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Effect of a Novel Food Rich in Miraculin on the Oral Microbiome of Malnourished Oncologic Patients with Dysgeusia

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Simple Summary: Patients suffering from taste disorders have been unable to find treatments in the pharmaceutical industry. In this study, a novel strategy has been presented to reduce side effects in patients suffering from cancer by administering dried miracle berries (DMBs), which contain the taste-modifying glycoprotein miraculin, as an adjuvant to medical-nutritional therapy. During a three-month pilot randomized, parallel, triple-blind, and placebo-controlled clinical trial, malnourished patients with cancer and dysgeusia received either a standard dose of DMB, a high dose of DMB, or a placebo. We analyzed the oral microbiome of patients who consumed a DMB or placebo tablet before each main meal. Patients with cancer and dysgeusia who consumed DMB regularly displayed changes to their oral microbiome, which may have contributed to the maintenance of an appropriate immune response.

Abstract: Background/Objectives: Dysgeusia contributes to the derangement of nutritional status in patients with cancer as well as worsening the quality of life. There has been a lack of effective treatments for taste disorders provided by the pharmaceutical industry. Methods: This was a pilot randomized, parallel, triple-blind, and placebo-controlled intervention clinical trial in which 31 malnourished patients with cancer and dysgeusia receiving antineoplastic treatment were randomized into three arms [standard dose of DMB (150 mg DMB/tablet), high dose of DMB (300 mg DMB/tablet) or placebo (300 mg freeze-dried strawberry)] for three months. Patients consumed a DMB or placebo

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tablet before each main meal. Using the nanopore methodology, we analyzed the oral microbiome of patients with cancer using saliva samples. Results: All patients with cancer and dysgeusia had dysbiosis in terms of lower bacterial diversity and richness. DMB consumption was associated with changes in oral microbiome composition. Neither selected bacteria nor taste perception, type of diet, and cytokine levels were associated with mucositis. Likewise, alcohol and tobacco consumption as well as general and digestive toxicity due to systemic therapy were not associated with specific changes of the oral microbiome, according to logistic binary regression. The standard dose of DMB resulted in a lower abundance of *Veillonella* compared with the high DMB dose and placebo at 3 months after intervention with DMB. In particular, some species such as *Streptococcus parasanguinis*, *Veillonella parvula*, and *Streptococcus mutans* were less abundant in the DMB standard-dose group. Additionally, the consumption of a standard dose of DMB revealed a negative association between the concentrations of TNF-α and the abundance of species such as *Streptococcus thermophilus*, *Streptococcus pneumoniae*, *Streptococcus dysgalactiae* and *Streptococcus agalactiae*. Conclusions: Accordingly, regular DMB consumption could modify the oral microbiome in patients with cancer and dysgeusia, which may contribute to maintaining an appropriate immune response. However, as the present pilot study involved a small number of participants, further studies are necessary to draw robust conclusions from the data.

Keywords: cancer; neoplasms; dysgeusia; malnutrition; oral microbiome; dried miracle berries; taste disorders

1. Introduction

Cancer is a group of diseases characterized by uncontrolled cell proliferation [1], affecting people in a multitude of ways, encompassing psychological, physiological, economic, and social aspects [2]. The 2020 data from the World Health Organization (WHO) indicate that 19.3 million new cancer cases were diagnosed, and the number of rising cancer cases might reach 30.2 million by 2040. Hence, seeking treatments and prevention can reduce those costs, improve patients' quality of life, and possibly increase survival rates.

Systemic cancer treatments such as chemotherapy, immunotherapy, and radiotherapy may lead to undesirable side effects that affect taste and smell [3,4]. Indeed, patients with cancer undergoing systemic treatments often experience taste disorders in the range of 20–86% [5–7]. Different types of taste disorders have been reported in patients with cancer depending on the type of antineoplastic treatment, the location of the cancer, and its characteristics [8,9]. Dysgeusia is the most frequent qualitative and quantitative taste dysfunction, including taste distortions with bitter, metallic, salty, or unpleasant tastes [10,11].

Additionally, antineoplastic therapies have also been associated with reduced saliva production [12–15]. Thus, xerostomia may also cause the saliva to be thicker and contain high concentrations of salt, influencing the sense of taste [12,16].

A reduction in quality of life in patients with cancer is observed when taste is impaired with further deterioration of their nutritional status [17]. In addition to reducing the patient's quality of life, dysgeusia can lead to weight loss during treatment and worsen the patient's prognosis [18]. The pharmaceutical industry has failed to provide effective treatments for dysgeusia. The treatments used for taste disorders are zinc supplementation, amifostine, selenium, lactoferrin, and cannabinoids; however, these treatments exhibit limited efficacy [19,20]. Indeed, to reduce dysgeusia and improve patient health and quality of life, novel strategies are needed [21,22].

The oral microbiota includes protozoans, fungi, yeast, bacteria, archaea, viruses, and phages [23,24] with bacteria being the most studied element [25]. Many factors affect individual oral microbiota, including age, diet, and lifestyle habits (e.g., smoking and alcohol consumption) [26,27]. Moreover, the oral microbiota plays a significant role in health, especially in terms of the oral cavity. Indeed, this microbial community is correlated with several oral pathologies, such as oral and other types of cancer [24]. The majority of studies

showing an association between derangement of the oral microbiota and oral diseases induced by cancer therapies were performed in patients undergoing radiotherapy [28,29]. Hence, a reduction in diversity and richness seems to be associated with the development of oral diseases [30].

Over the past decade, there has been a significant increase in research into the prevention and treatment of oral diseases [31]. This includes the identification of bioactive food components and the development of functional foods with health-promoting benefits [32]. Indeed, it is possible to increase patients' quality of life with the supplementation of functional foods. Consequently, concerning taste disorders, oncologic patients may switch from traditional therapies to innovative alternatives that can have a positive outcome [33].

Miraculin is a glycoprotein that converts sour flavors into sweet ones and increases enjoyment of meals [34,35]. This protein is present in *Synsepalum dulcificum*, which is a plant native to West Africa that is commonly known as the "miracle berry". The taste-modifying effect of this miracle fruit is effective under acidic conditions and lasts for approximately 30–60 min [36]. The freeze-dried extract of the miracle berries' pulp, rich in miraculin, is called dried miracle berries (DMBs) and was approved by the European Commission as a Novel Food in December 2021 [37]. DMBs are not only interesting because of the taste-modifying effects but also because of their bioactive ingredients, including fiber and phenolic compounds [38,39].

Only two small trials have reported the potential of this berry for improving dysgeusia caused by chemotherapy [18,40]. Recently, the research group generated clinical evidence on the efficacy of the DMB for the management of dysgeusia in a pilot randomized, parallel, triple-blind, and placebo-controlled clinical trial (the CLINMIR study) [41,42]. In that study, we observed improvements in electrochemical food perception, energy and nutrient intake, nutritional status, and quality of life in malnourished patients with cancer receiving antineoplastic treatment [41]. Nevertheless, no assessment of the oral microbiome and the relationship between microbial profile changes and the main outcome was conducted. As a result, the objective of the present study was to evaluate the oral microbiome of malnourished patients with cancer and dysgeusia following DMB consumption as a medical–nutritional adjuvant therapy as well as its relationship with other variables.

2. Materials and Methods

2.1. Ethical Statement

Scientific Research and Ethics Committee approval was obtained in June 2022 from the University Hospital La Paz (HULP, Code 6164). This study adheres to the Ethical Standards of the Declaration of Helsinki concerning recommendations that guide physicians conducting biomedical research on humans. All researchers should become familiar with and follow the ICH Harmonized Tripartite Guidelines to follow good clinical practice [43].

Participants signed the informed consent form; researchers informed them of the study characteristics and what participation in the trial entailed (verbally and in writing). We followed several legal requirements when processing personal information, including the Spanish Organic Law 3/2018 of 5 December and the General Data Protection Regulation of the European Union (EU) 2016/679 of 27 April 2016.

2.2. Experimental Design and Participants

The CLINMIR study is a pilot randomized, parallel, triple-blind, and placebo-controlled clinical trial. Using the number NCT05486260, the present protocol was registered at http://clinicaltrials.gov, accessed on 14 March 2024. The oncology service and the clinical nutrition unit at HULP in Madrid recruited 31 malnourished patients with cancer and dysgeusia [42].

Patients over 18 years of age who were treated for cancer using chemotherapy, radiotherapy, and/or immunotherapy and had lost 5% of their weight were defined as malnourished according to GLIM criteria [44], and those who suffered dysgeusia, mea-

sured by electrogustometry, were included in the study. Additionally, these patients should have a three-month life expectancy [41].

The study excluded participants who were involved in another clinical trial, received enteral or parenteral nutrition or had poorly controlled diabetes mellitus (HbA1c > 8%), hypertension or uncontrolled hyperthyroidism, severe digestive toxicity as a result of chemotherapy and radiotherapy, or who were suffering from severe kidney or liver disease (chronic renal failure, nephrotic syndrome, cirrhosis, etc.). Additionally, participants should not suffer from severe dementia, brain metastases, eating disorders, a history of severe neurological or psychiatric disorders that may interfere with treatment, alcoholism or substance abuse, or severe gastrointestinal diseases.

Malnourished patients with cancer and dysgeusia who were receiving active treatment were randomly assigned to one of three treatment arms. In a three-month study, each patient was instructed to dissolve a miraculin-based food supplement tablet five minutes before each main meal (breakfast, lunch, and dinner). Patients meeting the selection criteria were randomly assigned to one of the three groups in the clinical trial. The first group received a standard dose of DMB (150 mg DMB, equivalent to 2.8 mg of miraculin + 150 mg freeze-dried strawberry per orodispersible tablet). The second group had a higher dose of DMB (300 mg DMB, equivalent to 5.6 mg of miraculin). Finally, the third group took a placebo dose (300 mg of freeze-dried strawberry). All three treatments were isocaloric (Table S1). The clinical trial consisted of two phases. There were six face-to-face meetings in each. The selection phase had one selection visit, whereas the experimental phase had five visits. All participants were undergoing active treatment with at least chemotherapy, and taste alterations were measured by electrogustometry and taste strip tests [42]. To complete the three-month intervention period, the subjects received as many tablets as necessary during scheduled visits to the HULP. Participants were asked to return all packaging regardless of whether it was empty or partially consumed in order to assess compliance by comparing the number of tablets provided and returned. DMB was administered as an adjuvant medical–nutritional treatment [41,42,45].

