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Skin Antiaging and Skin Health Benefits of Probiotics Intake Combined with Topical Ectoin and Sodium Hyaluronate: A Randomized, Double-Blind, Placebo-Controlled Trial

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Abstract: Well-aging strategies include the use of both oral and topic products to nourish the skin from the inside out. A clinical-instrumental study was conducted on 96 subjects with mild to moderate facial aging to demonstrate the efficacy of probiotics (*Lactobacillus plantarum* PBS067, *Lactobacillus reuteri* PBS072 and *Lactobacillus rhamnosus* LRH020) intake combined with topical ectoin and sodium hyaluronate. The skin sebum content, hydration and moisturization, profilometry (wrinkle depth and skin smoothness), TEWL, elasticity (R0 and R2 parameters), radiance, and pores (size and impurities) were measured after 4 and 8 weeks of products use. These parameters were also integrated by a self-assessment questionnaire to align the measured and the perceived efficacy. One hour after serum application, wrinkle depth was reduced by 9.2%, while skin hydration increased by 16.6% and skin radiance by 19.1% following cream application. After 4 and 8 weeks of use, both the oral and topical products were shown to respect the skin's hydrolipid film, effectively improve skin parameters associated with aging, and detoxify the skin from impurities. The mechanism behind these improvements was linked to an enhancement of the skin microbiome. Our findings clearly demonstrate the efficacy of probiotic intake combined with topical ectoin and sodium hyaluronate in enhancing skin health and mitigating signs of skin aging.

Keywords: skin aging; probiotics; ectoin; sodium hyaluronate; clinical trial

1. Introduction

The human skin is a complex living ecosystem that hosts a rich variety of microorganisms, collectively known as the skin microbiota, which includes bacterial species such as *Cutibacterium*, *Corynebacterium*, *Staphylococcus*, and *Proteobacteria* [1–4]. The skin microbiota is essential to skin barrier function, helping to protect the skin and the deeper organs from inflammation, infections, and environmental stressors like UV radiation and pollution [1,5–10]. It also shares a bidirectional relationship with aging, as both changing with age and influencing the skin aging process [11–13]. This bidirectional relationship is part of the skin interactome, a novel integration the "genome-microbiome-exposome" that plays a significant role in skin aging and skin health [11,14].

Imbalance of the skin microbiota (i.e. dysbiosis) is often associated with skin conditions such as acne vulgaris, rosacea, psoriasis and atopic dermatitis, as well as pathological conditions including cutaneous wounds and ulceration, and melanoma [15–24]. The dysbiosis typically involves an increased colonization of pathogens, a reduced number of beneficial commensals, and a variation in both alpha (i.e. evenness and abundance of microbial species at sample level) and beta diversity (i.e.

similarity or difference in microbial communities between samples or conditions) [25–27]. The study of skin microbiota dysbiosis, as well as the normal microbiota, and skin aging is therefore of great interest for diagnosing skin diseases, identifying therapeutic options, and maintaining the skin barrier in optimal condition [28].

Growing evidence also highlights a crucial role of the gut microbiota in supporting skin health by strengthening the skin barrier function [29,30]. Disruption of gut integrity and imbalances in its microbiota can significantly impact skin homeostasis and allostasis, with a growing body of research highlighting a bidirectional interaction between the gut and the skin [29]. This interaction, termed the "gut–skin axis", highlights the complex relationship between the gut and the skin [31,32].

The interaction between the skin and the gut microbiomes underpins the combined use of food supplements and cosmetic products (in & out approach) to address skin aging on multiple levels, helping to nourish the skin from within while providing topical benefits [33–36]. This trial aimed to assess the antiaging efficacy of a patented [37] "in & out" approach providing targeted microbiome support through a combination of oral probiotics containing *Lactobacillus plantarum* PBS067, *Lactobacillus reuteri* PBS072 and *Lactobacillus rhamnosus* LRH020, supported by adjuvant topical products containing ectoin (an amino acid derivative with osmoprotectant properties), fruit water, triglycerides and a proprietary multi-molecular weight hyaluronate compound.

2. Materials and Methods

2.1. Trial Design and Ethics

The trial was a single-center, randomized (1:1:1:1 balanced randomization), double-blind, placebo-controlled with four treatment arms, conducted at Complife Italia S.r.l. facility in San Martino Siccomario (PV), Italy, from March to September 2023. It consisted of a screening visit, a baseline visit (W0) and two follow-up visits after 4 (W4) and 8 (W8) weeks of products use. During the baseline visit, the filler effect of the serum and the hydrating and glowing effect of the cream were tested 1 hour after products application. After the enrollment subjects were randomized to receive the active food supplement and the placebo cosmetic products (AFPC), the placebo food supplement and the placebo cosmetic products (PFPC), the active food supplement and the active cosmetic products (AFAC) and the placebo food supplement and the active cosmetic products (PFAC).

All trial procedures were conducted in accordance with the World Medical Association's (WMA) Helsinki Declaration and its amendments. The study protocol and all trial-related documents were approved by the "Comitato Etico Indipendente per le Indagini Cliniche Non Farmacologiche" (ref. no. 2002/01 by 02/02/2022). Each participant provided written informed consent (ICF) prior to the initiation of any study-related procedures.

The trial was designed to demonstrate the test product claim(s) in the current framework proposed by Commission Regulation (EU) No 655/2013. Endpoints were measured using techniques currently accepted in the cosmetic field while biases are minimized by procedure(s) standardization according to ISO 9001 Quality Management System.

2.2. Participants and Compliance with Treatment

Eligible participants included healthy male and female subjects aged 45 to 65 years with mild to moderate signs of facial aging and (self-declarative) sensitive skin. Exclusion criteria included natural or artificial UV exposure (e.g., tanning beds or facial lamps), dermatological skin conditions and medical histories that could interfere with study outcomes, pharmacological treatments interfering with the study outcomes (e.g. antibiotics), Botox treatment or intention to have Botox treatment during the study period, breastfeeding, pregnancy, and unwillingness to take the necessary precautions to avoid pregnancy (for women of childbearing potential). The complete inclusion and exclusion list is reported in the Supplementary Materials (Table S1).

Throughout the study period, participants were asked to maintain a journal documenting their food and drink consumption to ensure stability in their dietary habits. They were also instructed to

report any adverse events. Compliance with treatment was evaluated by counting and recording the number of the remaining (not used) stick packs after 4 and 8 weeks of treatment, with a compliance threshold set at ≥ 80%.

