to further elucidate the structural changes underlying the observed improvements in dermal density.

Age-related thinning of the dermis is associated with decreased fibroblast activity and reduced collagen synthesis [34]. In this study, increases in skin thickness and density in the probiotic group suggest enhanced extracellular matrix support. Previous studies have shown that short-chain fatty acids (SCFAs), key metabolites produced through microbial fermentation, may stimulate fibroblast activity, promote collagen production, and reinforce dermal architecture [35–37]. These pathways provide a plausible explanation for the structural benefits observed.

Skin barrier function also improved significantly, as evidenced by reductions in TEWL. A well-functioning barrier is essential for preventing water loss, maintaining hydration, and protecting against environmental aggressors. Together with the concurrent increases in superficial and deep skin hydration, these findings suggest an overall enhancement of barrier function and water-binding capacity of both the stratum corneum and deeper epidermal layers. This interpretation is consistent with previous studies reporting that probiotics may enhance ceramide synthesis, strengthen tight junctions, and modulate immune responses, all of which contribute to improved barrier integrity [33,38,39]. Although these mechanisms provide a plausible biological explanation for the reduction in TEWL observed in the present study, neither ceramide synthesis nor tight junction integrity was directly assessed. Therefore, the reduction in TEWL and the improvement in hydration should be interpreted as functional evidence of enhanced barrier performance rather than direct confirmation of the underlying biological mechanisms.

Oxidative stress is a major driver of skin aging, contributing to collagen degradation, inflammation, and photoaging. The marked increase in FRAP antioxidant capacity in the probiotic group may indicate strengthened cutaneous defenses against reactive oxygen species, consistent with previous findings on probiotic mediated antioxidant activity [7,14]. This biochemical improvement was accompanied by visible enhancements in skin radiance, a parameter strongly influenced by hydration, micro-relief, and oxidative balance [40]. Skin radiance is influenced by multiple aspects of skin health, including hydration, barrier

integrity, and oxidative balance. Improved hydration contributes to a smoother and healthier skin appearance, while a more balanced oxidative status may preserve cellular integrity and limit oxidative damage to structural skin components, thereby contributing to enhanced skin radiance [4,7]. The high proportion of participants showing improved radiance at T56 and T84 underscores the esthetic relevance of the instrumental findings.

Beyond their statistical significance, the observed improvements are also clinically relevant, as they involve multiple complementary aspects of skin aging, including wrinkle morphology, barrier function, hydration, dermal structure, and antioxidant capacity. Rather than acting on a single parameter, the probiotic formulation produced consistent improvements across structural, functional, biochemical, and clinical outcomes, suggesting a holistic effect on skin health. Furthermore, the persistence of several beneficial effects at the 28-day follow-up (T84) suggests that the observed changes may reflect sustained biological adaptations rather than transient responses to supplementation, although the underlying mechanisms remain to be elucidated. Finally, although the magnitude of change was moderate, these improvements were measurable and confirmed by expert clinical evaluation and participant self-assessment.

Strengths and Limitations of the Study

This study has some limitations that should be acknowledged. Participants were intentionally selected from a homogeneous, Caucasian population to reduce ethnic-related variability; while this strengthens internal validity, it limits generalizability to other ethnic groups. Moreover, inclusion was based primarily on visible wrinkles, without accounting for other determinants of skin aging such as UV exposure, smoking and menopausal status. The absence of gut microbiota profiling prevents direct confirmation of microbiome modulation as the mechanistic driver of the observed effects. Future studies incorporating metagenomic or metabolomic analyses would provide valuable insights into the gut–skin axis pathways involved. Dietary intake and inflammatory biomarkers were not assessed, limiting mechanistic insight into the pathways underlying the observed effects; moreover, only a Per-Protocol analysis was performed. Furthermore, although the FRAP assay provided valuable information on the antioxidant capacity of the skin, it does not directly quantify oxidative damage. Therefore, specific biomarkers of oxidative stress, such as lipid peroxidation products and protein carbonylation, were not assessed. Future studies should integrate these biomarkers, together with systemic inflammatory biomarkers, to provide a more comprehensive evaluation of the biological mechanisms underlying the observed clinical effects. Finally, the relatively short intervention period (56 days) and modest, single-center sample ($n = 66$) indicate that these findings should be considered preliminary and require independent replication in larger, multi-center, and ethnically diverse populations.

Despite these limitations, the study has notable strengths, including its randomized, double-blind, placebo-controlled design. Furthermore, the standardized skincare regimen, with no use of additional cosmetic products, minimized potential confounding factors and strengthened the attribution of the observed effects to the probiotic intervention. In addition, the use of multiple objectives—including validated instrumental techniques (3D profilometry, high-frequency ultrasound, corneometry, TEWL, FRAP assay) alongside expert clinical scoring—provided a comprehensive and objective evaluation of treatment efficacy. The inclusion of a follow-up period further allowed the assessment of the durability of the effects beyond the treatment window. Overall, the findings of this study support the potential of targeted oral probiotic supplementation to improve key structural, functional, and oxidative markers of skin aging.

5. Conclusions and Future Directions

This randomized, double-blind, placebo-controlled clinical trial provides evidence that targeted oral probiotic supplementation with *L. rhamnosus* LRH020, *L. plantarum* PBS067, and *L. reuteri* PBS072 significantly improves multiple validated markers of skin aging. The formulation reduced wrinkle depth and surface roughness, enhanced superficial and deep hydration, strengthened barrier integrity, increased dermal thickness and density, and markedly boosted cutaneous antioxidant capacity.

These results are consistent with a functional modulation of the gut–skin axis, a mechanism that remains to be directly confirmed in future studies with microbiome profiling, metabolomics, and inflammatory biomarkers detection.

In this trial, several benefits persisted 28 days after discontinuation, indicating a durable rather than transient effect; however, extending the follow-up period, and assessing whether the observed benefits are translatable to other ethnic populations remain important open questions to confirm the long-term relevance of probiotic-based approaches to skin health.

Although further specific investigation is needed to confirm and fully understand these results, the convergence of structural, functional, biochemical, and clinical outcomes highlights the potential of this probiotic combination as a promising strategy for promoting healthy skin aging.

Supplementary Materials: The following supporting information can be downloaded at: <https://www.mdpi.com/article/10.3390/cosmetics13040183/s1>, Table S1: inclusion and exclusion criteria; Table S2: Ingredient (INCI) list; Table S3: self-assessment questionnaire active group; Table S4: self-assessment questionnaire placebo group.

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Abbreviations

The following abbreviations are used in this manuscript:

PRO	Active probiotic product
PLA	Placebo
TEWL	Transepidermal water loss

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