2.3. Biological Samples and DNA Sequencing

A three-month trial was conducted. Saliva samples from each intervention group (standard dose, high dose, and placebo), were analyzed for the oral microbiome. We collected saliva samples at baseline (before supplementation with DMB), 1 month after treatment with DMB, and 3 months after intervention with DMB. These samples were placed in OMNIgene Oral OM-501 tubes (DNA Genotek Inc., Ottawa, ON, Canada).

2.3.1. Saliva DNA Extraction

DNA was extracted from saliva samples using a QIAamp DNA Microbiome Kit (Ref. ID: 51704, Qiagen Inc., Hilden, Germany) according to the manufacturer's instructions. DNA purity and integrity were assessed using spectrophotometry (NanoDrop, Thermo Fisher Scientific, Massachusetts, MA, USA).

2.3.2. Whole 16S rRNA Gene Sequencing and Taxonomic Assignment

Following the instructions of the 16S Barcoding kit ref SQK-16S024 (Oxford Nanopore Technologies, Oxford, UK), the 16S rRNA gene was PCR-amplified using redesigned 16S primers (27F and 1492R) with 5′ tags that facilitate the ligase-free attachment of Rapid Sequencing Adapters. A 0.2 mL PCR tube was filled with RNA-free water, 14 µL; 10 ng of input DNA, 10 µL; 16S barcodes at 10 ng/mL, 1 µL; and LongAmp Taq 2X Master mix, 25 µL, for a total volume of 50 µL per sample. The following conditions were used for PCR: denaturation at 95 °C for 1 min, 25 cycles of denaturation at 95 °C for 20 s, banding at 55 °C for 30 s, and extension at 65 °C for 2 min, which was followed by a final extension at 65 °C for 5 min.

A new 1.5 mL Eppendorf DNA LoBind tube was used to transfer the PCR product. By vortexing 30 µL of AMPure XP beads and mixing by pipetting, PCR products from

each sample were resuspended in AMPure XP beads (Beckman Coulter, ThermoScientific, Barcelona, Spain) and cleaned in 10 µL of 10 mM Tris-HCl pH 8.0 with 50 mM NaCl. Finally, all barcoded libraries from each sample were combined in the appropriate ratios to obtain a total concentration of 50–100 fmoles. The 16S amplicon of 1500 bp corresponds to those 50–100 fmoles. In the final step, 1 µL of rapid adapter tube was added to the previously mixed barcoded DNA with which each sample was identified. The mixture should be mixed gently by shaking the tube and centrifuged. A final volume of 11 µL is obtained by incubating the reaction for 5 min at room temperature.

A fresh tube of the library was prepared for loading into SpotON Flow Cell Mk R9 Version (ref FLO-MIN106D, Oxford Nanopore Technologies, Oxford, UK) using the Minion M1kc and M1kb sequencers (Oxford Nanopore Technologies, Oxford, UK). A total of 75 µL was used for the following components: sequencing buffer (SQB) 34 µL, loading beads (LB), mixed immediately before use 25.5 µL, RNA-free water 4.5 µL, and a previously prepared DNA library incubated at room temperature, 11 µL.

Once the raw data were generated, the base calling was performed with Guppy version 6.5.7 (Oxford Nanopore Technologies, Oxford, UK), and the resulting sequences were identified using Kraken2 (with Refseq Archaea, bacteria, viral, plasmid, human, UniVec_Core, protozoa, fungi & plant database) and further analyzed using QIIME (2-2020.8) [46]. The taxonomy was assigned to ASVs using the sklearn naïve Bayes taxonomy classifier (via q2-feature-classifier) [47] against SILVA 16S V3-V4 v132_99 [48] with a similarity threshold of 99%. The data filtering process excluded samples with fewer than 10,000 reads. There were two samples that were excluded from the analysis because of a low number of counts. The phylum, family, genus, and species levels were used for the interpretation of the results. Using the vegan library [49], the Shannon and Simpson's indices were used to examine the diversity of the samples, and the Chao1 index was used to estimate species richness. The beta-diversity measure (Bray–Curtis dissimilarity distance) was calculated using the vegan R package (version 2.6.4) [49]. When testing differences in beta diversity, we used the ADONIS-2 function from the vegan package using permutations for calculating *p*-values [49].

2.4. Plasma Cytokines

Tumor necrosis factor-alpha (TNF-α) and human proteolysis-inducing factor/dermcidin (PIF) were determined and analyzed as previously described [41,42,45]. To avoid more punctures and hospital visits than necessary, blood samples were collected by trained personnel at the HULP Extraction Unit in the morning (approximately 8:00 am) during blood tests before chemotherapy. Briefly, blood samples were collected in vacuum tubes, labeled, transported, and centrifuged for 10 min at 1500× *g*. Aliquots of blood samples were prepared and labeled according to a numerical code and stored at −80 °C [41], and they were collected at baseline (before supplementation with DMB) and 3 months after intervention with DMB. The X-MAP Luminex multiplex enzyme immunoassay platform (HSTCMAG-28SK-06, EMD Millipore Corporation, Michigan, MI, USA) was used to analyze TNF-alpha using specific antibodies as previously described [50]. The PIF was determined by enzyme-linked immunosorbent assay (ELISA) according to the manufacturer's instructions (CSB-E13626h, Cusabio, Wuhan, China).

2.5. Electrical Taste Perception

Electrogustometry was used to evaluate taste perception. To quantify objectively the human taste, electrical taste testing is an excellent method [51]. According to functional imaging studies, lingual electrical stimulation activates the same brain regions as chemical stimulation [52]. Patients who have cancer and taste distortion and who consume miraculin-based food supplements are expected to improve their taste perception by reducing their taste perception threshold (measured using decibel, dB) via electrical stimulation from baseline to one and three months after the intervention with DMB, as measured by electrical stimulation [41,45]. The threshold for an electric-induced taste stimulus was measured

using an electrogustometer (SI-03 Model, Sensonics International, Haddon Heights, NJ, USA). The electric stimulus is applied with an electrode placed on the tongue. A first stimulus (30 dB) is administered to familiarize the patient with the electrical stimulus. Once the threshold is checked, the stimulation starts at the zero-stimulus amplitude and increasingly progressively until the patient identifies the stimulus. To measure detection thresholds, the two-down one-up forced-choice single staircase procedure and a stimulus-response staircase was used [41,45].

2.6. Dietary Pattern Assessment

Food daily records were recorded for three days, one of which was a holiday (weekend day, a day off, or a day out of the usual routine). In the absence of weight recording, patients were advised to record household measurements (spoonful, cups, etc.) or to record household weights. During the review of all records, a nutritionist ensured that all the information collected was accurate and complete in the presence of the patient. With the help of DIAL software version 1 (Alce Ingeniera, Madrid, Spain), the energy and nutrients contained in foods, drinks, dietary supplements, and preparations were converted into energy and nutrients. The 72 h food daily record of dietary intake allowed us to transform food consumption into energy intake, water, macronutrient intake (proteins, fats (total fat, saturated fatty acids, monounsaturated fatty acids, and polyunsaturated fatty acids) as well as carbohydrates, fiber, and micronutrient intake (vitamins and minerals). Based on this information, it was possible to calculate the caloric and lipid profiles as well as the coverage of recommended intakes for the population. The data were analyzed using dietary reference values established by the European Food Safety Authority [53] and the Nutritional Objectives of the Consensus Document of the Spanish Community Nutrition Society [41,54].

2.7. Mucositis

At baseline, participants were scheduled for appointments. Participants underwent an oral examination with the use of mouth mirrors and a high-power headlamp at each appointment (5 visits) by a previously calibrated investigator. Oral mucositis clinical signs were recorded using the clinical component of the National Cancer Institute Common Terminology Criteria for Adverse Events version 3 (NCI-CTCAE v. 3) [55] and the clinical component of the Oral Mucositis Assessment Scale during the course of the intervention [56].

2.8. Statistical Analysis

A linear mixed model was used to evaluate the differences between placebo, DMB 150 mg, and DMB 300 mg, as well as the differences between visits: the effects of treatment, time, and their interaction (time $\times$ treatment) were considered. The linear mixed model was developed using the lme4 package [57] in the R program [58]. Further, general linear mixed models (GLMs) of covariance (ANCOVA) were used to evaluate differences between means for treatment, time, and treatment $\times$ time using baseline data as covariates (SPSS Inc., Chicago, IL, USA). Post hoc analyses were determined using the Bonferroni test. Compared to other programs, the lme4 package version 1.1-35.5 may be faster and more memory-efficient because it employs modern, efficient linear algebra methods, such as those in the Eigen package, and reference classes to prevent undue copying of large objects. Moreover, the software version 1.1-35.5 provides the ability to construct generalized linear mixed models, which maximizes the amount of information available when loss patients are included in some of the analyzed conditions [57].

This study examined the relationships between oral microbiome variables, inflammatory parameters, dietary variables, and electrical taste perception outcomes using Pearson's correlations. R Studio's corrplot function [59] was used to express associations by correcting multiple tests with the false discovery rate (FDR) procedure [60]. The graphs show only significant and corrected associations. In the graphs, red and blue lines indicate the correla-

tion values, with negative correlations highlighted in red (−1) and positive correlations highlighted in blue (+1). In this study, we examined only a subset of dietary variables (energy intake (%), lipids (%), saturated fatty acids (%), and monounsaturated fatty acids (%)) that were significantly correlated; a complete list of the dietary variables is available in our previous study [41].