2.3. Interventions and Randomization

The test item was a commercially available in & out patented (no. EP4151217) [37] treatment ("AW*vi* – The skin method") supplied by AW Srl (Sesto Fiorentino, FI, Italy). The treatment consisted of a food supplement (AW*vi* – The Skin Biotic), a cleanser (AW*vi* – The Gentle Cleanser), a serum (AW*vi* – The Power of Serum), and a cream (AW*vi* – The Active Cream).

Each treatment arm consisted of 24 subjects receiving the active food supplement and the placebo cosmetic products (group AFPC), the placebo food supplement and the active cosmetic products (group PFAC), the active food supplement and the active cosmetic products (AFAC), the placebo food supplement and the placebo cosmetic products (PFPC). The active food supplement treatment arm received (daily) 1 stick pack containing 10 mg *Lactobacillus plantarum* (PBS067-DSM 24937), 25 mg *Lactobacillus reuteri* (PBS072-DSM 25175), *Lactobacillus rhamnosus* (LRH020-LMG P-29513/DSM 25568), 927.015 Sorbitol, 1000 mg Fructo-oligosaccharides, 1.476 mg Vitamin A Acetate, 0.009 mg Biotin, 20 mg Silicon dioxide (E551). The placebo product formula was identical to the active formula, except that lactobacilli were replaced by an equivalent amount (51.5 mg) of maltodextrin.

The cosmetic products actives were sodium hyaluronate, ectoin, fruit water and triglycerides. The complete ingredient (INCI) list is reported in the Supplementary Materials (Table S2).

The placebo formulas (Supplementary Table S2) were the same as the active's formulas without the following active ingredients: ECTOIN and SODIUM HYALURONATE. Both the active and placebo products were applied as follows: massage the cleanser onto damp skin and rinse with water; apply the serum to dry and cleansed skin and once the serum is absorbed apply the cream. All the products were applied twice a day (morning and evening) all over the face (including the neck and the eye contour area).

2.4. Outcomes

2.4.1. Primary Outcomes

The primary outcomes of the trial were the measurement of the skin sebum content and the skin hydration (ability of the product to attract water when applied, humectant) and moisturization (increase of the water content by improvement of the skin barrier efficiency).

The skin sebum content ($\mu g/cm^2$) measurement was based on the "grease spot photometry" technique with a Sebumeter® SM 815 (Courage + Khazaka electronic GmbH, Cologne, Germany).

The skin hydration (1 hour after the cream application) and the skin moisturization (after 4 and 8 weeks of each tested treatment) were measured by the capacitance method using a Corneometer® CM 825 (Courage + Khazaka electronic GmbH, Cologne, Germany).

2.4.2. Secondary Outcomes

The secondary outcomes of the trial were related to the assessment of the efficacy of the products in improving skin signs related to aging. The secondary outcomes included the following measurements: wrinkle depth, skin smoothness, transepidermal water loss (TEWL), skin elasticity, skin radiance, pores (size and impurities) and skin microbiome.

The profilometric properties (wrinkle depth and skin smoothness) of the skin were measured in the "crow's feet" area by a PRIMOS[CR] small field device (Canfield Scientific Europe, BV, Utrecht, The Netherlands). Subjects repositioning between each visit was ensured by a stereotactic device (Canfield Scientific Europe, BV, Utrecht, The Netherlands).

Transepidermal water loss (TEWL) was measured with an open-chamber Tewameter® TM Hex (Courage + Khazaka electronic GmbH, Cologne, Germany) in the cheeks area.

The skin elasticity measurement was based on the suction method using Cutometer® MPA 580 equipped with a 2 mm diameter probe. The measured parameters included R0 (Uf) and R2 (Ua/Uf). R0, referred to as skin firmness, represents the maximum skin deformation (Uf) observed during the suction phase, while R2, known as overall elasticity, is defined as the ratio of skin distension during the elongation phase (Ua) to the maximum skin deformation (Uf) during the suction phase. Both parameters were measured in the cheeks area.

Skin radiance was measured, in the cheeks area, by the 8°gloss parameter taken with a colorimeter/spectrophotometer CM 700D (Konica Minolta, Milan, Italy).

The pore sizes and the protoporphyrin (microbial impurities) distribution were measured in the area near the nose by morphometric and colorimeter image analysis on digital pictures taken with VISIA®-CR (Canfield Scientific Europe, BV, Utrecht, The Netherlands).

Microbiome samples were obtained by swabbing a 3.5 x 5.5 cm area on the volunteers' face (cheek), outlined by a disposable adhesive template (Copan Spa, Brescia, Italy), by using a moistened FLOQswab® (Copan Spa, Brescia, Italy). For each participant, the collection site was brushed 10 times horizontally and 10 times vertically. Subsequently, the swabs were immersed in 1 mL of preservation medium (eNat®, Copan Spa, Brescia, Italy), and stored at room temperature for up to four weeks. Prior to isolating microbial DNA, the samples were prepared in accordance with the methodology previously described [38] and immediately stored at –20°C until further use.

Bacterial DNA was isolated using the QIAamp® DNA Microbiome Kit (Qiagen, Hilden, Germany), in accordance with the manufacturer's guidelines. The yield and quality of the extracted DNA were assessed using the Qubit™ 1X dsDNA HS Assay Kit (Invitrogen Co., Carlsbad, CA, USA) on a Qubit™ Flex Fluorometer (Thermo Fisher Scientific, Waltham, MA, USA). Polymerase chain reaction (PCR) amplification of the V1-V3 hypervariable regions of the bacterial 16S rRNA gene was performed using the Microbiota Solution A kit (Arrow Diagnostics, Genoa, Italy). The resulting products were purified using Agencourt AMPure XP magnetic beads (Beckman Coulter Inc., Brea, CA, USA). The amplicon size and purity of the PCR products, including a negative control, were determined by electrophoresis on 1.5% agarose gel in TAE buffer (1X). A further measurement was performed to ascertain the DNA concentration of the libraries, and samples were pooled in equimolar concentrations. The final amplicon libraries were sequenced on a MiSeq Illumina® sequencing platform using a MiSeq Reagent Kit v2 cartridge for 2 × 250 paired-end sequencing (Illumina Inc., San Diego, CA, USA).

2.4.3. Self-Assessment Questionnaire

At each checkpoint Subjects were asked to give their opinion on the tested product by answering a questionnaire about the product's perceived effects and satisfaction. The questionnaire was taken before any outcome measurement so as not to influence the participants' answers. Possible answers were "fully agree", "agree", "neither agree nor disagree", "rather don't agree", or "don't agree al all". Both "fully agree" and "agree" were included in the calculation of the responders.