Based on a Rivera–Pinto analysis, it is possible to identify microbial signatures, i.e., groups of microbes that can predict particular phenotypes of interest [61]. These microbial signatures correspond to an individual's unique microbiome and may be used to diagnose, prognosticate, or predict therapeutic responses. For the purpose of identifying microbial signatures, we can model the response variables (all variables derived from the oral microbiome) and select those that provide the highest accuracy in classification or prediction. As part of the Rivera–Pinto method and the Selbal algorithm, we evaluated specific signatures at the phylum and genus levels to select a sparse model that adequately explains the response variable. The microbial signatures were calculated using geometric means based on data collected from two groups of taxa. The name implies that these groups are those with relative abundances, or balances, that are related to the response variable of interest [61].

Finally, it was examined whether the presence or appearance of mucositis could be independently correlated with the response to the DMB treatment, the levels of cytokine, taste perception, dietary parameters, and the presence of selected species. Additionally, we examined clinical outcomes to determine whether they could be independently correlated with the presence of specific bacterial species. Our study evaluated the presence or absence of immunotherapeutic agents (including atezolizumad, becacizumab, paclitaxel, pembrolizumab, nivolumab, panitumumab, folfirinox, among others), general toxicity (including neutropenia, diarrhea, thrombocytopenia, among others), digestive toxicity, tobacco use and alcohol consumption. To achieve this, we used the SPSS statistical package version 29 (SPSS Inc., Chicago, IL, USA) to perform a binary logistic regression with the Wald regression backward option and a significance level of $p < 0.05$.

3. Results

Patients were recruited from November 2022 to May 2023, and 62 were assessed for eligibility. Thirty-one patients with cancer and dysgeusia were included in the study for meeting the selection criteria and were randomly assigned to one of three intervention groups, which were adjusted according to the type of cancer. The standard-dose DMB and placebo groups consisted of ten patients each, whereas the high-dose DMB group consisted of eleven patients. During the follow-up period, which extended from November 2022 to August 2023, ten participants dropped out of the study. The majority of these drop-outs were due to the taste distortion of non-sweet acidic foods (n = 6) and the difficulty that the prescription derived from the intervention added to their already complex antineoplastic treatment (n = 2). Furthermore, two placebo patients died. Finally, a total of 21 oncological patients completed the clinical trial. There were eight patients in the DMB standard-dose group, six in the placebo group, and seven in the high-dose group who completed the study. All variables were evaluated following the intention-to-treat principle [42]. The sample comprised 58.1% women and 41.9% men with a mean age of 60.0 ± 10.9 years. Baseline data for the population have been published previously elsewhere [41].

3.1. Beta Diversity

We compared saliva samples from each intervention group (standard dose, high dose, and placebo) at baseline (before supplementation with DMB), one month after DMB treatment, and three months after treatment with DMB. Treatment and time did not produce distinct microbial community structures (Bray–Curtis distance, $R^2 = 0.18$, $p = 0.890$ for treatment (Figure 1A) and $R^2 = 0.15$, $p = 0.920$ for time (Figure 1B)).

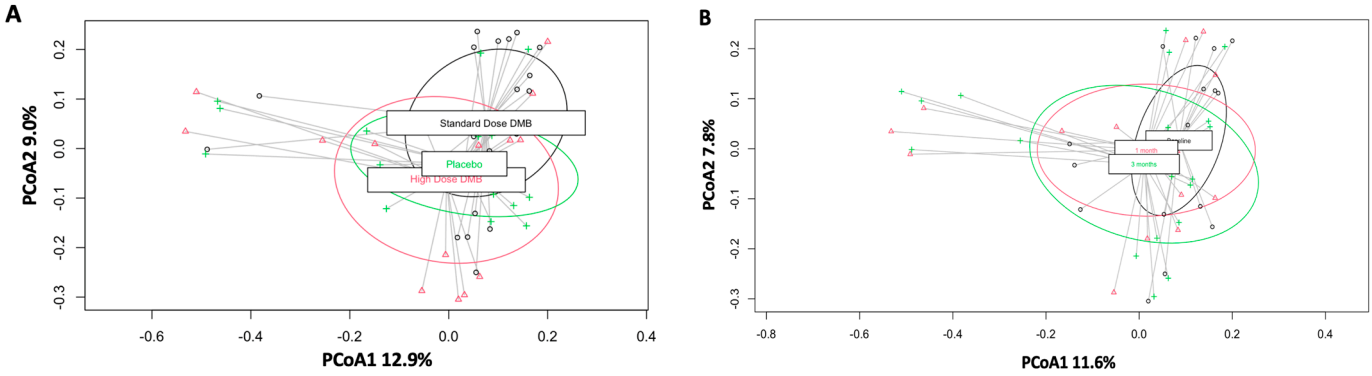

Figure 1. Plots of beta diversity metrics based on principal coordinate analysis. The R^2 and *p*-values are from the Adonis function measured using the vegan package. (**A**) Treatment, the black points and line correspond to standard-dose DMB, the red points and line correspond to high-dose DMB, and the green points and line correspond to placebo. (**B**) Time, the black points and line correspond to baseline, the red points and line correspond to 1 month, and the green points and line correspond to 3 months.

3.2. Phylum and Family Levels

In all groups, the relative abundances at the phylum level were similar at baseline, after one month, and after three months. *Bacillota* accounted for more than 98% of the relative abundance of the oral microbiome in our study. As for the other main phyla, *Actinobacteriota*, *Fusobacteria*, *Bacteroidota*, *Pseudomonadota*, and *Saccharibacteria*, there were no significant changes or trends among the groups (Table S2). According to Shannon and Simpson indices, related to bacterial diversity, there was no significant difference between the groups. Additionally, there were no differences among the groups in terms of the Chao1 index, which is related to bacterial species richness. The patients with cancer and dysgeusia showed dysbiosis in terms of diversity and richness compared with healthy individuals [62]. At the family level, we did not observe significant changes in the oral microbiome of patients with cancer either during treatment or time (Table S3).

3.3. Genus Level

At the genus level, *Streptococcus* was the most common in all studied groups (more than 63%). Overall, significant differences in the interaction between treatment and time were observed for *Enterococcus* ($p = 0.044$) and *Veillonella* ($p = 0.015$). Based on a post hoc analysis and Bonferroni correction, *Enterococcus* abundance was higher in the standard-dose DMB group than in the high-dose DMB and placebo groups at 3 months of intervention ($p = 0.044$, Table 1). *Veillonella* genus relative abundance decreased significantly over the treatment period in the standard-dose DMB group compared to the high-dose DMB and placebo groups (Table 1). In addition, we observed a trend of increase for *Granulicatella* ($p = 0.066$), *Bacillus* ($p = 0.061$), and *Staphylococcus* ($p = 0.053$) in DMB groups (Table 1). The remaining genera did not show any significant changes in either the treatment or time.

3.4. Species Level

Three species dominated the oral microbiome of patients with cancer, *Streptococcus pneumoniae*, *Streptococcus thermophilus* and *Veillonella parvula*. We observed significant differences in the interaction between treatment and time for *Granulicatella elegans*, *Streptococcus mutans*, *Streptococcus parasanguinis* and *Veillonella parvula* as well as a trend for *Gemella morbillorum* ($p = 0.054$), *Granulicatella adiacens* ($p = 0.069$), *Streptococcus australis* ($p = 0.063$), and *Streptococcus cristatus* ($p = 0.087$) (Table 2).

Table 1. Relative abundances at the genus levels of oral bacteria in malnourished patients with cancer and dysgeusia who received the standard dose of DMB (150 mg/tablet), the high dose of DMB (300 mg/tablet), or the placebo for 3 months.

	Standard-Dose DMB (150 mg) (*n* = 8)			**High-Dose DMB (300 mg) (*n* = 6)**			**Placebo (*n* = 7)**			***p*-Value**		
Genus	**Baseline**	**1 Month**	**3 Months**	**Baseline**	**1 Month**	**3 Months**	**Baseline**	**1 Month**	**3 Months**	**Treatment (T)**	**Time (t)**	**T × t**
Actinomyces	0.1 (0.02–0.3)	0.2 (0.03–0.3)	0.08 (0.02–0.3)	0.09 (0.04–0.2)	0.08 (0.04–0.2)	0.07 (0.04–0.1)	0.1 (0.07–0.6)	0.1 (0.04–0.7)	0.1 (0.05–0.6)	0.238	0.308	0.846
Aminipila	0.1 (0.01–0.5)	0.1 (0.02–0.8)	0.2 (0.03–0.5)	0.1 (0.06–0.4)	0.1 (0.03–0.3)	0.08 (0.07–0.2)	0.2 (0.006–1.6)	0.5 (0.2–2.8)	0.2 (0.1–4.7)	0.138	0.229	0.237
Atopobium	0.4 (0.1–0.7)	0.4 (0.1–0.7)	0.6 (0.1–1.1)	0.06 (0.02–0.1)	0.3 (0.3–0.3)	0.1 (0.07–0.1)	0.01 (0.01–0.01)	0.02 (0.02–0.02)	0.03 (0.03–0.03)	0.319	0.913	0.962
Bacillus	0.68 (0.3–1.6)	0.5 (0.4–1.1)	0.9 (0.5–1.2)	0.3 (0.1–0.9)	0.3 (0.2–0.5)	0.4 (0.2–2.5)	0.4 (0.3–1.1)	0.6 (0.4–0.8)	0.4 (0.3–0.4)	0.179	0.26	0.061
Bulleidia	0.1 (0.06–0.4)	0.1 (0.04–0.3)	0.07 (0.02–0.2)	0.06 (0.02–0.5)	0.07 (0.03–0.2)	0.1 (0.01–0.2)	0.08 (0.02–0.5)	0.2 (0.1–0.5)	0.1 (0.02–0.3)	0.328	0.752	0.74
Clostridioides	0.4 (0.02–1.0)	0.5 (0.01–3.1)	0.8 (0.02–2.9)	0.4 (0.01–4.8)	1.2 (0.7–1.9)	0.4 (0.06–0.7)	1.3 (0.1–2.9)	2.3 (0.1–6.4)	1.2 (0.1–4.4)	0.076	0.37	0.388
Enterococcus	0.5 (0.2–1.4)	0.4 (0.3–0.8)	0.5 [a] (0.4–0.9)	0.2 (0.03–0.7)	0.1 (0.04–0.4)	0.3 [b] (0.08–2.4)	0.3 (0.1–0.8)	0.5 (0.2–0.5)	0.3 [b] (0.1–0.3)	0.251	0.234	0.044
Gemella	4.8 (2.1–13.3)	5.9 (1.0–17.5)	8.6 (1.6–15.0)	6.3 (0.5–11.7)	3.7 (1.3–6.7)	3.4 (0.6–11.6)	4.4 (1.9–12.5)	6.0 (4.1–13.0)	8.6 (4.6–14.2)	0.605	0.512	0.309
Granulicatella	4.8 (1.6–14.7)	3.2 (2.2–8.0)	4.8 (1.4–9.2)	1.6 (0.09–7.4)	1.6 (0.3–4.8)	4.0 (0.8–23.4)	3.0 (1.4–8.8)	4.7 (1.3–7.4)	2.7 (1.3–3.1)	0.411	0.468	0.066
Herbinix	0.4 (0.1–1.0)	0.2 (0.07–1.6)	1.0 (0.3–1.3)	0.2 (0.01–0.9)	0.4 (0.3–1.9)	0.2 (0.03–0.5)	0.9 (0.07–1.7)	0.3 (0.09–4.2)	0.9 (0.1–2.5)	0.246	0.436	0.575
Lachnoanaerobaculum	1.3 (0.06–4.0)	1.4 (0.2–3.8)	0.9 (0.1–8.9)	0.1 (0.04–3.9)	0.9 (0.1–1.7)	0.5 (0.03–5.4)	1.1 (0.5–2.5)	2.0 (0.2–5.1)	1.3 (0.2–4.6)	0.464	0.632	0.835
Lachnoclostridium	0.5 (0.3–0.5)	0.4 (0.2–1.7)	1.2 (0.1–3.2)	0.6 (0.008–1.0)	0.5 (0.03–1.8)	0.3 (0.01–0.7)	0.3 (0.2–0.5)	0.5 (0.1–2.3)	0.4 (0.2–1.0)	0.376	0.304	0.172
Listeria	0.4 (0.1–1.1)	0.3 (0.2–0.6)	0.5 (0.3–0.8)	0.1 (0.01–0.7)	0.2 (0.06–0.3)	0.5 (0.5–0.5)	0.2 (0.08–0.7)	0.3 (0.1–0.4)	0.2 (0.07–0.2)	0.159	0.242	0.103
Mediterraneibacter	0.9 (0.2–2.1)	1.1 (0.3–2.5)	1.1 (0.2–9.7)	0.6 (0.02–4.2)	1.0 (0.2–1.7)	0.9 (0.09–6.0)	0.9 (0.5–3.4)	1.5 (0.7–2.7)	1.6 (0.3–2.7)	0.783	0.213	0.706
Megasphaera	0.3 (0.02–1.9)	0.2 (0.03–2.7)	0.1 (0.03–2.0)	0.4 (0.05–2.1)	0.8 (0.3–2.2)	1.0 (0.2–4.5)	0.6 (0.3–3.9)	0.5 (0.07–1.5)	0.3 (0.1–1.3)	0.709	0.724	0.129
Mogibacterium	0.4 (0.05–1.1)	0.3 (0.06–1.8)	0.5 (0.07–6.3)	0.3 (0.04–0.7)	0.3 (0.08–0.7)	0.2 (0.05–0.4)	1.0 (0.2–1.9)	0.4 (0.06–2.6)	0.6 (0.03–3.0)	0.405	0.7	0.705

Table 1. *Cont.*

	Standard-Dose DMB (150 mg) (*n* = 8)			High-Dose DMB (300 mg) (*n* = 6)			Placebo (*n* = 7)			*p*-Value		
Genus	**Baseline**	**1 Month**	**3 Months**	**Baseline**	**1 Month**	**3 Months**	**Baseline**	**1 Month**	**3 Months**	**Treatment (T)**	**Time (t)**	**T × t**
Novisyntrophococcus	0.1 (0.01–0.4)	1.2 (0.02–1.9)	1.8 (0.07–4.0)	3.2 (0.01–6.3)	1.1 (0.01–2.2)	5.4 (5.4–5.4)	0.6 (0.04–2.3)	2.8 (0.3–5.5)	0.6 (0.06–4.4)	0.317	0.366	0.249
Parvimonas	0.4 (0.04–2.8)	0.3 (0.06–3.4)	1.4 (0.02–5.2)	1.4 (0.3–2.0)	0.8 (0.3–2.5)	0.7 (0.2–1.6)	0.2 (0.01–6.1)	0.2 (0.003–6.9)	1.5 (0.05–17.7)	0.406	0.427	0.581
Romboutsia	0.2 (0.07–0.6)	0.09 (0.05–0.9)	0.5 (0.2–0.8)	0.3 (0.09–0.6)	0.3 (0.2–1.0)	0.2 (0.02–0.3)	0.4 (0.03–1.0)	0.1 (0.04–2.8)	0.5 (0.05–1.6)	0.385	0.536	0.557
Rothia	0.2 (0.02–0.3)	0.06 (0.01–0.2)	0.08 (0.03–0.3)	0.06 (0.02–0.5)	0.05 (0.02–0.1)	0.04 (0.009–0.1)	0.08 (0.01–0.3)	0.2 (0.01–0.3)	0.07 (0.01–0.7)	0.627	0.661	0.147
Schaalia	0.1 (0.07–0.8)	0.1 (0.02–0.9)	0.3 (0.2–0.4)	0.1 (0.02–0.4)	0.1 (0.03–0.3)	0.2 (0.07–0.2)	0.2 (0.08–1.1)	0.2 (0.07–0.5)	0.1 (0.05–0.2)	0.553	0.211	0.52
Staphylococcus	0.2 (0.1–0.5)	0.2 (0.1–0.4)	0.3 (0.3–0.3)	0.1 (0.03–0.5)	0.1 (0.07–0.3)	0.6 (0.6–0.6)	0.2 (0.07–0.3)	0.3 (0.2–1.6)	0.1 (0.1–0.3)	0.693	0.478	0.053
Streptococcus	69.5 (61.3–71.2)	64.4 (56.6–75.3)	68.7 (37.2–77.3)	72.0 (53.1–94.0)	76.3 (60.0–84.6)	69.0 (53.3–84.9)	65.0 (49.7–85.3)	63.0 (33.7–76.9)	68.0 (41.3–72.6)	0.236	0.525	0.789
Veillonella	9.3 (3.3–19.6)	6.6 (3.5–24.8)	2.4 [a] (0.06–5.5)	4.3 (1.4–5.6)	7.9 (3.8–23.7)	6.6 [b] (2.7–10.6)	10.5 (3.1–20.1)	5.4 (2.3–13.3)	5.6 [b] (4.1–8.0)	0.956	0.052	0.015
Shannon index	1.4 (1.2–1.5)	1.5 (1.1–2.0)	1.4 (1.1–2.6)	1.2 (0.4–2.1)	0.8 (0.6–1.7)	1.3 (0.8–1.7)	1.5 (0.8–1.9)	1.6 (1.1–2.6)	1.4 (1.1–2.2)	0.516	0.373	0.591
Simpson's index	0.5 (0.5–0.6)	0.6 (0.4–0.7)	0.5 (0.4–0.8)	0.5 (0.1–0.7)	0.4 (0.3–0.6)	0.5 (0.3–0.7)	0.6 (0.3–0.7)	0.6 (0.4–0.9)	0.5 (0.5–0.8)	0.673	0.428	0.714
Chao1 index	41.0 (22.8–60.0)	39.9 (32.0–57.2)	38.0 (24.3–64.0)	37.5 (21.0–47.0)	32.4 (4.0–79.0)	38.6 (30.2–52.0)	36.2 (22.0–49.0)	39.6 (24.8–66.0)	41.5 (23.8–54.7)	0.575	0.411	0.405

Values are presented as median and range. Different letters mean significant differences ($p < 0.05$) and were calculated following a general linear model of covariance followed by the Bonferroni correction for multiple tests.

Table 2. Relative abundances of species for oral bacteria in malnourished patients with cancer and dysgeusia who received the standard dose of DMB (150 mg), the high dose of DMB (300 mg), or the placebo for 3 months.