2.5. Sample Size

The sample size was determined using a two-sided significance level of 5% and a power of 80%. The calculation accounted for a 20% variation in the primary endpoints, attributable to both inter-individual variability and measurement technique errors. The sample size was calculated using PASS 11 statistical software (version 11.0.8, PASS, LLC, Kaysville, UT, USA). A sample size of 20 subjects per group was obtained. Four subjects more were added in each panel to take an anticipate dropout rate by 20%.

2.6. Randomization and Blinding

Subjects were randomized in a 1:1:1:1 ratio to receive either active or placebo treatments using a computer-generated randomization list (PASS 11, version 11.0.8, PASS, LLC, Kaysville, UT, USA) based on the "Efron's biased coin" algorithm. The randomization list was securely concealed within sequentially numbered, sealed, and opaque envelopes. The study followed established protocols to ensure a clear separation between the investigator and their collaborators and the staff administering the intervention. All the products were identical in size and shape.

2.7. Statistical Analysis

2.7.1. Analysis of Clinical Endpoints

The intragroup (vs. baseline) statistical analysis was performed on the raw data by repeated measures analysis of variance (RM-ANOVA) followed by Tukey-Kramer post hoc test or by t test of Student. The intergroup (active vs. placebo) statistical analysis was performed on the percentage variations by one-way analysis of variance followed by Tukey-Kramer post hoc test. Statistically significant data were reported as follows: * $p < 0.05$, ** $p < 0.01$, and *** $p < 0.001$.

2.7.2. Analysis of Microbiome Data

Raw sequencing data were processed using MicrobAT (Microbiota Analysis Tool) v.1.1.0 software (SmartSeq Srl, Novara, Italy) by comparison with the Ribosomal Database Project (RDP) database. Once filtered for read length (< 200 nt) and quality (average Phred quality score < 25) [39], the resulting sequences were aligned with a similarity threshold of ≥ 97% and assigned to taxonomic levels with a query coverage of 80% [40]. Features without taxa designation were ascribed to the corresponding unclassified group. A secondary analysis of the results was performed using MicrobiomeAnalyst Software [41,42], where features with low counts and variance were identified and removed while the remainder were analyzed according to their median abundance levels (minimum count 4) across samples.

Sample biodiversity calculated as the alpha diversity (diversity within the group) and the beta diversity (diversity between groups). For alpha diversity, two indices were employed: the Shannon index, which estimates the overall number of different taxa present; a higher Shannon index indicates a greater diversity of individual taxa. The Simpson index, often referred to as the "evenness" index, is based on a logarithmic scale and evaluates whether a few taxa dominate the microbiome (values close to 1) or if the abundance distribution is more balanced (values closer to 0). Beta diversity analysis was performed to evaluate differences in the overall taxonomic composition between groups. The distance matrix, generated by using the Bray-Curtis dissimilarity method, was visualized in two plots through principal coordinate analysis (PCoA). In these plots, each point represents the entire microbiota of a single sample. The statistical significance of the observed differences between microbial communities of each group was assessed using permutational analysis of variance (PERMANOVA) and reported as follows: * $p < 0.05$, ** $p < 0.01$, and *** $p < 0.001$.

3. Results

3.1. Participant Characteristics, Tolerability, and Compliance with Treatment

A total of 121 (n = 121) subjects were screened; 25 were excluded, with 16 not meeting the inclusion criteria and 9 declining to participate. The trial successfully randomized 96 subjects with 24 subjects per group. Ninety-four subjects completed the study, while two subjects in the AFAC group dropped out for personal reason not related to products use. The participants flow chart is reported in Figure 1.
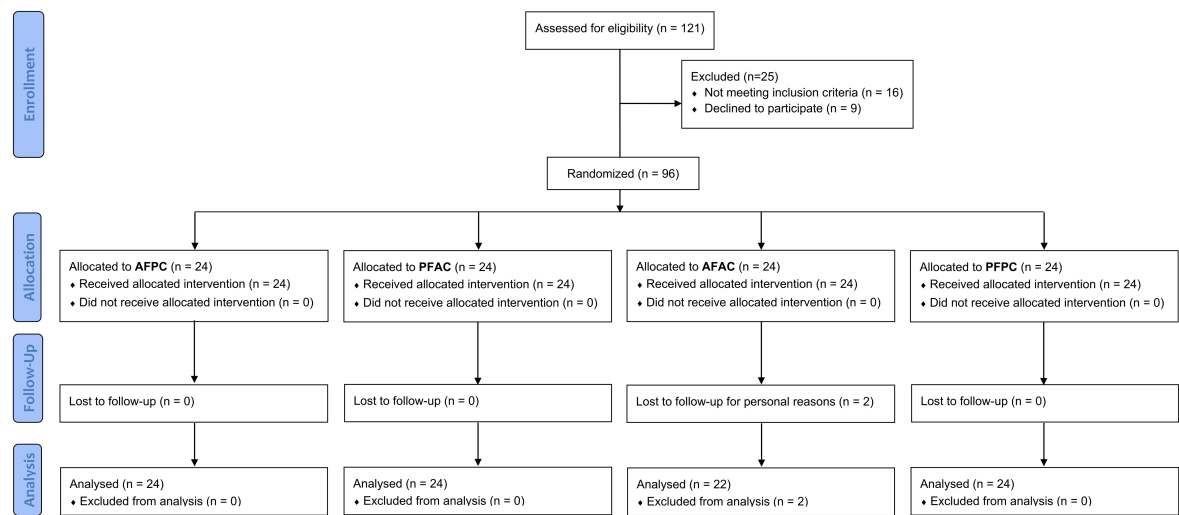
Enrollment

Assessed for eligibility (n = 121)

Excluded (n=25)
- Not meeting inclusion criteria (n = 16)
- Declined to participate (n = 9)

Randomized (n = 96)

Allocation

Allocated to **AFPC** (n = 24)
- Received allocated intervention (n = 24)
- Did not receive allocated intervention (n = 0)

Allocated to **PFAC** (n = 24)
- Received allocated intervention (n = 24)
- Did not receive allocated intervention (n = 0)

Allocated to **AFAC** (n = 24)
- Received allocated intervention (n = 24)
- Did not receive allocated intervention (n = 0)

Allocated to **PFPC** (n = 24)
- Received allocated intervention (n = 24)
- Did not receive allocated intervention (n = 0)

Follow-Up

Lost to follow-up (n = 0)

Lost to follow-up (n = 0)

Lost to follow-up for personal reasons (n = 2)

Lost to follow-up (n = 0)

Analysis

Analysed (n = 24)
- Excluded from analysis (n = 0)

Analysed (n = 24)
- Excluded from analysis (n = 0)

Analysed (n = 22)
- Excluded from analysis (n = 2)

Analysed (n = 24)
- Excluded from analysis (n = 0)

Figure 1. Participant flow chart. **AFPC** is the active food supplement and the placebo cosmetic products group; **PFAC** is the placebo food supplement and the active cosmetic products group; **AFAC** is the active food supplement and the active cosmetic products group; **PFPC** is the placebo food supplement and placebo cosmetic products group.