	Standard-Dose DMB (150 mg) (*n* = 8)			**High-Dose DMB (300 mg) (*n*= 6)**			**Placebo (*n* = 7)**			***p*-Value**		
Species	**Baseline**	**1 Month**	**3 Months**	**Baseline**	**1 Month**	**3 Months**	**Baseline**	**1 Month**	**3 Months**	**Treatment (T)**	**Time (t)**	**T × t**
Gemella haemolysans	0.3 (0.06–1.4)	0.2 (0.03–0.6)	0.3 (0.07–1.2)	0.6 (0.03–7.6)	0.5 (0.2–0.7)	0.5 (0.03–2.7)	0.7 (0.1–6.1)	0.7 (0.1–2.2)	1.8 (0.3–6.7)	0.105	0.297	0.517
Gemella morbillorum	0.1 (0.06–0.9)	0.2 (0.07–0.6)	0.3 (0.07–1.2)	0.4 (0.04–2.2)	0.4 (0.03–1.7)	0.08 (0.06–0.5)	0.3 (0.03–3.9)	0.6 (0.08–9.4)	2.5 (0.2–7.2)	0.103	0.17	0.054
Gemella sanguinis	3.7 (1.4–12.8)	5.3 (0.6–16.8)	7.5 (1.3–15.1)	1.0 (0.009–8.0)	1.9 (0.1–6.0)	1.1 (0.06–9.7)	2.2 (1.2–6.1)	3.2 (1.8–6.2)	4.0 (0.4–7.0)	0.312	0.352	0.769
Gemella sp. zg-570	0.06 (0.01–0.5)	0.06 (0.02–0.2)	0.1 (0.04–0.3)	0.3 (0.04–1.8)	0.3 (0.2–0.4)	0.4 (0.01–0.6)	0.2 (0.04–1.3)	0.4 (0.06–0.5)	0.4 (0.06–0.8)	0.102	0.3	0.584
Granulicatella adiacens	4.8 (1.6–14.7)	3.7 (2.2–8.0)	4.8 (1.4–9.2)	1.4 (0.09–7.4)	1.6 (0.3–4.1)	4.0 (0.8–23.6)	3.0 (1.4–8.8)	4.7 (1.3–7.5)	2.6 (1.3–3.1)	0.396	0.484	0.069
Granulicatella elegans	0.01 (0.004–0.1)	0.02 (0.01–0.1)	0.03 [a] (0.01–0.07)	0.02 (0.009–0.5)	0.6 (0.6–0.6)	0.01 [b] (0.006–0.02)	0.03 (0.01–0.2)	0.01 (0.01–0.3)	0.02 [b] (0.005–0.05)	<0.001	<0.001	<0.001
Streptococcus agalactiae	10.5 (5.1–15.4)	10.0 (5.6–14.2)	9.8 (6.0–13.9)	10.9 (5.5–14.1)	7.8 (6.6–11.9)	8.0 (5.9–11.4)	9.2 (7.3–12.6)	8.7 (6.2–14.2)	7.9 (7.3–16.3)	0.878	0.385	0.586
Streptococcus australis	0.1 (0.01–0.7)	0.07 (0.02–0.2)	0.06 (0.006–0.2)	0.03 (0.02–0.03)	0.03 (0.03–0.03)	0.06 (0.06–0.06)	0.01 (0.01–0.07)	0 (0–0)	0.6 (0.6–0.6)	0.218	0.301	0.063
Streptococcus cristatus	0.4 (0.04–1.6)	0.3 (0.06–1.5)	0.1 (0.03–3.0)	0.4 (0.1–4.0)	1.2 (0.1–1.5)	0.2 (0.04–0.7)	0.7 (0.06–3.4)	0.4 (0.06–0.7)	0.3 (0.05–2.2)	0.733	0.149	0.087
Streptococcus dysgalactiae	5.0 (3.3–6.3)	4.8 (2.9–7.5)	4.2 (2.5–5.8)	4.5 (3.3–5.6)	3.8 (3.3–6.0)	3.6 (2.7–5.3)	3.9 (2.8–6.2)	3.6 (3.1–5.5)	3.9 (3.1–6.3)	0.583	0.261	0.666
Streptococcus infantis	1.0 (0.7–2.4)	1.0 (0.6–2.8)	0.9 (0.4–2.3)	0.9 (0.5–1.2)	0.7 (0.5–1.3)	0.7 (0.6–1.0)	0.9 (0.7–1.6)	0.9 (0.6–1.7)	1.1 (0.7–1.2)	0.321	0.844	0.307
Streptococcus mutans	0.3 (0.2–2.9)	0.3 (0.2–3.5)	0.4 [a] (0.2–3.4)	1.9 (0.3–2.7)	2.2 (0.4–7.2)	0.6 [b] (0.3–1.8)	0.4 (0.2–1.2)	0.3 (0.2–0.3)	0.6 [b] (0.2–0.9)	0.022	0.125	0.012
Streptococcus parasanguinis	1.2 (0.09–3.9)	1.2 (0.5–1.6)	0.8 [a] (0.2–2.2)	0.3 (0.09–1.4)	0.6 (0.09–1.8)	1.5 [b] (0.1–2.5)	0.5 (0.2–1.3)	0.8 (0.09–3.6)	0.5 [c] (0.1–2.3)	0.78	0.746	0.018

Table 2. *Cont.*

	Standard-Dose DMB (150 mg) (*n* = 8)			High-Dose DMB (300 mg) (*n*= 6)			Placebo (*n* = 7)			*p*-Value		
Species	**Baseline**	**1 Month**	**3 Months**	**Baseline**	**1 Month**	**3 Months**	**Baseline**	**1 Month**	**3 Months**	**Treatment (T)**	**Time (t)**	**T × t**
Streptococcus pneumoniae	13.5 (5.5–17.3)	13.1 (6.1–24.2)	11.3 (6.8–20.3)	12.2 (4.8–17.4)	9.6 (5.3–15.5)	8.9 (5.2–14.5)	10.4 (6.8–15.5)	9.5 (6.8–14.0)	10.7 (6.8–21.5)	0.577	0.734	0.429
Streptococcus pyogenes	2.9 (2.5–3.4)	2.8 (2.1–3.3)	2.8 (1.4–3.1)	2.8 (2.0–4.4)	2.6 (2.1–3.6)	2.5 (2.1–3.6)	2.4 (2.0–3.5)	2.6 (1.6–3.7)	2.6 (2.3–3.4)	0.948	0.363	0.727
Streptococcus salivarius	2.9 (1.3–22.1)	2.6 (1.4–11.7)	2.4 (1.0–22.8)	3.1 (1.6–35.2)	4.2 (2.0–34.8)	12.6 (1.3–23.5)	3.8 (1.0–26.5)	6.3 (1.4–12.4)	10.0 (1.7–10.6)	0.57	0.758	0.713
Streptococcus sp. *HSISS1*	5.9 (1.0–12.6)	7.1 (0.1–12.7)	4.3 (0.5–11.5)	4.6 (0.4–12.8)	4.3 (1.3–6.5)	3.8 (1.8–9.7)	5.0 (2.1–10.0)	4.5 (0.3–9.4)	3.5 (1.5–8.8)	0.809	0.814	0.681
Streptococcus sp. *LPB0220*	1.8 (0.6–10.7)	3.3 (0.9–6.8)	1.3 (0.5–9.8)	3.5 (0.5–13.0)	5.9 (1.4–10.8)	8.0 (2.7–11.1)	5.9 (0.3–12.4)	0.8 (0.1–11.3)	1.6 (0.08–7.2)	0.446	0.802	0.308
Streptococcus suis	3.4 (2.0–6.3)	3.5 (1.9–4.8)	2.9 (1.5–4.9)	3.5 (1.6–8.4)	3.6 (2.0–7.6)	4.1 (1.3–6.2)	2.6 (1.4–7.0)	3.5 (1.1–4.9)	3.2 (1.6–4.5)	0.635	0.885	0.927
Streptococcus thermophilus	8.9 (7.7–10.1)	8.5 (7.3–10.3)	8.6 (3.9–9.5)	9.6 (6.4–16.7)	9.5 (6.9–16.8)	9.2 (6.4–13.5)	7.4 (6.4–12.9)	8.3 (4.4–10.1)	8.6 (5.6–10.2)	0.401	0.329	0.243
Lachnoanaerobaculum umeaense	1.4 (0.06–4.0)	1.4 (0.2–3.8)	0.9 (0.1–9.0)	0.1 (0.05–3.9)	0.9 (0.1–1.7)	0.5 (0.03–5.4)	1.1 (0.5–2.5)	2.0 (0.2–5.2)	1.6 (0.2–4.6)	0.458	0.62	0.868
Veillonella atypica	1.5 (0.01–10.2)	1.6 (0.02–13.7)	0.3 (0.03–3.0)	0.5 (0.07–3.3)	0.6 (0.6–6.2)	2.5 (1.0–4.0)	4.1 (0.3–10.7)	1.4 (0.1–8.4)	2.8 (1.0–4.3)	0.437	0.64	0.129
Veillonella nakazawae	0.7 (0.1–1.0)	0.5 (0.1–1.7)	0.3 (0.2–0.3)	0.1 (0.1–0.3)	0.3 (0.2–0.5)	0.3 (0.1–0.4)	0.1 (0.04–0.6)	0.4 (0.4–0.4)	0.05 (0.05–0.08)	0.158	0.683	0.946
Veillonella parvula	6.9 (2.8–9.6)	4.8 (2.9–15.8)	2.1 [a] (0.03–4.9)	3.4 (1.3–3.9)	5.1 (3.1–7.1)	4.7 [b] (0.5–7.9)	4.6 (1.3–9.6)	3.6 (0.5–8.9)	2.9 [b] (1.8–5.2)	0.608	0.053	0.043
Shannon index	3.1 (2.8–3.3)	3.1 (2.7–3.5)	3.0 (2.9–3.6)	3.1 (2.6–3.5)	3.1 (2.6–3.4)	3.0 (2.9–3.2)	3.2 (2.7–3.5)	3.2 (2.9–3.7)	3.3 (2.8–3.4)	0.473	0.851	0.948
Simpson's index	0.9 (0.9–0.9)	0.9 (0.9–1.0)	0.9 (0.9–1.0)	0.9 (0.8–0.9)	0.9 (0.8–0.9)	0.9 (0.9–0.9)	0.9 (0.9–0.9)	0.9 (0.9–1.0)	0.9 (0.9–0.9)	0.584	0.998	0.895
Chao1 index	48.0 (21.0–89.0)	40.1 (21.9–80.0)	42.6 (26.6–65.7)	39.2 (27.2–51.8)	46.5 (26.0–69.3)	33.1 (24.1–52.3)	37.0 (26.6–55.7)	36.7 (30.0–59.0)	36.3 (26.5–59.0)	0.339	0.339	0.588

Values are presented as median and range. Different letters mean significant differences ($p < 0.05$) and were calculated following a general linear model of covariance followed by the Bonferroni correction for multiple tests.

Granulicatella elegans exhibited the greatest variation ($p < 0.01$). Patients with cancer receiving a standard dose of DMB were found to have a greater presence of that bacteria in their oral microbiome compared with patients receiving a high dose of DMB and placebo. There were substantial changes in two species of *Streptococcus*, *Streptococcus mutans* ($p = 0.012$) and *Streptococcus parasanguinis* ($p = 0.018$). For *Streptococcus mutans*, we did not find statistical differences among groups at baseline; however, we observed higher abundances in the standard-dose DMB group compared to both the high dose of DMB and placebo groups at 3 months after intervention with DMB. The levels of *Streptococcus parasanguinis* in the DMB groups exhibited an inverse pattern, with lower abundances in the standard dose DMB group, whereas the abundances in the placebo group remained unchanged. Within the DMB groups, *Veillonella parvula* exhibited opposite variations, with significantly lower abundances in the standard dose DMB group compared with both the high-dose DMB and the placebo groups (Table 2).

3.5. Microbiome Balance

The Rivera–Pinto method [61] was employed to ascertain the microbiome balance at the end of the trial. The analysis revealed that the standard-dose DMB group was characterized by lower balance scores for *Streptococcus mutans* and *Bacillus* when compared to *Gemella* sp. zg-570 and *Veillonella* genera (Figure 2A). On the contrary, *Gemella* sp. zg-570 and *Veillonella* genera were more associated with the placebo group than with the standard-dose DMB group (Figure 2A). Concerning the high-dose DMB group versus the placebo group, *Gemella sanguinis* and *Streptococcus lutetiensis* were most associated with the placebo group (Figure 2B). Thus, at the higher dose of DMB, lower balance scores were associated with lower relative abundances of *Streptococcus intermedius* and *Streptococcus agalactiae* when compared to *Gemella sanguinis* and *Streptococcus lutetiensis* (Figure 2B).

3.6. Analysis of the Relationships between Oral Microbiome, Inflammatory Cytokines, Nutritional Status, and Electrical Taste Perception in the Three Groups

There were significant correlations between the abundance of several species in the standard dose DMB group and a variety of outcomes, as shown in Figure 3A. The abundance of *Streptococcus* genus, particularly species of *Streptococcus thermophilus*, *Streptococcus pneumoniae*, *Streptococcus dysgalactiae* and *Streptococcus agalactiae*, correlated negatively with the concentration levels of TNF-α in patients with cancer. Furthermore, energy intake (%) and lipid percentage of energy, as well as monounsaturated fatty acids (MUFAs) and polyunsaturated fatty acids (PUFAs) expressed as percentages of energy, were positively correlated with those bacteria. Additionally, the MUFAs and PUFAs percentages of energy were positively associated with *Veillonella parvula* levels (Figure 3A). The group that received the highest dose of DMB showed several correlations with other outcomes of the study (Figure 3B). Indeed, the relative abundance of *Streptococcus pneumoniae*, *Streptococcus dysgalactiae*, and *Streptococcus agalactiae* was positively correlated with energy intake. Saturated fatty acids were positively associated with *Streptococcus pneumoniae* and *Streptococcus dysgalactiae* levels. Lastly, *Granulicatella adiacens* was negatively associated with TNF-α concentration (Figure 3B). In the placebo group, the relative abundance of *Granulicatella adiacens* was negatively correlated with the energy intake. The levels of *Streptococcus thermophilus* were negatively correlated with electric taste perception on both the right and left sides. The PUFAs intake was positively associated with *Streptococcus agalactiae* (Figure 3C). Neither selected bacteria (*Granulicatella adiacens*, *Streptococcus agalactiae*, *Streptococcus dysgalactiae*, *Streptococcus pneumoniae*, and *Veillonella parvula*) nor electrogustometry, the type of diet, or cytokine levels were associated with mucositis, according to the binary logistic regression. There were only two variables that remained in the equation without reaching statistical significance: TNF-α levels ($p = 0.109$) and saturated fatty acids ($p = 0.142$). Moreover, no independent correlation was found between the presence of selected species and clinical outcomes evaluated as immunotherapeutic agents used for the cancer treatment (Wald, 0.011; *p*-value, 0.918; OR, 1.010; 95% CI of OR, 0.832–1.227), general toxicity (Wald,

0.605; *p*-value, 0.437; OR, 0.928; 95% CI of OR, 0.768–1.121), digestive toxicity (Wald, 0.931; *p*-value, 0.335; OR, 1.877; 95% CI of OR, 0.520–6.748), or consumption of tobacco (Wald, 1.438; *p*-value, 0.230; OR, 2.302; 95% CI of OR, 0.586–9.182) and alcohol (Wald, 1.042; *p*-value, 0.307; OR, 0.674; 95% CI of OR, 0.316–1.438).

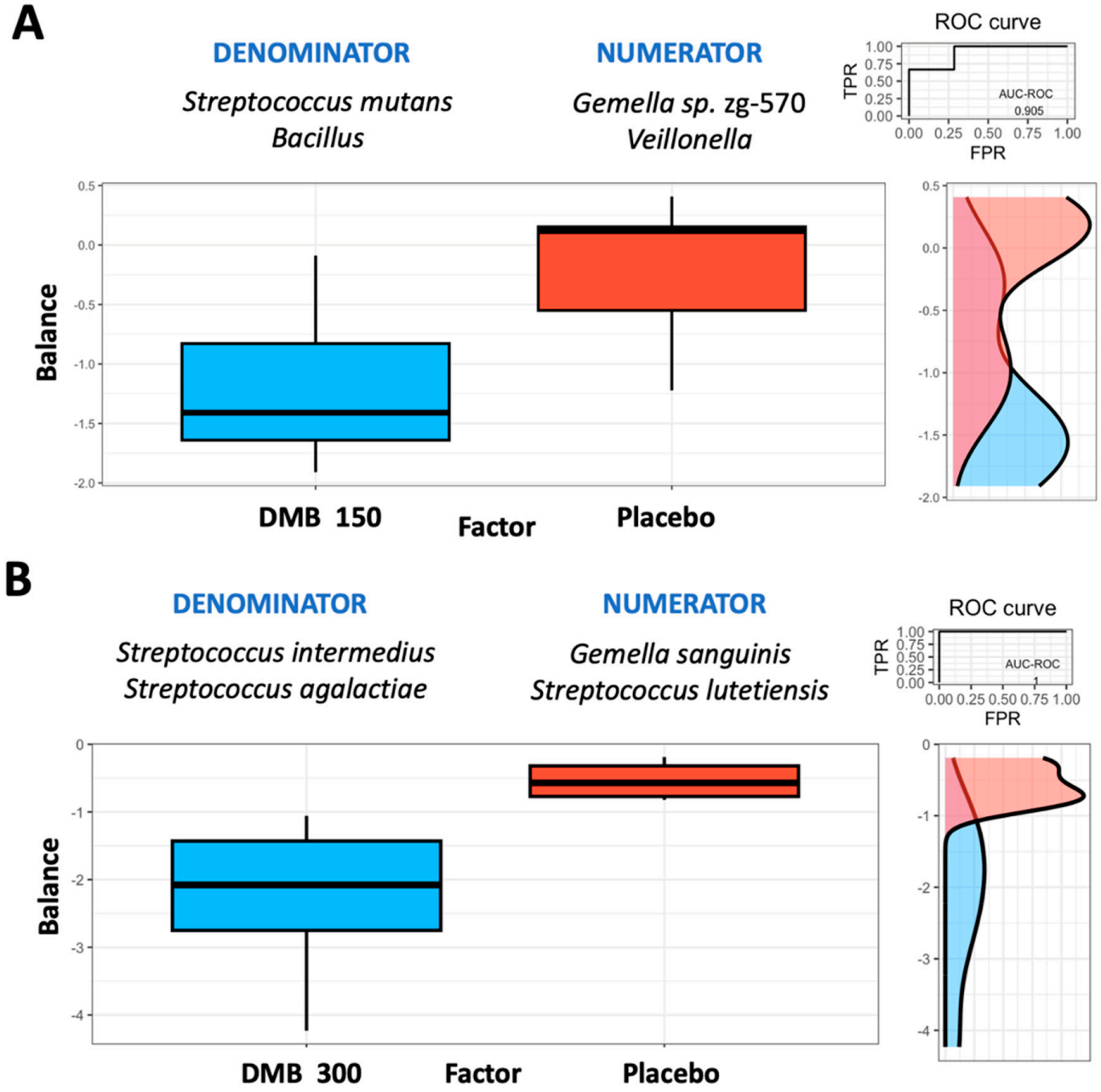

Figure 2. Group microbial balances are presented in an overview. It is indicated at the top of the plot that groups of taxa constitute the global balance. Box plots illustrate the distribution of balance scores for the DMB 150 mg (standard dose) and placebo groups (**A**) and the DMB 300 mg (high dose) and placebo groups (**B**). On the right, the ROC curve with its AUC value and the density curve are displayed.