The enrolled subjects were male and female Caucasian aged between 45 and 65 years old with mild to moderate signs of facial aging and sensitive skin. Additional demographics and the baseline characteristics of the participants are shown in Table 1 and clearly indicate the absence of covariates.

Table 1. Baseline and demographic characteristics.

	AFPC (n = 24)	PFAC (n = 24)	AFAC (n = 22)	PFPC (n = 24)	Units
Age	55.9 ± 1.1	55.4 ± 1.2	53.3 ± 1.3	56.3 ± 1.3	Years
Sex					
Male	8.3% (2)	4.2% (1)	4.5% (1)	8.3% (2)	% (no.)
Female	91.7% (22)	95.8% (23)	95.5% (21)	91.7% (22)	% (no.)
Skin sebum content	83.5 ± 8.3	88.8 ± 8.0	96.5 ± 11.1	88.9 ± 8.2	$\mu g/cm^2$
Skin moisturization	46.0 ± 1.0	47.2 ± 1.5	47.9 ± 1.3	46.5 ± 1.4	cu
Skin profilometry					
Wrinkle depth	305.8 ± 24.3	300.9 ± 22.7	283.2 ± 17.8	275.2 ± 14.6	μm
Skin smoothness (Sa)	34.4 ± 1.7	36.0 ± 1.4	36.6 ± 2.0	33.8 ± 1.6	μm
TEWL	13.1 ± 0.8	13.9 ± 0.8	13.7 ± 0.6	12.2 ± 0.5	$g \times h^{-1} \times m^{-2}$
Skin elasticity					
Skin firmness, R0	0.3910 ± 0.0119	0.3833 ± 0.0103	0.3880 ± 0.0096	0.3689 ± 0.0102	mm
Overall elasticity, R2	0.5975 ± 0.0106	0.6135 ± 0.097	0.6152 ± 0.086	0.6273 ± 0.0084	au
Skin radiance	10.18 ± 0.45	10.25 ± 0.42	9.78 ± 0.49	10.50 ± 0.44	au
Pores					
Size	233.8 ± 3.5	222.5 ± 1.9	227.6 ± 3.6	226.2 ± 4.0	pixel no.
Impurities	57924.2 ± 12357.7	88589 ± 16729.5	91527.6 ± 18868.7	71706.5 ± 20629.9	pixel no.

Continuous data are expressed as mean ± SEM; categorical data are expressed as counts and percentages. cu corneometric units; au arbitrary units.

All tested products were well tolerated, with no adverse effects reported either by the investigator or the participant. Dietary habits remained consistent throughout the study period and did not represent a covariate between the groups.

Compliance with treatment was above 90% in each group (AFPC: 97.4 ± 0.5%; PFAC: 97.8 ± 0.5%; AFAC: 97.2 ± 0.5%; PFPC: 97.3 ± 0.4%).

3.2. Short Term Results With Topical Products Apllication

The wrinkle depth in the "crow's feet area" (Figure 2a) was 293.1 ± 14.1 μm in the active group (n = 48) and 290.5 ± 14.2 μm in the placebo group (n = 48). One hour after applying the active serum, wrinkle depth statistically significantly ($p < 0.001$) decreased by 9.2% compared to a 3.9% reduction ($p < 0.01$) in the placebo group. The effect size of the serum in reducing wrinkle depth was then 2.4 times greater in the active group compared to the placebo group ($p < 0.05$).

The skin hydration 1-hour after the application (Figure 2b) of the active cream was statistically significantly ($p < 0.001$) increased by 16.6% (55.2 ± 0.9 c.u. at T1h vs. 47.6 ± 1.0 at T0 cu). The placebo group also showed a statistically significant increase in skin hydration of 9.2% ($p < 0.001$), with the effect of the active cream being 1.8 times greater ($p < 0.001$).

A statistically significant increase of skin radiance (Figure 2c) was recorded with both the active (+19.1%, $p < 0.001$) and the placebo cream (+13.1%, $p < 0.001$) after 1 hour, with a 1.5 times greater effect for the active cream ($p < 0.05$).
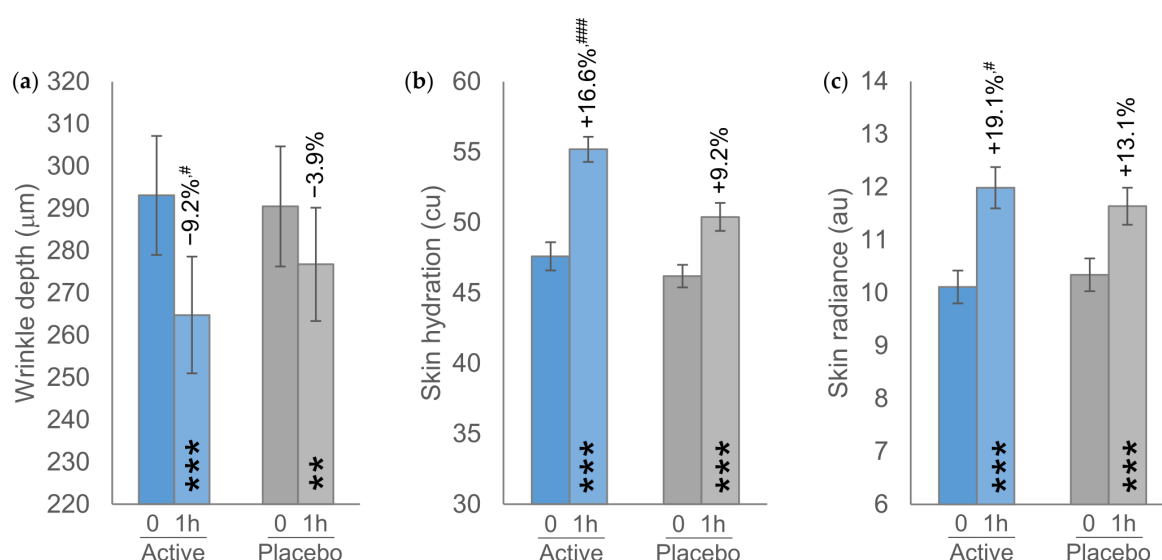
Figure 2. (**a**) Wrinkle depth in the "crow's feet area". (**b**) Skin hydration. (**c**) Skin radiance. The intragroup (vs. T0) statistical analysis is indicated inside the bars with the symbol *, while the intergroup (between treatment groups) statistical analysis is shown above the bars with the symbol. The significance levels are as follows: */# $p < 0.05$, **/## $p < 0.01$, and ***/### $p < 0.001$.

3.3. Primary Endpoints

In the AFPC group, skin moisturization statistically significant increased by 10.7% ($p < 0.001$) and 17.0% ($p < 0.001$), after 4 and 8 weeks of treatment, respectively (Table 2). A similar (p < 0.05) increase in skin moisturization was observed in the PFAC group, with 12.8% ($p < 0.001$) at W4 and 14.5% at W8 ($p < 0.001$). In the AFAC group the increase in skin moisturization was higher both at W4 (+15.5%, $p < 0.001$) and W8 (+21.4%, $p < 0.001$), although the differences were not statistically significant ($p < 0.05$) compared to the AFPC and the PFAC groups. These variations were 2.9 and 3.2 times greater than the results obtained in the PFPC placebo group. A slight but statistically significant increase was also observed in the PFPC placebo group, with increases of +5.4% ($p < 0.01$) at 4 weeks and +6.6% ($p < 0.001$) at 8 weeks. Although the increase in skin moisturization peaked at W4 in PFAC and PFPC groups, it was further increased at W8 in both the AFPC ($p < 0.05$) and AFAC ($p < 0.001$) groups. Statistical differences between the PFPC placebo group and the active groups were as follows: $p < 0.05$ for AFPC at week 8, $p < 0.05$ for AFAC at week 4, and $p < 0.01$ for AFAC at week 8.

All the products did not alter ($p > 0.05$) the skin sebum content neither at W4 and W8 (Table 2). A small but statistically significant decrease in skin sebum content ($p < 0.01$) was observed in the AFAC group at week 8, with sebum levels remaining within the normal range.

Table 2. Primary endpoints. Data are mean ± SEM. The intragroup (vs. baseline) statistical analysis is denoted by the symbol * and is reported near the raw data; while the intergroup (active groups vs. PFPC placebo group) statistical analysis is denoted by the symbol # and is reported near the percentage variation, as follows: * $p < 0.05$, **/## $p < 0.01$, ***/### $p < 0.001$.

	T0	T4	T8
Skin moisturization (cu)			
AFPC (n = 24)	46.0 ± 1.0	50.7 ± 1.0*** (+10.7%)	53.4 ± 1.3*** (+17.0%)#
PFAC (n = 24)	47.2 ± 1.5	52.8 ± 1.7*** (+12.8%)	53.6 ± 1.4*** (+14.5%)
AFAC (n = 22)	47.9 ± 1.3	55.0 ± 1.3*** (+15.5%)#	57.9 ± 1.4*** (+21.4%)##
PFPC (n = 24)	46.5 ± 1.4	48.9 ± 1.5** (+5.4%)	49.5 ± 1.4*** (+6.6%)
Skin sebum content (μg/cm^2)			
AFPC (n = 24)	83.5 ± 8.3	77.2 ± 8.2 (−6.0%)	76.5 ± 7.6 (−6.2%)
PFAC (n = 24)	88.8 ± 8.0	80.6 ± 7.0 (−4.2%)	81.3 ± 6.5 (−4.0%)
AFAC (n = 22)	96.5 ± 11.1	87.7 ± 10.3 (−8.1%)	77.6 ± 7.2** (−9.7%)
PFPC (n = 24)	88.9 ± 8.2	81.4 ± 6.6 (−3.3)	81.4 ± 6.8 (−4.3)

AFPC is the active food supplement and the placebo cosmetic products group; PFAC is the placebo food supplement and the active cosmetic products group; AFAC is the active food supplement and the active cosmetic products group; PFPC is the placebo food supplement and placebo cosmetic products group.

3.4. Secondary Endpoints

3.4.1. Skin Profilometry

Wrinkle depth in the "crow's feet" area decreased in all subjects treated with active products (Table 3). In the AFPC group wrinkle depth decreased by 9.3% ($p < 0.01$) after 4 weeks and 13.8% ($p < 0.001$) after 8 weeks of treatment. A similar reduction was observed in the PFAC group, with an 8.1% decrease at week 4 ($p < 0.01$) and 9.9% decrease at week 8 ($p < 0.01$). In the AFAC group, wrinkle depth reduction at week 4 (−10.3%, $p < 0.01$) was comparable to that observed in the AFPC and PFAC groups, but at week 8, it was significantly greater (−16.5%, $p < 0.001$) and showed a significant increase compared to week 4 ($p < 0.05$). In the PFPC placebo group, winkle depth was slightly reduced, although this change was not statistically significant ($p < 0.05$). Compared to the PFPC placebo group, the change in wrinkle depth in the AFAC group was 3.8 times greater at week 4 and 5.1 times greater at week 8 ($p < 0.05$ vs. PFPC at W8).

An improvement in skin smoothness (Sa parameter, Table 3) was observed only in the AFAC group, with a reduction of 5.1% at week 4 ($p < 0.05$) and 5.8% at week 8 ($p < 0.01$). Although the Sa parameter also decreased in the AFPC and the PFAC group, this variation was not statistically significant ($p > 0.05$). In contrast, the PFPC placebo group showed a statistically significant worsening of +1.6% in skin smoothness at week 8 (p < 0.05).

3.4.2. Transepidermal Water Loss (TEWL)

A statistically significant decrease of TEWL (Table 3) was recorded in both the PFAC (−5.3%, $p < 0.05$ at W4; −7.2%, $p < 0.01$ at W8) and AFAC (−6.6%, $p < 0.01$ at W4; −10.8%, $p < 0.001$ at W8) groups after 4 and 8 weeks of treatment. Moreover, a statistically significant ($p < 0.01$) decrease of 6.1% in TEWL was observed in the AFPC group at week 8. TEWL remained unchanged ($p < 0.05$) in the PFPC

placebo group. At week 8, the TEWL reduction in the AFAC group was 5.8 times greater compared to the change in TEWL in the PFPC placebo group.