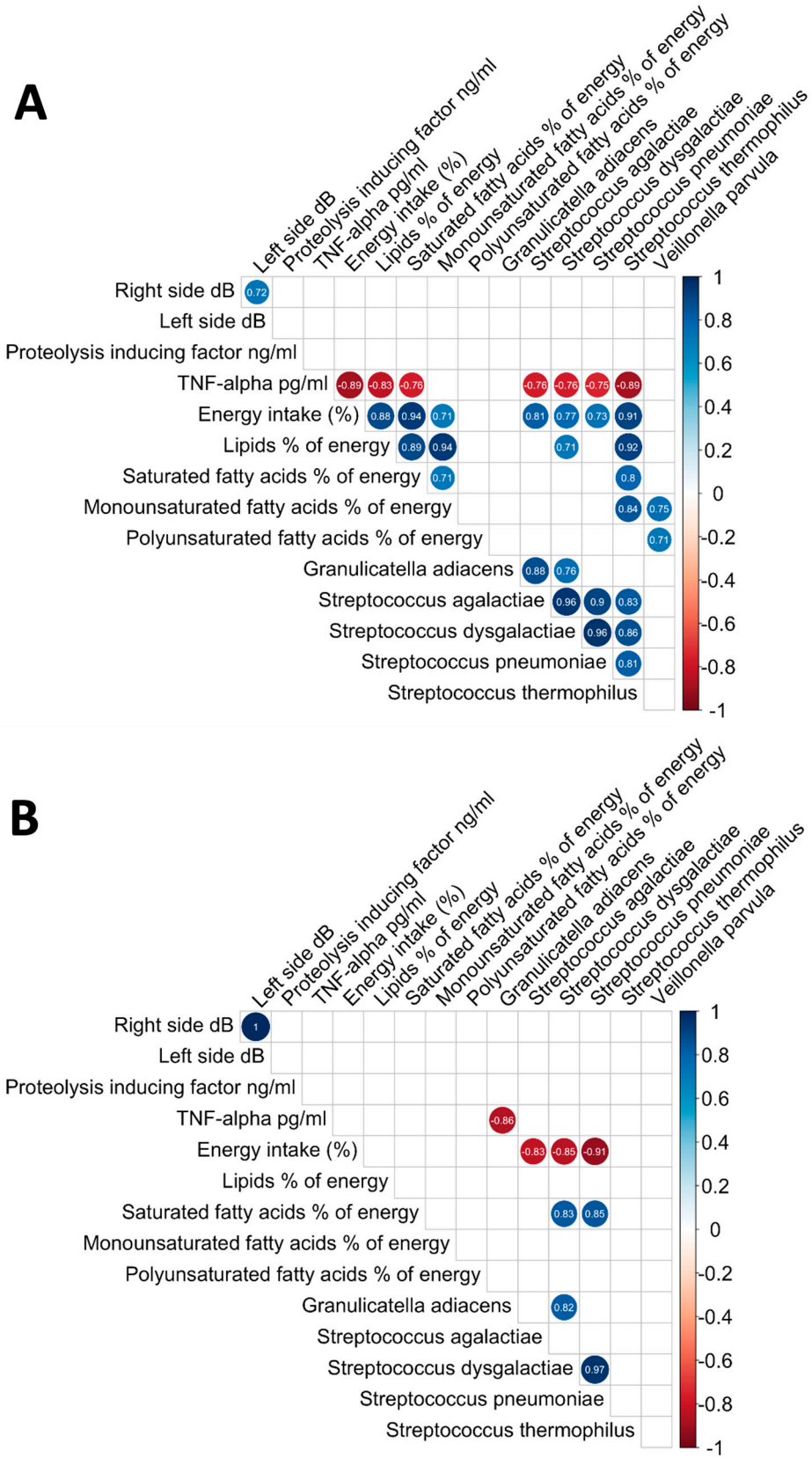

Figure 3. *Cont.*

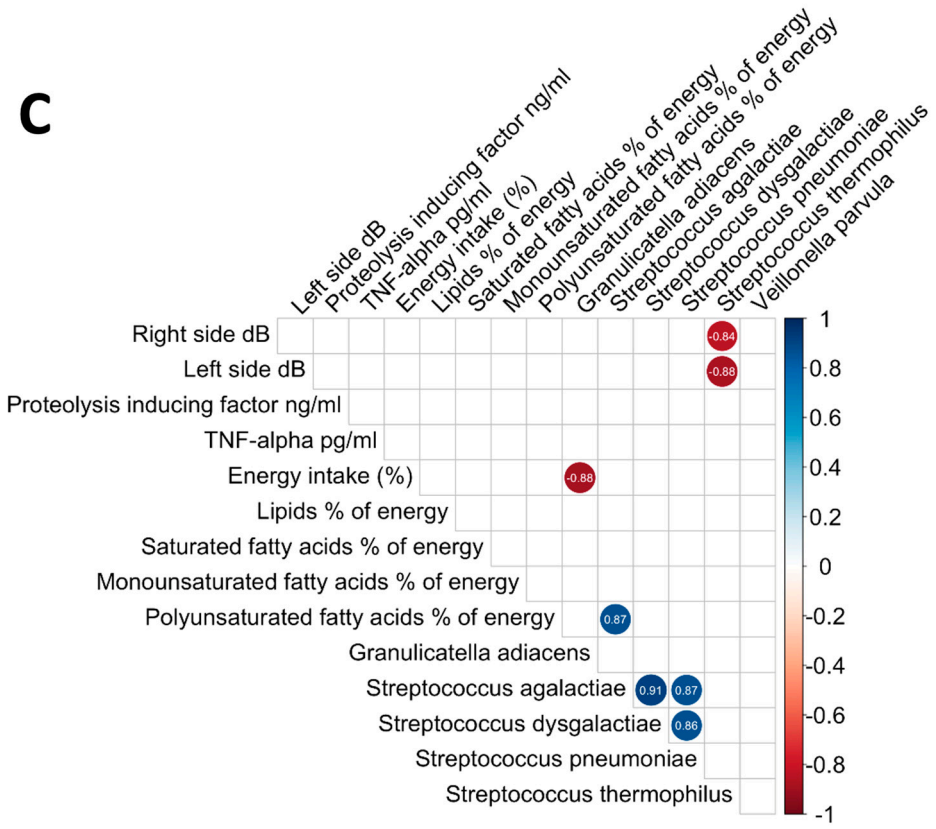

Figure 3. Correlations between the oral microbiome, inflammatory cytokines, nutritional status, and electrical taste perception. (**A**). DMB 150 mg (standard dose), (**B**). DMB 300 mg (high dose), and (**C**). placebo. Right and left side measures refer to electrogustometry variables; dB, the threshold of taste perception measured in decibels.

4. Discussion

In the present study, patients with cancer, malnourished and with dysgeusia presented dysbiosis. We have shown that the regular consumption of 150 mg of DMB (standard dose), as an adjuvant to medical–nutritional treatment, changed the oral microbiome composition in these patients receiving antineoplastic treatment. In particular, the consumption of the standard dose of DMB resulted in a greater relative abundance of *Enterococcus* and a lower abundance of the *Veillonella* genus than the consumption of a high dose of DMB or placebo. Additionally, some species such as *Granulicatella elegans*, *Granulicatella adiacens*, *Streptococcus mutans*, and *Gemella morbillorum* exhibited greater relative abundances in patients receiving the standard dose of DMB. However, lower abundances of *Streptococcus parasanguinis*, *Veillonella parvula*, *Streptococcus australis* and *Streptococcus cristatus* were detected. The consumption of the standard dose of DMB revealed a link between several *Streptococcus* species and lower TNF-α plasma levels as well as higher energy and plasma MUFAs and PUFAs dietary intake.

The oral microbiota is composed of a multitude of bacterial species, including *Actinomycetota* (formerly *Actinobacteria*), *Bacteroidota* (*Bacteroidetes*), *Bacillota* (*Firmicutes*), *Fusobacteriota* (Fusobacteria), *Pseudomonadota* (*Proteobacteria*), *Saccharibacteria* and *Spirochaetota* (*Spirochaetes*) [24]. These bacterial species are generally conserved across individuals, making up the bulk of the oral microbiota [63], which plays a relevant role in oral health [64]. The mechanism by which commensals promote healthy oral microbiota involves outcompeting pathogens for colonization [65]. Microbes temporarily overpower the immune system in the event of reduced diversity and richness, which is described as dysbiosis [66,67]. Nevertheless, the oral microbiome serves more than just a local function, as microbes are capable of communicating with the entire body [68]. The presence of oral dysbiosis can

contribute to the maintenance of chronic low-grade systemic inflammation by causing a localized inflammatory state within the oral cavity [69].

Cancer can be affected by microbes in a variety of ways, including contact-dependent effects occurring locally at the mucosal surface or within the tumor microenvironment. The second type of effect is contact-independent effects, which are caused by the metabolites produced by microbes and the vesicles of their outer membranes that circulate in the blood [70,71]. Thus, several types of cancer may be associated with specific patterns of salivary and fecal microbiomes as well as circulating microbial DNA in blood plasma [72].

Alterations in the oral microbiome that are associated with cancer treatments cause dysbiosis, which is a condition characterized by an imbalanced status of the oral microbial community [73]. Thus, the dysbiosis of the oral microbiome is characterized by an increase in the prevalence of pathogenic microbial species at the expense of commensal microorganisms and a markedly reduced richness and diversity of species [74,75]. Taste disorders are frequently reported by oncologic patients undergoing antineoplastic treatments [3,4]. Consequently, taste disorders have a significant impact on eating behaviors and, as a result, have a major influence on overall health [64]. In addition, this alteration might cause oral microbiome dysbiosis during cancer therapies, which is characterized by a markedly reduced richness and diversity of species [75]. Here, we observed that the richness and diversity, measured by the Shannon and Simpson indices, along with the Chao1 index, were reduced in our patients with cancer compared to the levels reported in healthy individuals [62], indicating basal dysbiosis in all groups.

Gemella was the fourth most prevalent genera in our study, which is followed by *Streptococcus*, *Veillonella*, and *Granulicatella*. *Gemella sanguinis* was the most prevalent *Gemella* species in our study. A significantly greater enrichment of the *Gemella* genus in oral squamous cell carcinoma has been reported. In oral leukoplakia and oral squamous cell carcinoma, *Streptococcus* sp. NPS 308, *Streptococcus agalactiae*, *Gemella hemolysans*, and *Gemella morbillorum* were slightly increased [76]. A high relative abundance of *Gemella morbillorum* is present in oral cavity squamous cell carcinoma tumor tissues compared with the paired adjacent normal tissues [77]. Here, we observed a tendency for *Gemella morbillorum* to be more abundant in the standard dose of DMB group; however, lower levels were observed in the high dose of DMB group, indicating a different profile that was dose-dependent for this bacterium. In vitro studies with three oral squamous cell carcinoma cell lines (CAL27, SCC4, and SCC25) have demonstrated the positive effects of oral commensals belonging to the *Streptococcus* genus [78]. In our patients with cancer, these species make up more than 63% of the total oral microbiome. According to the above data, these bacteria are capable of acting as anticancer agents.