Table 3. Skin profilometry and TEWL. Data are mean ± SEM. The intragroup (vs. baseline) statistical analysis is denoted by the symbol * and is reported near the raw data; while the intergroup (active groups vs. PFPC placebo group) statistical analysis is denoted by the symbol # and is reported near the percentage variation, as follows: * $p < 0.05$, **/## $p < 0.01$, ***/### $p < 0.001$.

		T0	T4	T8
Wrinkle depth (μm)				
	AFPC (n = 24)	305.8 ± 24.3	273.1 ± 20.0** (−9.3%)	258.4 ± 19.3*** (−13.8%)
	PFAC (n = 24)	300.9 ± 22.7	271.6 ± 19.3** (−8.1%)	266.0 ± 19.9** (−9.9%)
	AFAC (n = 22)	283.2 ± 17.8	253.5 ± 17.6** (−10.3%)	233.0 ± 15.3*** (−16.5%)#
	PFPC (n = 24)	275.2 ± 14.6	266.6 ± 14.4 (−2.7%)	263.8 ± 13.5 (−3.3%)
Skin smoothness (μm)				
	AFPC (n = 24)	34.4 ± 1.7	32.9 ± 1.3 (−3.0%)	33.3 ± 1.5 (−2.2%)
	PFAC (n = 24)	36.0 ± 1.4	34.5 ± 1.2 (−3.6%)	34.2 ± 1.4 (−4.8%)
	AFAC (n = 22)	36.6 ± 2.0	34.7 ± 1.9* (−5.1%)	34.4 ± 2.0** (−5.8%)
	PFPC (n = 24)	33.8 ± 1.6	34.3 ± 1.7 (+1.9%)	34.2 ± 1.7* (+1.6%)
TEWL (g×h^{-1}×m^{-2})				
	AFPC (n = 24)	13.1 ± 0.8	12.8 ± 0.7 (−1.2%)	12.1 ± 0.6** (−6.1%)
	PFAC (n = 24)	13.9 ± 0.8	13.0 ± 0.7* (−5.3%)	12.7 ± 0.7** (−7.2%)
	AFAC (n = 22)	13.7 ± 0.6	12.7 ± 0.5** (−6.6%)	12.2 ± 0.5*** (−10.8%)#
	PFPC (n = 24)	12.2 ± 0.5	12.3 ± 0.6 (+0.7%)	11.9 ± 0.5 (−1.9%)

3.4.3. Skin Elasticity

Treatment with the active products positively affected skin firmness (R0 parameter) and elasticity (R2 parameter), with improvements observed after both 4 and 8 weeks of treatment (Table 4). The R0 parameter in the AFPC group decreased by 2.1% ($p < 0.05$) at week 4 and was further decreased by 4.7% ($p < 0.001$ vs. baseline and $p < 0.05$ vs. placebo PCPC group) after 8 weeks of treatment, respectively. The 8-weeks reduction was statistically significantly higher than the 4-weeks variation ($p < 0.05$). In the PFAC group, the decrease was 3.9% ($p < 0.01$) at week 4 and 6.5% ($p < 0.001$) at week 8, while in the AFAC group, it decreased by 5.1% ($p < 0.001$) at week 4 ad 7.7% ($p < 0.001$) at week 8. Skin firmness in the placebo PFPC group remained unchanged ($p < 0.05$) after both 4 and 8 weeks of treatment. At week 8, the reduction in the R0 parameter was statistically significantly higher in PFAC ($p < 0.01$) and AFAC ($p < 0.001$) groups compared to the PFPC placebo group. At week 8, the reduction in the R0 parameter in the PFAC group was 38.6 times greater compared to the change in the R0 parameter in the PFPC placebo group, while it was 45.5 times greater in AFAC group.

A similar trend was observed for the R2 parameter, which improved across all active groups (AFPC, PFAC, and AFAC) after both 4 and 8 weeks of treatment (Table 4). The results in the AFAC group were 9.4 times ($p < 0.05$ vs. PFPC group) higher than those in the placebo group at week 4 and

5.5 times ($p < 0.001$ vs. PFPC group) higher at week 8. Interestingly, the R2 parameter at week 8 showed a further increase compared to week 4 in both the AFPC (+5.4% at W8 vs. +2.4% at W4, $p < 0.001$) and AFAC (+4.7% at W8 vs. +7.1% at W4, $p < 0.05$) groups. R2 parameter was unchanged ($p > 0.05$) in the placebo PFPC group.

Table 4. Skin elasticity. Data are mean ± SEM. The intragroup (vs. baseline) statistical analysis is denoted by the symbol * and is reported near the raw data; while the intergroup (active groups vs. PFPC placebo group) statistical analysis is denoted by the symbol # and is reported near the percentage variation, as follows: * $p < 0.05$, **/## $p < 0.01$, ***/### $p < 0.001$.

	T0	T4	T8
Skin firmness – R0 (mm)			
AFPC (n = 24)	0.3910 ± 0.0119	0.3816 ± 0.0100* (−2.1%)	0.3719 ± 0.0110*** (−4.7%)
PFAC (n = 24)	0.3833 ± 0.0103	0.3683 ± 0.0108** (−3.9%)	0.3590 ± 0.0121*** (−6.5%)##
AFAC (n = 22)	0.3880 ± 0.0096	0.3679 ± 0.0100*** (−5.1%)	0.3576 ± 0.0093*** (−7.7%)###
PFPC (n = 24)	0.3689 ± 0.0102	0.3660 ± 0.0098 (−0.6%)	0.3682 ± 0.0113 (−0.2%)
Overall elasticity – R2 (au)			
AFPC (n = 24)	0.5975 ± 0.0106	0.6107 ± 0.0088* (+2.4%)	0.6286 ± 0.0094*** (+5.4%)#
PFAC (n = 24)	0.6135 ± 0.0097	0.6295 ± 0.0101* (+2.6%)	0.6393 ± 0.0117*** (+4.2%)
AFAC (n = 22)	0.6152 ± 0.0086	0.6434 ± 0.0077*** (+4.7%)#	0.6573 ± 0.0066*** (+7.1%)###
PFPC (n = 24)	0.6273 ± 0.0084	0.6300 ± 0.0087 (+0.5%)	0.6345 ± 0.0097 (+1.3%)

3.4.4. Skin Radiance

The skin radiance (Table 5) showed a positive increase across all active products, with a slight improvement also observed in the PFPC placebo group. in the AFPC group the skin radiance was improved by 10.4% ($p < 0.001$) after 4 weeks and by 19.2% ($p < 0.001$) after 8 weeks of treatment. Skin radiance demonstrated a steady enhancement from week 4 to week 8 in both the AFPC group (+19.2% at W8 vs. +10.4% at W4, $p < 0.001$) and the PFAC group (+22.5% at W8 vs. +12.3% at W4, $p < 0.01$). The results obtained in the AFAC group were 5.3 times ($p < 0.001$ vs. PFPC group) higher than those in the placebo group ($p < 0.001$ vs. PFPC group) at week 4 and 5.0 times higher at week 8. While the change in skin radiance was statistically significant in the PFPC group, it was smaller (+5.0% at W4, $p < 0.05$ and + 6.3% at W8, $p < 0.001$) than that observed in all active groups.

3.4.5. Pores Size and Impurities (Protoporphyrin Distribution)

The size of skin pores did not show any statistically significant change ($p > 0.05$) in any study group, either after 4 weeks or 8 weeks of treatment (Table 5).

The protoporphyrin distribution was decreased in all the active study groups (Table 5). In the AFPC group the distribution of the protoporphyrin was decreased by 8.7% ($p < 0.05$) after 4 weeks and by 8.8% ($p < 0.05$) after 8 weeks. A similar decrease was also observed in the PFAC group (−4.9% at W4, $p < 0.05$; −6.0% at W8, $p < 0.05$) and in the AFAC group (−9.6% at W4, $p < 0.05$; −9.8% at W8, $p < 0.01$), while no change was noted in the placebo PFPC group.

Table 5. Skin radiance and pores (size and impurities). Data are mean ± SEM. The intragroup (vs. baseline) statistical analysis is denoted by the symbol * and is reported near the raw data; while the intergroup (active

groups vs. PFPC placebo group) statistical analysis is denoted by the symbol # and is reported near the percentage variation, as follows: * $p < 0.05$, **/## $p < 0.01$, ***/### $p < 0.001$.

		T0	T4	T8
Skin radiance (au)				
	AFPC (n = 24)	10.18 ± 0.45	11.11 ± 0.41*** (+10.4%)	11.93 ± 0.43*** (+19.2%)
	PFAC (n = 24)	10.25 ± 0.42	11.39 ± 0.41** (+12.3%)	12.46 ± 0.57*** (+22.5%)##
	AFAC (n = 22)	9.78 ± 0.49	12.18 ± 0.61*** (+26.3%)###	12.70 ± 0.66*** (+31.5%)###
	PFPC (n = 24)	10.50 ± 0.44	11.00 ± 0.47* (+5.0%)	11.09 ± 0.45*** (+6.3%)
Pore size (pixel no.)				
	AFPC (n = 24)	233.8 ± 3.5	229.6 ± 3.4 (−1.7%)	231.6 ± 3.8 (−0.8%)
	PFAC (n = 24)	222.5 ± 1.9	220.7 ± 2.7 (−0.7%)	229.7 ± 3.5 (+0.9%)
	AFAC (n = 22)	227.6 ± 3.6	224.4 ± 3.3 (−1.1%)	225.7 ± 3.1 (−0.4%)
	PFPC (n = 24)	226.2 ± 4.0	230.4 ± 4.2 (+2.1%)	226.3 ± 3.4 (+0.3%)
Impurities (pixel no.)				
	AFPC (n = 24)	57924.2 ± 12357.7	50363.4 ± 8999.1* (−8.7%)	51013.1 ± 10155.9* (−8.8%)
	PFAC (n = 24)	88589.3 ± 16729.5	81241.0 ± 14329.2* (−4.9%)	82071.6 ± 15742.2* (−6.0%)
	AFAC (n = 22)	91527.6 ± 18868.7	81647.6 ± 17474.8* (−9.6%)	78297.3 ± 15552.1** (−9.8%)
	PFPC (n = 23)	71706.5 ± 20629.9	66015.2 ± 18190.0 (−2.8%)	68071.7 ± 20090.5 (−3.6%)

3.4.6. Skin Microbiome

The microbial composition of the most abundant genera and their parent taxa included *Cutibacterium* (52.4%), *Corynebacterium* (3.2%), *Kocuria* (0.5%), *Acetobacter* (0.6%), *Achromobacter* (0.3%), *Staphylococcus* (21.0%), *Gemella* (0.3%), *Streptococcus* (2.0%), and *Anaerococcus* (0.9%).

Both alpha (Shannon and Simpson index) and beta diversity indexes remained unchanged ($p > 0.05$) in AFPC, PFAC, PFPC group (Figure 3). In contrast, a statistically significant difference between week 4 and 8 was observed in the AFAC group for both the Shannon ($p < 0.01$ at phylum level) and the Simpson index ($p < 0.01$ at phylum and $p < 0.05$ at genus level) but not for the beta diversity index ($p > 0.05$).