Veillonella parvula is elevated in oral cancer [79], especially in oral squamous cell carcinoma, by promoting the expression of inflammatory cytokines, including interleukins (IL-6, IL-8) and TNF-α [80]. We found that the regular consumption of a standard dose of DMB decreases the abundance of *Veillonella parvula*, which is an important oncologically related bacteria after 3 months of treatment [80]. The lower relative abundance of this bacteria in the standard-dose DMB group could be related to the association with the TNF-α levels observed in the present study. After 3 months of intervention, the high-dose DMB group presented increased *Veillonella parvula*, and the dose here might have contradictory results.

At the species level, we also found that the relative abundance of *Granulicatella elegans*, *Granulicatella adiacens*, and *Streptococcus mutans* was greater in patients receiving the standard dose of DMB. However, lower abundances of *Streptococcus parasanguinis*, *Streptococcus australis* and *Streptococcus cristatus* were detected.

Regarding the genus *Granulicatella*, we observed a significant variation in the abundance of *Granulicatella elegans* with an increase in the standard dose DMB group and a decrease in the high-dose DMB group. This is of interest, since a positive association has been found between the abundance of *Granulicatella elegans* and inflammation [81]. In

the case of *Granulicatella adiacens*, higher levels at both standard and high doses of DMB were observed.

Microbiome-associated pathology can arise from changes in general bacterial composition, such as those found in periodontitis, and from the colonization and overgrowth of keystone species [82]. The infiltration of immunosuppressive cells and the interference of immune killer cells by commonly occurring oral microorganisms, such as *Fusobacterium nucleatum* and *Porphyromonas gingivalis*, can prevent tumor cells from being observed and cleared by the immune system [83–85]. There is evidence that a high abundance of *Fusobacterium nucleatum* in the oral microbiome of patients with colorectal cancer is associated with tumor metastasis, recurrence, chemo-resistant cancer, and reduced radiotherapy efficacy [86–89]. In our study, the absence of periodontitis and gingivitis in these patients with cancer may explain why the aforementioned species were not detected in the samples. It is still necessary to conduct large cohort and case-control studies to validate the hypothesis that periodontal disease contributes to the initiation and development of cancer [90].

Further, in our study, neither of the highly represented bacteria was associated with the presence or absence of mucositis at the end of the study based on binary logistic regression. It is also important to note that no independent correlation was found between the presence of selected species and clinical outcomes evaluated as immunotherapeutic agents used for cancer treatment, general toxicity, digestive toxicity, or the consumption of tobacco and alcohol.

The oral *Streptococcus* genus plays a key role in oral dysbiosis and a wide range of clinical conditions, including dental caries, gingivitis, periodontal disease, and oral cancer [91]. We found that *Streptococcus thermophilus*, *Streptococcus pneumoniae*, *Streptococcus dysgalactiae*, and *Streptococcus agalactiae* were negatively associated with the plasma levels of TNF-α in malnourished patients with cancer receiving a standard dose of DMB, which might be associated with the overall improvement of systemic inflammation after DMB supplementation. There are some strains of *Streptococcus* that have inherent antitumor activity or that can activate the immune system of the host to fight tumors [92]. Several studies have suggested that the frequent and/or excessive consumption of sugar (especially sucrose) contributes to tooth decay [93]. *Streptococcus mutans* is thought to play a critical role in the metabolism of sucrose, producing lactic acid, which is capable of demineralizing enamel [94]. A possible relationship between *Streptococcus* species, diet, and caries etiology is illustrated here. As a result of our study, the evaluated *Streptococcus* species were positively associated with energy intake, lipid percentage of energy, plasma MUFAs and PUFAs, expressed as energy percentages, indicating that diet can have a significant impact on the main genus of the oral microbiome.

Moreover, the percentages of energy-related MUFAs and PUFAs were positively associated with *Veillonella parvula* levels. This finding is of interest, since we observed an improvement in the quality of life in malnourished patients with cancer who received a standard dose of DMB [41]. Therefore, the oral intake of DMB might improve the pattern of oral bacteria and in turn reduce inflammation. In the case of the group that received high doses of DMB, we observed that the relative abundances of *Streptococcus pneumoniae*, *Streptococcus dysgalactiae*, and *Streptococcus agalactiae* were positively correlated with energy intake. Saturated fatty acids were positively associated with *Streptococcus pneumoniae* and *Streptococcus dysgalactiae* levels. The abundance of *Granulicatella adiacens* was negatively associated with the plasma TNF-α concentration, which could be related to a slight anti-inflammatory effect. It should be necessary to evaluate a complete panel of cytokines to assess the potential systemic inflammation associated with oral dysbiosis. It is necessary to evaluate a panel of cytokines in order to assess the systemic inflammation associated with oral dysbiosis. Finally, there was no correlation between changes in the oral microbiome of the DMB groups and electric taste perception. It was only in the placebo group that *Streptococcus thermophilus* abundance was negatively correlated with electric taste perception.

Streptococcus mutans is involved in the etiology of several oral diseases [95–97]. In particular, *Streptococcus mutans* is implicated as the main etiologic agent of caries [98–100]. Dysbiosis of the dental plaque microbiome is associated with an abundance of biofilm-forming, acid-producing, and acid-tolerant species. In the standard-dose DMB group, the relative abundance of *Streptococcus mutans* increased slightly, whereas the increase in the placebo group was twice as high. This slight increase in the standard-dose DMB group might be linked to increased energy consumption by those patients.

In contrast, a lower relative abundance of *Streptococcus mutans* was observed in patients receiving high doses of DMB. Nevertheless, compared with patients in the standard dose group, these patients did not show an improvement in quality of life [41]. While *Streptococcus mutans* is associated with caries, *Streptococcus cristatus* plays a relevant role in the development of periodontitis, a common oral disease, and it may contribute to the pathogenicity of the oral microbiome [101,102]. We observed that *Streptococcus cristatus* was lower in all three groups post-intervention, which was possibly as a result of the absence of periodontitis in the present population.

Overall, the changes in the oral microbiota observed in patients having the standard dose of DMB differed from those having the higher dose. This could be due to the fact that the latter group manifested a sweet taste for a longer period of time after the consumption of the DMB tablet, compared to the former, which in turn results in a lower dietary intake [41].

5. Conclusions

To identify innovative therapies for the treatment of taste disorders in patients with cancer, we conducted this pilot randomized, parallel, triple-blind, and placebo-controlled clinical trial aimed at providing a novel strategy for reducing the side effects of chemotherapy, radiotherapy, and immunotherapy, including alterations in body composition, nutritional status, and quality of life [41]. All patients presented dysbiosis in terms of bacterial diversity and richness compared with healthy individuals. Here, we showed that the regular consumption of a standard dose of DMB, as an adjuvant to medical–nutritional treatment, could modify the oral microbiome composition in malnourished patients with cancer receiving antineoplastic treatment. Thus, we reported that the relative abundances of *Enterococcus* were higher in patients with cancer receiving a standard dose of DMB at 3 months after intervention. However, lower abundances of *Streptococcus parasanguinis*, *Veillonella parvula*, and *Streptococcus mutans* were detected. Furthermore, DMB consumption was negatively associated with *Streptococcus thermophilus*, *Streptococcus pneumoniae*, *Streptococcus dysgalactiae*, and *Streptococcus agalactiae* and with TNF-α plasma concentrations in patients with cancer and taste disorders. The presence of *Streptococcus thermophilus* and *Veillonella parvula* was positively associated with plasma MUFAs with only *Veillonella parvula* being associated with plasma PUFAs. Overall, DMB intake could modify the oral microbiome in patients with cancer and dysgeusia, which may contribute to a better immune response. There is still a need for further studies with a large number of patients and variables to be measured.

Supplementary Materials: The following supporting information can be downloaded at https://www.mdpi.com/article/10.3390/cancers16193414/s1, Table S1: Nutritional composition of the food supplement enriched in miraculin (DMB) and placebo; Table S2. Cancer types and chemotherapy characteristics of the general population; Table S3: Distribution of phyla and alpha diversity indices for microbiota of saliva from cancer patients of the CLINMIR study; Table S4: Distribution of selected families for microbiota of saliva from cancer patients of the CLINMIR study.

Author Contributions: Conceptualization, B.L.-P., A.G. and S.P.-M.; methodology, B.L.-P., T.H. and J.F.-B.; software, J.P.-D.; validation, F.J.R.-O., A.I.Á.-M. and M.B.-H.; formal data analysis, J.P.-D., F.J.R.-O. and M.B.-H.; investigation, B.L.-P. and L.A.-C.; resources, S.P.-M.; data curation, L.A.-C.; writing—original draft preparation, J.P.-D., F.J.R.-O., M.B.-H. and A.G.; writing—review and editing, J.P.-D., F.J.R.-O., M.B.-H. and A.G.; supervision, S.P.-M. and A.G.; project administration, B.L.-P.; funding acquisition, S.P.-M. All authors have read and agreed to the published version of the manuscript.

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Institutional Review Board Statement: The study was conducted under the Declaration of Helsinki and approved by the Ethics Committee of La Paz University Hospital (protocol code 6164, 23 June 2022).

Informed Consent Statement: Informed consent was obtained from all subjects involved in the study.

Data Availability Statement: A reasonable request should be made to the corresponding author for access to the datasets used and/or analyzed in the current study.

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Conflicts of Interest: The authors declare that they have no commercial or financial relationships that could be construed as potential conflicts of interest.

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