In the AFPC group the linear discriminant analysis effect size (LDA LEfSe) at week 8 revealed a statistically significant decrease in *Achromobacter* (−0.37%, LDA score = 4.23, $p < 0.001$) and an increase in *Gemella* (+0.26%, LDA score = −4.16, $p < 0.05$) relative abundances. In the PFAC group there was an increase in the relative abundance of *Acetobacter* (+0.70%, LDA score = −4.23, $p < 0.05$) and *Acinetobacter* (+0.59%, LDA score = −4.48, $p < 0.05$). In the AFAC group there was an increase of *Cutibacterium* (+10.32%, LDA score = −5.65) even if not statistically significant ($p > 0.05$). This variation should be related to the increase in the Simpson index value (both at genus and phylum level). In the PFPC group, the microbiome was unchanged.
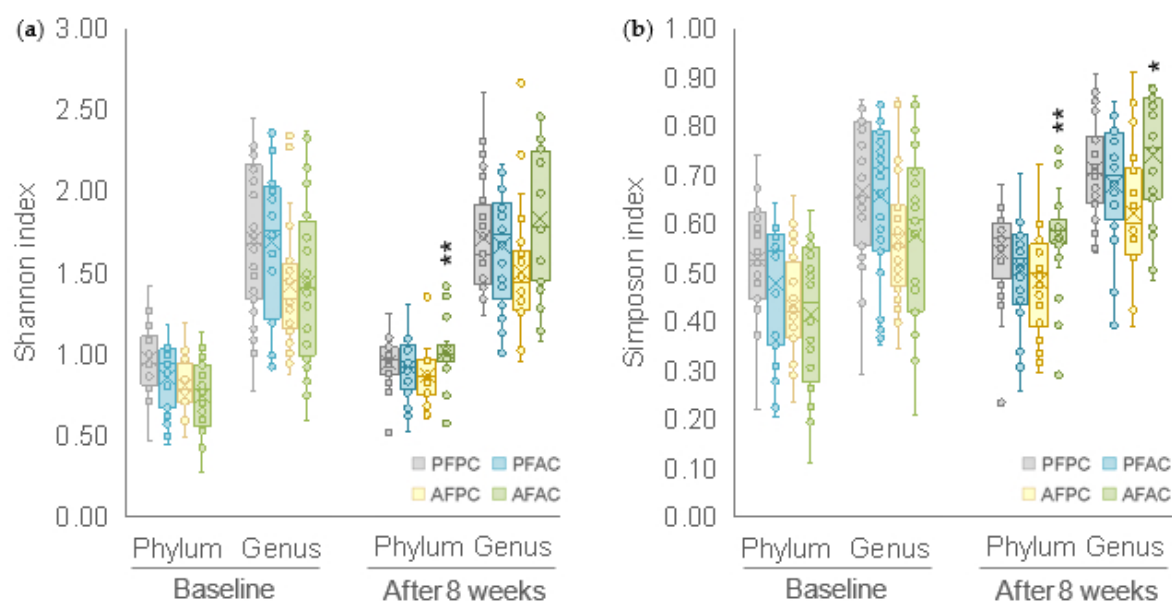
Figure 3. (**a**) Shannon index. (**b**) Simpson index. The intragroup (vs. T0) statistical analysis is indicated above the bars with the symbol * as follows: * $p < 0.05$ and ** $p < 0.01$.

3.4.7. Self-Assessment Questionnaire

All the active products, both topical and oral, received positive evaluations from the study participants. Detailed results for each group are provided in the supplementary material (Table S3).

4. Discussion

Skin aging is a complex and multifactorial process in which cellular senescence (i.e. cellular proliferation arrest) and skin inflammaging are driven by the alteration of the skin barrier properties [43–46]. In recent years the alteration of the skin barrier was also correlated with skin microbiota alterations [1,5–10]. Supporting the skin barrier, respecting its hydrolipidic film and protecting the skin microbiota is then crucial to maintain skin longevity and well-being over time [47,48].

The results of this randomized, double-blind, placebo-controlled trial involving 96 subjects showed that the product use has high level of compliance with treatment without adverse effects during an eight weeks period of use.

The short-term effects observed one hour after a single application of the cosmetic products revealed statistically significant improvements: wrinkle depth decreased by 9.2% with serum application, while skin hydration and radiance increased by 16.6% and 19.1%, respectively, with cream application.

In the long term, all active products effectively maintained the skin hydrolipidic film. The skin sebum content remained stable, except for a slight decrease observed in the AFAC group at week 8, likely due to a sebum normalizing effect in subjects with oily skin. On the contrary the skin moisturization was increased in all the groups including the placebo PFCP group. Interestingly, skin moisturization showed a consistent improvement from week 4 to week 8 in the active food supplement groups (AFPC and AFAC), while it reached its maximum efficacy in both the active cosmetic product (PFAC) and placebo (PFPC) groups. These findings clearly highlight the synergistic effect of the food supplement and its role in enhancing the efficacy of the cosmetic product.

The product's efficacy in preserving skin lipids while enhancing the hydric component of the skin's hydrolipidic film was reflected in its positive impact on the skin microbiome. In the active cosmetic product (PFAC) group, increased skin moisturization correlated with a rise in the relative abundance of transient microbiota members, specifically Proteobacteria (*Acetobacter* and *Acinetobacter*), which are associated with higher epidermal water content [1,4]. Interestingly the active food supplement group (AFPC) showed an increase in fermentative bacteria from the *Gemella* genus,

which may reflect a manifestation of the gut-skin axis [50]. These findings were evident in the active food supplement and cosmetic products (AFAC) group as a promotion of a better bacterial distribution.

Long-term effects observed at 4- and 8-weeks post-treatment initiation revealed statistically significant improvements from baseline to the 8-week follow-up in the active-active group. These included reductions in wrinkle depth, transepidermal water loss (indicating improved skin barrier function and condition), protoporphyrin distribution (associated with impurity removal), and skin sebum content. Concurrently, enhancements were noted in skin smoothness, moisturization, radiance/brightness, elasticity, and firmness.

The beneficial role of probiotics, particularly for their skin anti-aging properties, is increasingly acknowledged in scientific literature. In a recent clinical trial on *Lactobacillus plantarum* HY7714 Lee et al. reported its efficacy in reducing epidermal moisture loss and wrinkle depth while enhancing skin gloss and elasticity [51]. Our results confirm the beneficial effects of probiotics also for antiaging properties.

5. Conclusions

The results of this randomized, double-blinded, placebo-controlled study of the Skin Method shows positive short term and long-term results in the active cosmetic products of serum and cream (which contain the proprietary hyaluronate, ectoin, active waters and triglycerides) compared with the placebo group (active waters and triglycerides only).

The effects are seen as early as 1 hour after a single application including improvement in wrinkles, increase in hydration and radiance. The effects on primary and secondary outcomes were significantly improved at 4 and 8 weeks after commencing treatment.

There were superior improvements in skin moisturization, wrinkles, smoothness, radiance, elasticity and firmness and transepidermal water loss in the active cosmetic and food supplement group compared with the other three groups. Sebum content and impurities removal improved at 8 weeks only in the active cosmetic and food supplement group. There were no differences noticed regarding pore size in any group.

A strong increase in the efficacy of the cosmetic products was seen with the addition of the active food supplement, which contains *L. plantarum*, *L reuteri* and *L rhamnosus*, D-biotin and Vitamin A, compared with the placebo food supplement which contains active components of D-biotin and Vitamin A only. This finding confirms the efficacy of probiotics (AFPC group) in improving the measured skin health parameters and highlights their synergistic interaction with cosmetic products.

Improvements were self-reported by many subjects, up to 95%, for all parameters, and the proportion of subjects reporting improvements was higher in the active cosmetics and active supplement group compared with the other groups.

The use of the combined use of oral probiotics and topic products is fully supported by the results of this study, with a food supplement supporting active topical products, results in significant improvements as early as 1 hour, with further improvements over 8 weeks, and with superior results than using the food supplement or topical products individually.

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